Toxicology of silica nanoparticles: an update

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Received: 8 May 2017 / Accepted: 18 May 2017 / Published online: 1 June 2017
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Abstract Large-scale production and use of amorphous silica nanoparticles (SiNPs) have increased the risk of human exposure to SiNPs, while their health effects remain unclear. In this review, scientific papers from 2010 to 2016 were systematically selected and sorted based on in vitro and in vivo studies: to provide an update on SiNPs toxicity and to address the knowledge gaps indicated in the review of Napierska (Part Fibre Toxicol 7:39, 2010). Toxicity of SiNPs in vitro is size, dose, and cell type dependent. SiNPs synthesized by wet route exhibited noticeably different biological effects compared to thermal route-based SiNPs. Amorphous SiNPs (particularly colloidal and Stöber) induced toxicity via mechanisms similar to crystalline silica. In vivo, route of administration and physicochemical properties of SiNPs influences the toxicokinetics. Adverse effects were mainly observed in acutely exposed animals, while no significant signs of toxicity were noted in chronically dosed animals. The correlation between in vitro and in vivo toxicity remains less well established mainly due to improper—unrealistic—dosing both in vitro and in vivo. In conclusion, notwithstanding the multiple studies published in recent years, unambiguous linking of physicochemical properties of SiNPs types to toxicity, bioavailability, or human health effects is not yet possible.

Keywords Amorphous silica nanoparticles · Pyrogenic · Colloidal · Stöber · Oxidative stress · Toxicity

Introduction

Nanosilica, also known as the nanoform (<100 nm) of silicon dioxide or silica nanoparticles (SiNPs), possesses distinct physico-chemical characteristics compared to its bulk...
form; smaller size materials have an increased surface-to-volume ratio and a higher surface reactivity (Oberdörster 2010; Napierska et al. 2010). Due to their appealing properties, SiNPs are now extensively used in agriculture, food, and consumer products including cosmetics (Napierska et al. 2010; Khot et al. 2012; Kasaai 2015; Brinch et al. 2016). Until 2012, nearly 1.5 million tons of SiNPs had already been placed in the global market (Liljenström et al. 2013) and SiNPs became one of the three most produced nanomaterials (NMs) worldwide in 2013. Among the 846 nano-based products listed in a consumer products inventory, approximately 100 claim to contain SiNPs (Vance et al. 2015). Moreover, amorphous SiNPs are being synthesized with highly tunable biocompatibility and stability, and considered as a very promising candidate for various bio-medical applications such as gene carrier, drug delivery, and molecular imaging (Tang and Cheng 2013; Bitar et al. 2012).

In recent years, large-scale industrial production and global commercialization of SiNPs have resulted in increased risk of human exposures at workplaces (Kim et al. 2014a; Oh et al. 2014). Food additive silica (E551) is also in the nano size range (Dekkers et al. 2011), indicating that the general population is probably more exposed than initially anticipated. Moreover, in view of the efforts to use NM in medical applications, SiNPs could also be intentionally introduced into the human body for disease diagnosis and treatments (Croissant et al. 2017). Such growing potentials for exposure raised a global concern regarding the safety and potential adverse health effects of SiNPs.

Human health effects associated with silica exposure, especially crystalline silica (0.5–10 µm), have widely been studied. Occupational exposure to crystalline silica induces silicosis in workers (a fibrotic lung disease) and is also associated with lung cancer, emphysema, and pulmonary tuberculosis (Leung et al. 2012). Conversely, natural amorphous silica is generally considered as less harmful, since the toxicological potential of silica has so far been linked to its crystallinity. Recent studies have revealed that amorphous SiNPs can be as reactive as crystalline particles (Turci et al. 2016). In vivo, amorphous SiNPs are, however, cleared more rapidly from the lung, which may contribute to explain their lower pathogenic potential (Arts et al. 2007). The human health effects of nanosilica remain to be clarified and toxicologists believe that exposure to SiNPs, due to their small size, may bring different adverse effects compared to micron-sized silica (Napierska et al. 2010).

The comprehensive review of Napierska et al. (2010) suggested that exposure to SiNPs (1–100 nm) induced toxic effects in vitro (immortalized mammalian cell lines) and in vivo (rats and mice). Physico-chemical properties such as size, surface area, and surface features were found to play a key role in the toxicity of SiNPs. Importantly, Napierska concluded that physico-chemical properties of SiNPs differ based on their production method and, therefore, may cause different biological effects. However, no definite conclusions were made due to insufficient or no data available for,

- Detailed physico-chemical characterization of different types of SiNPs;
- Comparison of the toxicity of different types of SiNPs (based on their production process);
- Comparison of the toxicity mechanisms of amorphous SiNPs and crystalline silica;
- Exposure via different routes and adverse effects of chronic exposure in vivo;
- Correlation of in vitro and in vivo studies and
- Physico-chemical properties for the safer design of SiNPs.

Therefore, the aim of this review is to summarize the toxicity studies of SiNPs published after the Napierska review (2010), critically discuss the outcomes, and to evaluate how these data gaps have been addressed (Fig. 1).

**Methodology**

The selection criteria was similar to the method described in Vriens et al. (2017), which was used to construct the MOD-ENP-TOX nanotoxicity database.

**Exclusion criteria**

To specifically focus on adverse health effects of SiNPs, papers reporting on other interventions such as ecotoxicity, synergistic effects, SiNPs doped with other materials, and therapy-based outcomes were excluded.

**Inclusion criteria**

Papers reporting,

1. physico-chemical characteristics such as primary size, shape, composition, and crystallinity;
2. toxicological endpoints such as cytotoxicity, apoptosis/necrosis, genotoxicity, oxidative stress, immunotoxicity, and autophagy using immortalized cell lines or primary cells (experimental in vitro studies); and
3. toxic effects in laboratory-animals, more specifically in vivo experiments using rats and mice.

**Literature search**

We searched two databases for papers published from June 2010 (after Napierska et al. 2010) to December 2016. In
In the PubMed “http://www.ncbi.nlm.nih.gov/PubMed” and EMBASE “https://www.embase.com” databases, the following keyword combinations were used: “silica nanoparticle” OR “silica NP” AND “toxicity” NOT “review”. We retrieved a list of 859 and 405 articles in English, respectively. In a second step, duplicates were removed and the titles were screened to identify studies that best matched with our search terms, leaving 611 relevant papers. In a
third step, we excluded papers that met the exclusion criteria and left 128 most relevant papers. Finally, 82 papers reporting a minimum set of physico-chemical characterization and toxic effects were selected for the main content of the review. Notably, only one study was found on the toxicity of crystalline nanosilica (Chu et al. 2012), but it was finally excluded due to insufficient data on the size of particles. As a result, the review is dealing only with amorphous SiNPs.

Induction of oxidative stress is considered as the major mechanism involved in SiNPs toxicity (Wang et al. 2009; Ye et al. 2010a, b) and, therefore, the in vitro section of the review was structured according to toxic endpoints and its association with oxidative stress. For in vivo, studies were sorted based on exposure route and modalities, since they can significantly influence the toxicokinetics of SiNPs. Throughout the review, the following abbreviations were used to indicate the different types of silica nanoparticles: SiNPs when it is not clear which type was used, C-SiNPs for colloidal silica; S-SiNP for stöber silica; M-SiNPs for mesoporous silica; Pr-SiNPs for precipitated silica; and Py-SiNPs for pyrogenic silica.

The table summarizing in vitro studies (Table 1) was sorted according to the type of SiNPs (colloidal, stöber, mesoporous, pyrogenic, precipitated, and not specified) and cell types. Table 2 (in vivo studies) was organized according to the type of SiNPs and exposure routes.

In vitro studies

Cytotoxicity

Oxidative stress (over production of reactive oxygen species, i.e., ROS) induced by NPs could damage the cellular components and lead to cell death via apoptosis (Fu et al. 2013). Therefore, studies reporting on cytotoxicity and oxidative stress were summarized in this section.

Cytotoxicity associated with oxidative stress

Duan et al. (2013a) showed that S-SiNPs (62 nm) induced time- (6, 12, and 24 h) and dose-dependent (25–100 µg/ml) reduction in cell viability (assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, i.e., MTT), loss of membrane integrity (lactate dehydrogenase (LDH) release) and apoptosis (Annexin V/PI staining) in human umbilical vein endothelial cells (HUVECs). Apoptosis was also induced in lung (A549) and skin epithelial cells (A431) treated with Pr-SiNPs (15 nm). A dose-dependent increase (25–200 µg/ml for 72 h) in cytotoxicity (MTT and LDH), ROS production (assessed by dichlorodihydrofluorescein assay, i.e., DCFH-DA), lipid peroxidation (measurement of malondialdehyde, i.e., MDA), and apoptosis (caspase 3 and 9 activity) was observed in both cell lines. The lung cells showed, in general, a slightly higher toxic response compared to skin cells (Ahamed 2013).

SiNPs (20 and 80 nm) induced P53-mediated apoptosis in human fetal lung fibroblasts (HFL-1). At the dose of 500 µg/ml, 20 nm SiNPs induced a threefold increase in DCF fluorescence compared to 80 nm. In addition, increased expression of P53, upregulation of cytochrome C (CytC) and caspase 9, and downregulation of anti-apoptotic protein B-cell lymphoma 2 (bcl2) was observed in cells treated with 1000 µg/ml for 48 h (Xu et al. 2012). Another study with lung fibroblasts also showed that SiNPs (20 nm) could reduce cell viability (MTT) by inducing apoptotic cell death (fluorescence microscopy) in a dose-dependent manner (250–1000 µg/ml for 48 h) (Zhang et al. 2011). Athinarayanan et al. (2014) isolated SiNPs (10–50 nm) from commercial food products processed with food additive silica (E551) and exposed human lung fibroblasts (WI-38 cell line) with increasing doses (25–400 µg/ml). After 24 h, they observed cytotoxicity (MTT) in a dose-dependent manner and ROS production (DCFH-DA) at 50 µg/ml.

Cytotoxicity not associated with oxidative stress

Py-SiNPs (12 and 40 nm) induced a significant size and dose- (31.3, 93.8, and 156.3 µg/cm² culture well) dependent cytotoxicity (LDH, Sulphorodhamine B assay (SRB) and water-soluble tetrazolium-1(WST-1)) in human colon carcinoma cell line (HT29), while no induction of ROS (DCFH-DA) was observed (Gehrke et al. 2013). In the study by Napierska et al. (2012a), 50 µg/ml (24 h) of 16 nm iron-doped S-SiNPs and pure S-SiNPs induced strong cytotoxicity (MTT and LDH) in a human endothelial cell line (EA.hy926), but a significant increase in oxidative stress markers (GSH depletion, malondialdehyde (MDA formation), induction of heme oxygenase-1, glutathione reductase, and NADPH oxidase-1) was observed only for iron-doped SiNPs.

Conclusion: cytotoxicity

Cytotoxicity of SiNPs was investigated using different cell lines and incubation times, making the comparison between studies difficult. However, from Table 3, it is clear that all types of SiNPs induced cytotoxicity. Significant (compared to untreated cells) cytotoxic effects were observed only at or above the concentration of 25 µg/ml. Furthermore, it can be clearly seen that SiNPs induced oxidative stress and mediated apoptosis mainly via the intrinsic or mitochondrial pathway (caspase-dependent pathway) in a size- and dose-dependent manner. ROS-mediated toxicity is believed to be an important mechanism of NP toxicity.
Table 1 In vitro studies on SiNPs toxicity

| Type of SiNPs | Cell line | Cell type | Particle primary size | Source                        | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References         |
|--------------|-----------|-----------|-----------------------|-------------------------------|---------------|-------------------|----------|---------------------|---------|---------------------|
| C-SiNPs      | V79       | Hamster lung fibroblast | 9, 15, 30, and 55 nm | AkzoNobel AB                 | 10–600 µg/ml | 24 h              | Cell viability | Tryphan blue exclusion and colony formation assay | Reduction with 15 nm NPs | Maser et al. (2015) |
|              | A549      | Human type II alveolar epithelial | 9, 15, 30, and 55 nm | Sigma-Aldrich                | 0.6–600 µg/ml | 4–20 h            | Genotoxicity | Comet assay           | Increase with 15 nm NPs |                      |
| C-SiNPs      | H441      | Human distal lung epithelial | 30 nm                  | Sigma-Aldrich                | 0.6–600 µg/ml | 4–20 h            | Cell viability | MTS assay             | Reduction at 600 µg/ml in monocultures | Kasper et al. (2011) |
|              | ISO-HAS-1 | Human endothelial                  |                        | Sigma-Aldrich                | 0.6–600 µg/ml | 4–20 h            | Cytotoxicity | LDH assay             | Increase at 600 µg/ml in all cultures |                      |
|              | ISO-HAS-1 | Human endothelial                  |                        | Sigma-Aldrich                | 0.6–600 µg/ml | 4–20 h            | Cellular barrier integrity | TEER measurement        | Reduction at 600 µg/ml in all cultures |                      |
| Co-culture:  | H441/ISO- | Human distal lung epithelial + human endothelial | 30 nm                  | Micromod Partikeltechnologie | 100 µg/ml    | 4-20 h            | Pro-inflammatory response(s) | ELISA                  | Increase of sICAM-1, IL-6 and IL-8 |                      |
|              | HAS-1     | Human distal lung epithelial + human endothelial | 30 nm                  | Micromod Partikeltechnologie | 100 µg/ml    | 4-20 h            | Cell morphology | Hoechst staining            | Cells rounded at 600 µg/ml |                      |
|              | A549      | Human type II alveolar epithelial | 70 nm—without or without lung surfactant | Micromod Partikeltechnologie | 100 µg/ml    | 4-20 h            | Cytotoxicity | LDH assay             | Increase in monolayers | Kasper et al. (2015) |
|              | A549      | Human type II alveolar epithelial | 70 nm—without or without lung surfactant | Micromod Partikeltechnologie | 100 µg/ml    | 4-20 h            | Pro-inflammatory response(s) | ELISA                  | Increase of IL-8 in monocultures and co-cultures |                      |
| Co-culture:  | A549 and ISO- | Human type II alveolar epithelial + human endothelial | 70 nm—without or without lung surfactant | Micromod Partikeltechnologie | 100 µg/ml    | 4-20 h            | Apoptosis        | Western blot                 | Increase of apoptotic proteins |                      |
|              | HAS-1     | Human type II alveolar epithelial + human endothelial | 70 nm—without or without lung surfactant | Micromod Partikeltechnologie | 100 µg/ml    | 4-20 h            | Apoptosis        | Western blot                 | Increase of apoptotic proteins |                      |
|              | A549      | Human type II alveolar epithelial | 70 nm—without or without lung surfactant | Micromod Partikeltechnologie | 100 µg/ml    | 4-20 h            | Apoptosis        | Western blot                 | Increase of apoptotic proteins |                      |
| C-SiNPs      | Caco2     | Human colon epithelial            | 15 nm (Levasil 200/40%) and 55 nm (Levasil 50/50%) | H.C. Starck | 0.03–156.3 µg/cm² | 24, 48 and 72 h | Cell viability | XTT assay             | Dose-dependent reduction with 15 nm NPs | Tarantini et al. (2015a, b) |
|              |           | Human colon epithelial            | 15 nm (Levasil 200/40%) and 55 nm (Levasil 50/50%) | H.C. Starck | 0.03–156.3 µg/cm² | 24, 48 and 72 h | Oxidative stress | DCFH-DA assay            | Dose-dependent increase with 15 nm SiNPs |                      |
|              |           | Human colon epithelial            | 15 nm (Levasil 200/40%) and 55 nm (Levasil 50/50%) | H.C. Starck | 0.03–156.3 µg/cm² | 24, 48 and 72 h | Pro-inflammatory response(s) | ELISA                  | Increase of IL-8 with 15 nm NPs only at 156.3 µg/cm² |                      |
|              |           | Human colon epithelial            | 15 nm (Levasil 200/40%) and 55 nm (Levasil 50/50%) | H.C. Starck | 0.03–156.3 µg/cm² | 24, 48 and 72 h | Apoptosis        | Caspase-3 assay            | Dose-dependent increase with 15 nm NPs |                      |
|              |           | Human colon epithelial            | 15 nm (Levasil 200/40%) and 55 nm (Levasil 50/50%) | H.C. Starck | 0.03–156.3 µg/cm² | 24, 48 and 72 h | Genotoxicity     | Micronuclei induction      | Dose-dependent increase with 15 nm NPs |                      |
|              |           | Human colon epithelial            | 15 nm (Levasil 200/40%) and 55 nm (Levasil 50/50%) | H.C. Starck | 0.03–156.3 µg/cm² | 24, 48 and 72 h | Genotoxicity     | γH2AX fluorescence         | Increase with 15 nm NPs |                      |
|              |           | Human colon epithelial            | 15 nm (Levasil 200/40%) and 55 nm (Levasil 50/50%) | H.C. Starck | 0.03–156.3 µg/cm² | 24, 48 and 72 h | NP internalization | TEM                      | NPs detected in lysosomes and in endocytic compartments |                      |
### Table 1 continued

| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|---------------|------------------|----------|-------------------|---------|------------|
| C-SiNPs      | HepG2     | Human liver epithelial | 19, 43, 68, and 498 nm | Laboratory synthesis | 12–200 µg/ml | 24 h | Cell viability | CCK-8 assay | Size and dose-dependent reduction | Li et al. (2011) |
|              |           |           |                       | | | | | Cytotoxicity | LDH assay | Size and dose-dependent increase | |
|              |           |           |                       | | | | | Oxidative stress | DCFH-DA assay | Size-dependent increase | |
|              |           |           |                       | | | | | Genotoxicity | Comet assay | Size-dependent increase | |
|              |           |           |                       | | | | | Apoptosis | Apoptosis assay | Size-dependent increase | |
|              |           |           |                       | | | | | Cell cycle arrest | Flow cytometry | Size-dependent increase | |
|              |           |           |                       | | | | | Cell morphology | H & E staining | Cellular shrinkage, chromatin condensation and vacuolar degeneration detected | |
|              | RAW 264.7 | Mouse blood macrophage | 20 and 100 nm (uncoated or l-arginine coated) | E&B Nanotech | 10–640 µg/ml | 24 h | Cell viability | WST-8 assay | Size and surface charge-dependent reduction | Kim et al. (2014b) |
| C-SiNPs and M-SiNPs | J774A.1 | Mouse macrophage | 100 nm | Laboratory synthesis | 0.1–1000 µg/ml | 24 and 72 h | Cell viability | MTT assay | Reduction only for C-SiNPs SiNPs | Lee et al. (2011) |
|              |           |           |                       | | | | | Apoptosis | Annexin V/PI staining | Increase of caspase-3 activation | |
|              |           |           |                       | | | | | Pro-inflammatory response(s) | RT PCR and western blot | Increase of TNF-α, IL-6 and IL-1β | |
|              |           |           |                       | | | | | Pathway analysis | RT PCR and western blot | Activation of MAPKs and NF-kB | |
|              |           |           |                       | | | | | Cell viability | WST-1 assay | ED50: 6–9 µg/ml and 15–22 µg/ml in J774 and 3T3, respectively | Rabolli et al. (2011) |
|              |           |           |                       | | | | | In vitro dosimetry | ISDD simulation | Similar delivered doses for all particle sizes | |
|              |           |           |                       | | | | | Genotoxicity | Lymphoma assay | Mutations detected at 100 and 150 µg/ml | Demir and Castranova (2016) |
|              |           |           |                       | | | | | C-SiNPs | Polysciences | 50–2000 µg/ml | 24 and 48 h | Cell viability | FACS | Dose, time and size-dependent reduction | Mendoza et al. (2014) |
|              | PBMCs     | Lymphocytes, monocytes and dendritic cells | 10 and 100 nm | Polysciences | 50–2000 µg/ml | | | Oxidative stress | GSH depletion | Size and dose-dependent increase | |
|              |           |           |                       | | | | | Oxidative stress | Western-blot | Size-dependent increase in proteins with free radicals only with 10 nm | |
|              |           |           |                       | | | | | Pro-inflammatory response(s) | Multiplex bead array | Size and dose-dependent increase in cytokines | |
|              |           |           |                       | | | | | Cell morphology | Immuno electron microscopy | Size-dependent increase in cell damage | |
| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|-------------|------------|-----------|-----------------------|--------|--------------|------------------|----------|----------------|---------|-------------|
| C-SiNPs     | HUVEC      | Human vein endothelial | 10, 50, 150, and 500 nm | Polysciences | 10 nm–10 μg/ml | 1 h (10 nm) and 3 h (50–500 nm) | Oxidative stress | Fluorescent microscopy, Cytometric bead array | Free radical increase | Corbalan et al. (2011) |
| C-SiNPs     | Platelets  | Mouse     | 50 nm                  | Polysciences | 1, 1.5 and 25 μg/ml | 30 min | Platelet aggregation | Aggregation assay | Dose-dependent increase | Nemmar et al. (2015) |
| C-SiNPs     | Platelets  | Human     | 10, 50, 150, and 500 nm | Polysciences | 1–200 μg/ml | 15 min | Platelet aggregation | Aggregation assay | Size-dependent increase | Jose Corbalan et al. (2012) |
| S-SiNPs     | A549       | Human type II alveolar epithelial | 2.1, 16.4, 60.4, and 104 nm | Laboratory synthesis | 5 μg/cm² of plate surface | 12–24 h | Cytotoxicity | LDH assay | Size-dependent increase | Napierska et al. (2012b) |
| THP-1       | Biculture: A549/THP-1 | Human monocyte | | | | | | | | |
| Triculture: A549/THP-1/EA.hy926 | Human co-cultures | | | | | | | | |
| S-SiNPs     | Co-culture: NCI-H441/ISO-HAS-1 with or without THP-1 cells | Lang co-cultures | 15, 35, and 80 nm | Laboratory synthesis | 50 and 100 μg/ml | 72 h | Oxidative stress | DCFH-DA assay | Increase in the presence of THP-1 | Farcal et al. (2012) |
| | | | | | | | | | | |

**Table 1 continued**
| Type of SiNPs | Cell line | Cell type       | Particle primary size | Source         | Exposure dose | Exposure duration | Endpoint                  | Assay(s)/method(s)                          | Results                                      | References                  |
|--------------|-----------|-----------------|-----------------------|----------------|---------------|------------------|--------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| S-SiNPs      | HepG2     | Human liver epithelial | 43 nm                | Laboratory synthesis | 25–200 µg/ml  | 3 and 24 h       | Oxidative stress         | DCFH-DA assay                          | Dose-dependent increase                     | Sun et al. (2011)                        |
|              |           |                  |                       |                |               |                  | Mitochondrial membrane potential | Probe measurements               | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Apoptosis                | Annexin V/PI staining and western blot | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Cell morphology          | TEM                                      | NPs detected in cytoplasm, mitochondria and lysosomes |                              |
| S-SiNPs      | HepG2     | Human liver epithelial | 62 nm                | Laboratory synthesis | 25–100 µg/ml  | 24 h             | Cell viability           | MTT assay                                | Dose-dependent reduction                   | Yu et al. (2014)                        |
|              |           |                  |                       |                |               |                  | Oxidative stress         | DCFH-DA assay                          | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Autophagy                | MDC staining                            | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Autophagy                | Immunoblot                             | Dose-dependent increase of LC3-II/LC3-I |                              |
|              |           |                  |                       |                |               |                  | NP internalization       | LSCM                                     | NPs detected in cytoplasm and mitochondria |                              |
| S-SiNPs      | LC-02     | Human liver epithelial | 50 nm                | Laboratory synthesis | 50–200 µg/ml  | 24 h             | Cell viability           | CCK-8 assay                                | Dose-dependent reduction                   | Wang et al. (2013)                       |
|              |           |                  |                       |                |               |                  | Oxidative stress         | DCFH-DA assay                          | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Apoptosis                | Annexin V/PI staining            | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Mitochondrial damage     | Mitotracker/laser confocal microscopy | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Cell morphology          | Hoechst staining                      | Dose-dependent increase in cell damage     |                              |
| S-SiNPs      | HaCaT     | Human keratinocyte | 50 nm                | Laboratory synthesis | 25–500 µg/ml  | 4 h               | Cell viability           | MTT assay                                | Dose-dependent reduction                   | Liang et al. (2014)                      |
|              |           |                  |                       |                |               |                  | Cytotoxicity             | LDH assay                               | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Oxidative stress         | DCFH-DA assay                          | Dose-dependent increase                     |                              |
|              |           |                  |                       |                |               |                  | Oxidative stress         | GSH depletion                          | Increase                                  |                              |
|              |           |                  |                       |                |               |                  | Apoptosis                | Hoechst/PI staining                   | Increase                                  |                              |
|              |           |                  |                       |                |               |                  | NP internalization       | TEM                                     | Detected in cytoplasm                      |                              |
| Type of SiNPs | Cell line | Cell type         | Particle primary size | Exposure dose | Endpoint                         | Assay(s)/method(s)          | Results                                                                 | References |
|-------------|----------|------------------|-----------------------|---------------|----------------------------------|----------------------------|------------------------------------------------------------------------|------------|
| S-SiNPs     | EA.hy926 | Human endothelial | 16 (pure or iron-doped) and 60 nm | Laboratory synthesis | 25 and 50 µg/ml | 24 h Cell viability | MTT assay Reduction at 50 µg/ml with 16 nm NPs | Napierska et al. (2012a) |
|             |          |                  |                       | Source        | Exposure duration | Cytotoxicity          | LDH assay Increase at 50 µg/ml with 16 nm NPs                    |            |
|             |          |                  |                       |               |                    | Oxidative stress     | DCFH-DA assay Dose-dependent increase only with Fe doped NPs       |            |
|             |          |                  |                       |               |                    | GSH depletion         | Dose-dependent increase only with Fe doped NPs                      |            |
|             |          |                  |                       |               |                    | Oxidative stress     | LPO assay Dose-dependent increase of MDA formation with Fe doped NPs |            |
|             |          |                  |                       |               |                    | Oxidative stress     | RT-PCR assay Increase with 16 nm NPs                               |            |
|             |          |                  |                       |               |                    | NP internalization   | TEM NPs found in cytoplasm                                         |            |
| S-SiNPs     | HUVEC    | Human vein       | 62 nm                 | Laboratory synthesis | 25–100 µg/ml | 6, 12 and 24 h Cell viability | MTT assay Dose-dependent reduction                                | Duan et al. (2013a) |
|             |          | endothelial      |                       | Source        | Exposure duration | Cytotoxicity          | LDH assay Dose-dependent increase                                  |            |
|             |          |                  |                       |               |                    | Oxidative stress     | DCFH-DA assay Dose-dependent decrease                               |            |
|             |          |                  |                       |               |                    | Oxidative stress     | LPO assay Dose-dependent increase of MDA formation                 |            |
|             |          |                  |                       |               |                    | Oxidative stress     | SOD assay Dose-dependent decrease                                  |            |
|             |          |                  |                       |               |                    | Oxidative stress     | GSHPx assay Dose-dependent decrease                                |            |
|             |          |                  |                       |               |                    | Apoptosis             | Annexin V/PI staining Dose-dependent decrease                      |            |
|             |          |                  |                       |               |                    | Mitochondrial membrane potential | Probe measurements Dose-dependent increase                        |            |
|             |          |                  |                       |               |                    | Genotoxicity          | Comet assay Dose-dependent upregulation of chk 1 and down regulation Cdc25c, Cyclin B1, Cdk2 |            |
|             |          |                  |                       |               |                    | Cell cycle analysis   | Western blot Dose-dependent increase                              |            |
|             |          |                  |                       |               |                    | NP internalization    | LSCM and TEM NPs detected in cytoplasm                             |            |
|             |          |                  |                       |               |                    | Autophagy             | MDC staining Dose-dependent increase of LC-3 II/LC3-I              | Duan et al. (2013b) |
|             |          |                  |                       |               |                    | LSCM and TEM          | Dose-dependent increase in cellular uptake and autophagic vacuoles, autophagosomes and autolysosomes detected |            |
|             |          |                  |                       |               |                    | Expression of         | ELISA Dose-dependent increase in the expression of CRP, TNF-α, IL-1β and IL-6 |            |
|             |          |                  |                       |               |                    | inflammatory factors  | NO, NOS, eNOS and iNOS measurements Dose-dependent increase of iNOS and decrease of NO, NOS and eNOS |            |
|             |          |                  |                       |               |                    | Oxidative stress     | Western blot Dose-dependent decrease of p-mTOR/mTOR, p-P13 K/P13 K and p- Akt/Akt |            |
|             |          |                  |                       |               |                    | Pathway analysis      | Western blot Dose-dependent increase                              |            |
\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Type of SiNPs & Cell line & Cell type & Particle primary size & Source & Exposure dose & Exposure duration & Endpoint & Assay(s)/method(s) & Results & References \\
\hline
S-SiNPs & HUVEC & Human vein endothelial & 58 nm & Laboratory synthesis & 12.5–100 µg/ml & 24 h & Cell viability & MTT assay & Dose-dependent reduction & Guo et al. (2015) \\
& & & & & & & Cytotoxicity & LDH assay & Dose-dependent increase & & \\
& & & & & & & Oxidative stress & DCFH-DA assay & Dose-dependent increase & & \\
& & & & & & & Oxidative stress & GSH depletion & Dose-dependent increase & & \\
& & & & & & & Oxidative stress & LPO assay & Dose-dependent increase & & \\
& & & & & & & Oxidative stress & SOD assay & Dose-dependent decrease & & \\
& & & & & & & Oxidative stress & GSH-Px assay & Dose-dependent decrease & & \\
& & & & & & & Inflammatory factors & ELISA & Increase of IL-1β, IL-8, TNFα, ICAM-1, VCAM-1, and MCP-1 & & \\
& & & & & & & Oxidative stress & NO, NOS, eNOS and iNOS measurements & Differential expression of NO, iNOS and eNOS activity and downregulation of ET-1 & & \\
& & & & & & & Pathway analysis & RT-PCR and western blot & Dose-dependent increase of Nrf-2, p-ERK, p-JNK, p-p38 MAPK, and NF-kB & & \\
\hline
S-SiNPs & HUVEC & Human vein endothelial & 62 nm & Laboratory synthesis & 25–100 µg/ml & 24 h & Cellular uptake & LSCM & Dose-dependent increase & Duan et al. (2014b) \\
& & & & & & & Cytoskeleton damage & Cell cytoskeleton staining & Weakening of F actin at 100 µg/ml & & \\
& & & & & & & Mitochondrial membrane potential & Probe measurements & Dose-dependent increase & & \\
& & & & & & & Autophagy & TEM & Autophagic ultrastructures detected & & \\
& & & & & & & Autophagy & Western blot & Increase of LC3-II/LC3-I & & \\
& & & & & & & Inflammatory factors & Western blot & Decrease of ICAM-1 and VCAM-1 & & \\
& & & & & & & Pathway analysis & Western blot & Dose-dependent decrease of p-mTOR/mTOR, p-P13K/P13K and p-Akt/Akt & & \\
\hline
\end{tabular}
\end{table}
| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|---------------|------------------|----------|-------------------|---------|-------------|
| S-SiNPs     | HUVEC     | Human vein endothelial | 58 nm | Laboratory synthesis | 12.5–100 µg/ml | 24 h | Cell viability | MTT assay | Size and dose-dependent reduction | Guo et al. (2016) |
| S-SiNPs and M-SiNPs | MPMCs | Human peritoneal mast cells | 25 nm | Laboratory synthesis | 100 µg/ml | 24 h | Hemolysis of RBCs | Hemolysis assay | Increase with non-porous NPs | Maurer-jones et al. (2010) |
| S-SiNPs and M-SiNPs | RAW 264.7 | Mouse macrophage | 25 and 100 nm | Laboratory synthesis | 10–150 µg/ml | 24 h | Hemolysis of RBCs | Hemolysis assay | Increase only with M-SiNPs | Sergent et al. (2012) |
| S-SiNPs and M-SiNPs | A549 | Human type II alveolar epithelial | 115 nm (with or without amine modification) | Laboratory synthesis | 10, 50, 100, 250 and 500 µg/ml | 24 h | Cell viability | WST-8 assay | Dose-dependent reduction only in RAW 264.7. Amine modified SiNPs were less toxic | Yu et al. (2011) |
| | RBCs | Human red blood cells | 58 nm | Laboratory synthesis | 12.5–100 µg/ml | 24 h | Cell viability | MTT assay | Size and dose-dependent reduction | Guo et al. (2016) |
| S-SiNPs and M-SiNPs | RAW 264.7 | Human alveolar epithelial | 25 and 100 nm | Laboratory synthesis | 10–150 µg/ml | 24 h | Cell viability | SRB | No reduction | Sergent et al. (2012) |
| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|---------------|-----------------|----------|------------------|---------|------------|
| M-SiNPs      | NRK-52E   | Rat kidney epithelial | 198 nm | Laboratory synthesis | 25–1000 µg/ml | 3 and 24 h | Cell viability | MTT assay | Dose-dependent reduction | Chen et al. (2015) |
|              |           |           |                       |        |               |                 | Cytotoxicity | LDH assay | Dose-dependent increase |           |
|              |           |           |                       |        |               |                 | Expression of inflammatory factors | Western blot | Dose-dependent increase of FN, TGF-β, and ICAM-1 (50–400 µg/ml) |           |
|              |           |           |                       |        |               |                 | Pathway analysis | Immuno-fluorescence staining | Dose-dependent increase in the expression of NF-κB p65 (50–400 µg/ml) |           |
|              | HEK293    | Human kidney epithelial | 100 nm, 2.3 nm pore size | Laboratory synthesis | 100 µg/ml | 24, 48 and 72 h | Cellular morphology | TEM | Cells shrunk and nucleus condensed | Zhang et al. (2015) |
|              |           |           |                       |        |               |                 | Oxidative stress | Fluorescent dihydroethidium | No effect |           |
|              |           |           |                       |        |               |                 | Oxidative stress | RT PCR | No effect |           |
|              |           |           |                       |        |               |                 | Chromosomal aberrations | FISH assay | No effect |           |
|              |           |           |                       |        |               |                 | Mutations | EGFR and KRAS | No effect |           |
|              |           |           |                       |        |               |                 | Genotoxicity | Human mRNA micro array | 579 genes upregulated and 1263 genes downregulated |           |
|              | EA.hy926  | Human endothelial | 48 nm (surface functionalized with PET and TMS) | Laboratory synthesis | 20–1000 µg/ml at a flow rate of 30 µl/min using a micro-fluidic device | – | Cell viability | Trypan blue | No effect | Kim et al. (2014a) |
|              |           |           |                       |        |               |                 | Platelet adhesion | Adhesion assay | Increase at 1000 µg/ml |           |
|              |           |           |                       |        |               |                 | Platelet aggregation | Aggregation assay | Increase at 1000 µg/ml |           |
|              |           |           |                       |        |               |                 | Morphology | Fluorescent fixed cell imaging | Large aggregate formation at 1000 µg/ml |           |
| Py-SiNPs and S-SiNPs | A549 | Human type II alveolar epithelial | 12 (Py-SiNPs) and 50 nm (S-SiNPs) | Py-SiNPs—Evonik; S-SiNPs—Landsberg am Lech | 52 µ g/cm² and 117 µ g/cm² (ALI) 15.6 µ g/cm² (submerged) | ALI (5 and 7 h) Submerged (24 h) | Cytotoxicity | LDH assay | Dose-dependent increase | Panas et al. (2014) |
|              |           |           |                       |        |               |                 | Pro-inflammatory response | ELISA | Increase in IL-8 |           |
|              |           |           |                       |        |               |                 | Expression of inflammatory factors | Western blot | Increase in the expression of COX-2 and phosphorylated p38; stronger effects observed in submerged culture |           |
| Pr-SiNPs     | A549      | Human type II alveolar epithelial | 15 nm | Nanoamor | 25–200 µg/ml | 72 h | Cell viability | MTT assay | Dose-dependent reduction | Ahamed (2013) |
|              | A431      | Human skin epithelial |          |           |        |                 | Cytotoxicity | LDH assay | Dose-dependent increase |           |
|              |           |           |                       |        |               |                 | Oxidative stress | DCFH-DA assay | Dose-dependent increase |           |
|              |           |           |                       |        |               |                 | Oxidative stress | LPO assay | Dose-dependent increase of MDA formation |           |
|              |           |           |                       |        |               |                 | Oxidative stress | GSH depletion | Dose-dependent increase |           |
|              |           |           |                       |        |               |                 | Apoptosis | RT PCR | Dose-dependent increase of caspase 3 and caspase 9 |           |
| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|----------------------|--------|---------------|------------------|----------|-------------------|---------|------------|
| Pr-SiNPs (NM-200) and Py-SiNPs (NM-203); NRT-808, NRT-817, NRT-820 and NRT-944 | Immortalized balb/3T3 Mouse fibroblast | 10–25, 5–30, 15,35, 80, and 90 nm | NM-JRC repository; NRT-Laboratory synthesis | 1–100 µg/ml | 72 h | Cell viability | MTT assay | Reduction with 15 nm NPs | Uboldi et al. (2012) |
| Two Py-SiNPs samples (different sizes) one Pr-SiNPs and two C-SiNPs (different sizes) | V79 Hamster lung fibroblast | 20–80 nm | SiNPs | 12.5–100 µg/cm² | 24 h | Cell viability | WST-1 assay | Size-dependent reduction | Guichard et al. (2016) |
| Py-SiNPs(12 and 40 nm) and S-SiNPs (200 nm) | HT-29 Human colon epithelial | 12, 40, and 200 nm | Py-SiNPs—Evonik; S-SiNPs—Angstrom Sphere | 0.03–156.3 µg/cm² | 24 h | Cell viability | WST-1 assay | Size-dependent reduction | Gehrique et al. (2013) |
| Py-SiNPs | C2BBe1 Human colon epithelial | 12 nm | Sigma-Aldrich | 10 µg/cm² | Acute—24 h Chronic—29 passages | Cell viability | MTT assay | No effect | McCracken et al. (2013) |
### Table 1 continued

| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|---------------|-------------------|----------|-------------------|---------|------------|
| Py-SiNPs and Pr-SiNPs | GES-1 | Human gastric epithelial | 10–50 nm | Evonik and Haihua | 10–600 µg/ml | 24, 48 and 72 h | Cell viability | CCK-8 assay | Size and dose-dependent reduction | Yang et al. (2014b) |
| | | Human colon epithelial | | | | | | LDH assay | Size and dose-dependent increase | |
| | | | | | | | DCFH-DA assay | No effect | |
| | | | | | | | Apoptosis assay | No effect | |
| | | | | | | | Cell cycle arrest | Cell cycle assay | Increase at S phase for GES cells and G2/M for caco2 cells | |
| | | | | | | | TEM | NPs detected in cytoplasm | |
| | | | | | | | Ultramicrotome and TEM | Increase in cell damage | |
| | | | | | | | TEM | Cellular uptake | Increase in cell damage | |
| | | | | | | | HEER measurement | No effect | |
| | | | | | | | | Cellular barrier integrity | | |
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| Pr-SiNPs | HePG2 | Human liver epithelial | 14 nm | Nanoamor | 1–200 µg/ml | 72 h | Cell viability | MTT and NRU assay | Dose-dependent reduction at 25–200 µg/ml | Ahmad et al. (2012) |
| | | | | | | | Oxidative stress | DCFH-DA assay | Dose-dependent increase | |
| | | | | | | | Oxidative stress | LPO assay | Dose-dependent increase MDA formation | |
| | | | | | | | Apoptosis | GSH depletion | Dose-dependent increase | |
| | | | | | | | Apoptosis | RT PCR and western blot | Up regulation of p53, bax, and caspase 3 and down regulation of bcl 2 | |
| | | | | | | | Apoptosis | Caspase-3 assay | Dose-dependent increase | |
| | | | | | | | Genotoxicity | Cytokinesis block micronuclei induction | No effect | Tavares et al. (2014) |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

*Abbreviations: CCK-8*: cell counting kit-8; *LDH*: lactate dehydrogenase; *DCFH-DA*: 2',7'-dichlorodihydrofluorescein diacetate; *ELISA*: enzyme-linked immunosorbent assay; *MTT*: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *NRU*: neutral red uptake assay; *LPO*: lipid peroxidation assay; *TEER*: transepithelial electrical resistance; *RT-PCR*: real-time polymerase chain reaction; *TEM*: transmission electron microscopy.*
| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|--------------|------------------|----------|-------------------|---------|------------|
| S-SiNPs      | Primary Microglial cells | Rat macrophage like cells | 150–200 nm | Laboratory synthesis | 0.0728–7.28 µg/ml | 24 h | Cell viability | MTS assay | No effect | Choi et al. (2010) |
|              |           |           |                       |        |              |                  | Cytotoxicity | LDH assay | No effect |          |
|              |           |           |                       |        |              |                  | Oxidative stress | DCFH-DA assay | No effect |          |
|              |           |           |                       |        |              |                  | NO production | DAF-FM assay | No effect |          |
|              |           |           |                       |        |              |                  | Pro-inflammatory response(s) | Luminex | Reduction in TNF-α. A small amount of IL-β detected |          |
|              |           |           |                       |        |              |                  | Inflammatory factors | RT-PCR | Increase in COX-2 |          |
| Reverse microemulsion | HK-2 | Human kidney epithelial | 20 and 100 nm | Laboratory synthesis | 5–500 µg/ml | 24,48 and 72 h | Cell viability | WST-1 and clonogenic assay | Size, dose and time-dependent reduction | Passagne et al. (2012) |
| LLC PK-1     | Porcine kidney epithelial |           |                       |        |              |                  | Oxidative stress | LPO assay | Dose-dependent increase of MDA formation |          |
|              |           |           |                       |        |              |                  | Oxidative stress | Fluorescent dihydroethidium | Dose-dependent increase |          |
| SiNPs        | A549      | Human type II alveolar epithelial | 20 nm (with or without amine or carboxyl coated) | Laboratory synthesis | 200–1000 µg/ml | 12–48 h | Cell viability | PI staining | Reduction above 250 µg/ml | Nowak et al. (2014) |
|              |           |           |                       |        |              |                  | Autophagy | MDC staining | Threefold and fivefold increase at 100 and 1000 µg/ml, respectively |          |
|              |           |           |                       |        |              |                  | Cellular uptake | TEM | Upregulation of anti-oxidant genes |          |
|              |           |           |                       |        |              |                  | Cellular uptake | PI staining | Reduction above 250 µg/ml |          |
| SiNPs        | BEAS-2B   | Human bronchial epithelial | 20–40 nm | Sigma-Aldrich | 1 µg/ml | 24 h | Cell viability | MTT assay, flow cytometry | Reduction in cell viability | Eom and Choi (2011) |
|              |           |           |                       |        |              |                  | Oxidative stress | DCFH-DA assay | Increase in ROS production |          |
|              |           |           |                       |        |              |                  | Pathway analysis | Western blot | Increase in HO-1, Nrf-2 and ERK phosphorylation |          |
| SiNPs        | HFL-1     | Human lung fibroblast | 20 and 80 nm | Laboratory synthesis | 250–2000 µg/ml | 48 h | Cell viability | MTT assay | Size and dose-dependent reduction | Xu et al. (2012) |
|              |           |           |                       |        |              |                  | Oxidative stress | DCFH-DA assay | Size and dose-dependent increase |          |
|              |           |           |                       |        |              |                  | Apoptosis | Flow cytometry | Size and dose-dependent increase |          |
|              |           |           |                       |        |              |                  | Apoptotic pathway analysis | Western blot | Increase of p53 and differential expression of cytochrome C, Bax, Bcl-2, caspase, β-actin and COX IV |          |
| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/ method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|---------------|-------------------|----------|----------------------|---------|------------|
| SiNPs MRC-5  | Human lung fibroblast | 4–13 nm | NaBond Technologies | 12.5–62.5 µg/ml for 24, 48 and 72 h (cell viability) Other assays—62.5 µg/ml | Cell viability Oxidative stress Advanced oxidation protein products | MTT assay DCFH-DA assay GSH depletion Western blot | Time and dose-dependent reduction Time-dependent increase Time-dependent increase | Voicu et al. (2015) |
| SiNPs A549   | Human type II alveolar epithelial | 30 nm | Nanoamor | 0.0–100 µg/ml | 24 h | Cell viability Oxidative stress | MTT assay DCFH-DA assay GSH depletion Western blot | Dose-dependent reduction in A549 Dose-dependent increase in A549 Differential expression of anti-oxidant proteins Increase of Nrf 2 in A549 | Michael Berg et al. (2013) |
| SiNPs HFL-1  | Human lung fibroblast | 20 nm | Nanjing High Technology of Nano | 250–1500 µg/ml | 48 h | Cell viability Apoptosis | MTT assay Catalase assay | Dose-dependent increase Dose-dependent increase Dose-dependent increase | Zhang et al. (2011) |
| SiNPs HaCaT  | Human keratinocyte | 15, 30, 100, and 5000 nm | Wan Jing New Material Co. Ltd and Sigma-Aldrich (5000 nm) | 0.5–100 µg/ml | 24 h | Cell viability Oxidative stress Apoptosis Genotoxicity | CCK-8 assay DCFH-DA assay Annexin V/PI staining 8-OH-dG γH2AX fluorescence Comet assay | Size and dose-dependent reduction Size and dose-dependent increase Size and dose-dependent increase Size and dose-dependent increase | Gong et al. (2012) |
| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|---------------|-------------------|----------|-------------------|---------|-------------|
| SiNPs        | HaCaT     | Human keratinocYTE | 70, 300, and 1000 nm | Micromod Partikeltechnologie | 10–1250 µg/ml | 24 h | Cytotoxicity | LDH assay | Dose-dependent increase | Nabeshi et al. (2011a) |
|              |           |           |                       |        |               |                   |          | Oxidative stress | DCFH-DA assay | Dose-dependent increase |
|              |           |           |                       |        |               |                   |          | Oxidative stress | Hydroxyl fluorescein assay | Dose-dependent increase |
|              |           |           |                       |        |               |                   |          | Genotoxicity | 8-OH-dG | Increase in tail length and tail moment |
|              |           |           |                       |        |               |                   |          | Cellular uptake | Assessment using Cytochalasin D and apocynin | Internalization of NPs by NADPH oxidase independent endocytosis |
| SiNPs        | HaCaT     | Human keratinocYTE | 70, 300, and 1000 nm | Micromod Partikeltechnologie | 100 µg/ml | 24 h | NP internalization | TEM | 70 nm NPs into the nucleus and 300 and 1000 nm SiNPs only in endosomes | Nabeshi et al. (2011b) |
| SiNPs        | HepG2     | Human liver epithelial | 7, 20, and 50 nm | Shanghai Cabot Chemical | 20–640 µg/ml | 24, 48 and 72 h | Genotoxicity | Comet assay | Increase in tail length with 70 nm NPs | Lu et al. (2011) |
| SiNPs:       | Kupffer cells | Rat liver macrophage | 15 nm | Sigma-Aldrich | 50–800 µg/ml Supernatant was incubated with BRL cells | 24 h | Cell viability | CCK-8 assay | Dose-dependent reduction in BRL | Chen et al. (2013) |
|              |           |           |                       |        |               |                   |          | Cytotoxicity | LDH assay | Dose-dependent increase in BRL |
|              |           |           |                       |        |               |                   |          | Oxidative stress | DCFH-DA assay | Dose-dependent increase in KCs |
|              |           |           |                       |        |               |                   |          | Oxidative stress | GSH depletion | Dose-dependent increase in KCs |
|              |           |           |                       |        |               |                   |          | Pro-inflammatory response | ELISA | Dose-dependent increase of TNF-α in KCs |
|              |           |           |                       |        |               |                   |          | NO production | Griess reagent | Dose-dependent increase of NO in KCs |
|              |           |           |                       |        |               |                   |          | Cellular morphology | Phase contrast microscopy | Cell damage observed for BRL |
|              |           |           |                       |        |               |                   |          | Cell viability | WST-8 assay | Dose-dependent reduction |
| SiNPs RAW 264.7 | Mouse macrophage | 12 nm | Sigma-Aldrich | 200 and 400 µg/ml | 24 h | Genotoxicity | Hoechst/PI staining | Deformation of nuclei at both concentrations | Hashimoto and Imazato (2015) |
|              |           |           |                       |        |               |                   |          | Genotoxicity | Comet assay | Deformation of nuclei at both concentrations |
|              |           |           |                       |        |               |                   |          | Genotoxicity | Micronuclei induction | Increase |
|              |           |           |                       |        |               |                   |          | Cellular uptake | SEM and TEM | NPs detected in vesicles and in nucleus |
Table 1 continued

| Type of SiNPs | Cell line | Cell type | Particle primary size | Source | Exposure dose | Exposure duration | Endpoint | Assay(s)/method(s) | Results | References |
|--------------|-----------|-----------|-----------------------|--------|--------------|------------------|----------|-------------------|---------|------------|
| SiNPs        | Human peripheral lymphocytes | Human | 10–20 nm | Sigma-Aldrich | 50–100 µg/ml | 24 h | Cell viability | MTT assay | Dose-dependent reduction | Rajiv et al. (2015) |
| SiNPs        | Primary microglial cells | Rat brain macrophage like cells | 20 nm | Sigma-Aldrich | 250 and 500 mg/ml | 24 h | Cell viability | MTT assay | No reduction | Xue et al. (2012) |
| SiNPs        | PC12       | Rat neuron like cells | 25± nm | Saint Louis | 25–200 µg/ml | 24 h | Pro-inflammatory response | ELISA | Mild increase of cytokines such as IL-6, TNF-α and IL-1β | Xie and Wu (2016) |
| SiNPs        | RAW 264.7  | Mouse macrophage | 10, 30, 300, and 1000 nm (with and without amine modification) | Micromod Partikeltechnologie | 6.25–100 µg/m (with LPS and PGN) | 24 h | Cell viability | WST-8 | Dose and size-dependent reduction (only for bare SiNPs) | Uemura et al. (2016) |

*TEER* Transepithelial electrical resistance, *RT-PCR* Reverse transcription polymerase chain reaction, *ISDD* In vitro sedimentation, diffusion and dosimetry model, *FACS* Fluorescence-activated cell sorting, *LSCM* Laser scanning confocal microscopy, *AO/EB* Acridine orange/ethidium bromide, *FISH* Fluorescence in situ hybridization, *EGFR* Epidermal growth factor receptor, *KRAS* Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, *HPRT* Hypoxanthine phosphoribosyl transferase, *HSCA* High content screening and analysis, *DAM-FM* 4-amino-5-methylamino-2′,7′-difluorescein, *PI* Propidium iodide, *Nrf 2* Nuclear factor erythroid 2-related factor 2
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|--------------|---------------|----------------------|--------|-------------------|---------------|------------------|-------------------|---------------------|---------------------|----------|------------|
| C-SiNPs      | Oral          | 20 and 100 nm        | E&B Nano-tech | Rat; SD (female and male) | 500 and 1000 mg/kg bw | 1 h–10 days | Animal | Clinical signs | Observation | No animal death | Lee et al. (2014) |
|              |               |                      |        |                   |               |                 |                   | Lungs, liver, brain, kidneys, testis and spleen | Tissue distribution | Molybdenum blue method | | |
|              |               |                      |        |                   |               |                 |                   | NP Localization | TEM | NPs detected in lungs, liver, spleen and kidneys | | |
|              |               |                      |        |                   |               |                 |                   | Urine and feces | Excretion kinetics | Molybdenum blue method | Most NPs excreted via feces | |

| C-SiNPs      | Oral          | 20 and 100 nm (uncoated or L-arginine coated) | E&B Nano-tech | Mice; C57BL/6 | 750 mg/kg bw | Daily for 14 days | Blood | Blood cell count | Serum analyzer | Increase | Kim et al. (2014b) |
|              |               |                      |        |                   |               |                 |                   | Pro-inflammatory response(s) | Multiplex analysis | | |
|              |               |                      |        |                   |               |                 |                   | Spleen | Oxidative stress | DCFH-DA assay | Increase | |
|              |               |                      |        |                   |               |                 |                   | Oxidative stress | SOD and GPx assay | No effect | |
|              |               |                      |        |                   |               |                 |                   | Oxidative stress | Griess reagent/NO production | Increase | |

| C-SiNPs      | Oral          | 12 nm                 | ABC Nano-tech | Rat; SD | Acute-1959 and 2061; Sub-acute-489.8, 979.5, 1959; Sub-chronic: 244.9, 489.8 and 975.9 mg/kg bw | Acute—14 days Sub-acute—daily for 14 days Sub-chronic: daily for 13 w | Blood | Blood cell count | Hematology | No effect | Yun et al. (2015) |
|              |               |                      |        |                   |               |                 |                   | Organ damage biomarkers | Serum analyzer | | |
|              |               |                      |        |                   |               |                 |                   | Urine | Urinalysis | urine analyzer | No effect | |
|              |               |                      |        |                   |               |                 |                   | Eye | Ophthalmology | Morphological examination | No effect | |
|              |               |                      |        |                   |               |                 |                   | Major organs | Tissue distribution | ICP-MS | NPs not detected | |
|              |               |                      |        |                   |               |                 |                   | Urine and feces | Excretion kinetics | ICP-MS | Most NPs excreted via feces | |
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|--------------|----------------|-----------------------|--------|-------------------|---------------|------------------|------------------|---------------------|-------------------|---------|------------|
| C-SiNPs      | Oral gavage    | 20 and 80 nm, coated with L-arginine | E&B Nanotech | Rats; Crl:CD(SD) | 500, 1000 and 2000 mg/kg bw | Daily for 90 days | Animal | Clinical signs | Observation | No animal death | Kim et al. (2014c) |
|              |                |                       |        |                   |               |                  | Blood            | Blood cell count | Hematoanalyser   | No effect |           |
|              |                |                       |        |                   |               |                  | Organ damage biomarkers | Serum analyser | No effect |           |
|              |                |                       |        |                   |               |                  | Eye              | Ophthalmology | Observation of ocular fundus | No effect |           |
|              |                |                       |        |                   |               |                  | All major organs | Necropsy | Weighing organs | No effect |           |
|              | Intratracheal  | 9, 15, 30 and 55 nm   | AkzoNobel AB | Rat; Wistar (female) | 360 μg in 500 ml of saline | 3 days | Blood | Blood cell count | Hematoanalyser | Increase of polymorphonuclear neutrophils and lymphocytes | Maser et al. (2015) |
|              |                |                       |        |                   |               |                  | Lungs            | Histopathology | H & E staining | Mild increase of granulomatous inflammation |           |
|              | Dermal         | 20 nm, coated with L-arginine | E&B Nanotech | Rat; Sprague-Dawley (SD) | 500 mg, 1000 mg, and 2000 mg/kg bw | Daily for 90 days | Animal | Clinical signs | Observation | No animal death | Ryu et al. (2014) |
|              |                |                       |        |                   |               |                  | Blood            | Blood cell count | Hematoanalyser   | No effect |           |
|              |                |                       |        |                   |               |                  | Organ damage biomarkers | Serum analyser | No effect |           |
|              |                |                       |        |                   |               |                  | All major organs | Necropsy | Organ weighing | No effect |           |
|              |                |                       |        |                   |               |                  | Histopathology   | H & E staining | No effect |           |
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay (s)/method(s) | Results | References |
|--------------|----------------|-----------------------|--------|--------------------|--------------|-------------------|-------------------|---------------------|----------------------|---------|------------|
| C-SiNPs      | Dermal         | 20 and 100 nm (uncoated or L-arginine coated) | E&B Nano-tech | Rat | 2000 (coated) and 1000 mg/kg bw (uncoated) | Daily for 28 d | Brain | Blood–brain barrier damage | Evans blue staining | No effect | Shim et al. (2014) |
| C-SiNPs      | Intraperitoneal | 50 nm | Polysciences | Mice; Male Sprague–Dawley | 0.25 mg/kg bw | 24 h | Blood | Blood cell count | Hematocytometer | Increase of leukocytes | Nemmar et al. (2016) |
| C-SiNPs      | Intravenous    | 64 nm | Laboratory synthesis | Mice; ICR (male and female) | 0, 29.5, 103.5, and 177.5 mg/kg bw | 14 days | Blood | Genotoxicity | Comet assay | Increase of LDH, ALT and AST | Yu et al. (2013) |

**Additional Notes:**
- C-SiNPs: Carbon-coated silicon nanoparticles
- Dermal: Skin exposure
- Intraperitoneal: Intraperitoneal injection
- Intravenous: Intravenous injection
- TEM: Transmission electron microscopy
- EDX: Energy-dispersive X-ray spectroscopy
- NPs: Nanoparticles
- Si: Silicon
- ICP-OES: Inductively coupled plasma-atomic emission spectroscopy
- LPO: Lipid peroxidation
- SOD: Superoxide dismutase
- Catalase assay
- ELISA: Enzyme-linked immunosorbent assay
- H & E: Hematoxylin & eosin staining
- CD-68: Cluster of differentiation 68 staining
- TEM: Transmission electron microscopy
- ICP-OES: Inductively coupled plasma-atomic emission spectroscopy
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|--------------|----------------|----------------------|--------|-------------------|---------------|------------------|-------------------|-------------------|-------------------|---------|------------|
| S-SiNPs     | Intratracheal  | 50 nm, with or without amine modification | Laboratory synthesis | Mice; C57BL/6 (male) | 4 and 20 mg/kg bw | 24 h | Lungs | Inflammation | BALF cell count and LDH assay | Dose-dependent increase of total cell number, macrophages, neutrophils and LDH release | Morris et al. (2016) |
| S-SiNPs     | Intratracheal  | 58 nm | Laboratory synthesis | Mice; C57 (male) | 2 mg/kg bw | Once every 3 days for 45 days | Testis | Histopathology | H & E staining | Decrease in mature sperm and primary spermatozoa | Zhang et al. (2016) |
|             |                |                 |        |                   |               |                  |                   | Oxidative stress | ROS/RNS production | Dose-dependent increase |               |
|             |                |                 |        |                   |               |                  |                   |                  | Meiotic regulating factors | Cell cycle arrest observed |               |
|             |                |                 |        |                   |               |                  |                   |                  | Oxidative stress | Increase |           |
|             |                |                 |        |                   |               |                  |                   |                  | Sperm quality evaluation | Microscopy | No effect |
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|--------------|----------------|-----------------------|--------|--------------------|---------------|-------------------|-------------------|----------------------|-------------------|---------|------------|
| S-SiNPs      | Intratracheal  | 43 nm                 | Laboratory synthesis | Mice; BALB/c (female) | 0, 7, 21, and 35 mg/kg bw | Once every 3 days for 15 days | Lungs, liver and heart | Histopathology | H & E staining | Increase of Inflammation | Organ damage biomarkers | Serum analyzer | Increase of BUN, CREA, uric acid, and AST | NP localization | TEM | NPs detected in cytoplasm and lysosomes | Increase |
|              |                |                       |        |                    |               |                   |                   |                      |                   |                      |                      |                        |                        | Yang et al. (2016) |
| S-SiNPs      | Intravenous    | 62 nm                 | Laboratory synthesis | Mice; ICR | 29.5, 103.5, and 177.5 mg/kg bw | 14 days | Heart | Autophagy | LC3 and VEGFR2 positive staining | TEM | Increase of LC3 | Increase of autophagic ultrastructures | Cell cytoskeleton staining | MMP measurements | Weakening of F actin | Dose-dependent decrease | LC3-II/LC3-I ratio | Increase | Duan et al. (2014a, b) |
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|--------------|----------------|-----------------------|--------|--------------------|---------------|------------------|-------------------|---------------------|---------------------|---------|------------|
| M-SiNPs     | Intragastrical | Spherical 83 nm, short rods (AR 1.75) and long rods (AR 5) | Laboratory synthesis | Mice; ICR (male) | 40 mg/kg bw | 14 days | Blood | Blood cell count | Hematocytometer | No effect | Li et al. (2015) |
|             |                |                       |        |                    |               |             |       | Organ damage biomarkers | Serum analyzer | Increase of LDH release and CREA | Gross tissue damage in kidneys | Shape-dependent distribution and clearance in organs |
|             |                |                       |        |                    |               |             |       | Histopathology | H & E staining | | |
|             |                |                       |        |                    |               |             |       | Tissue distribution and excretion kinetics | ICP-OES and TEM | | |
|             |                |                       |        |                    |               |             |       | | | |
| M-SiNPs     | Intravenous    | 1.5 (short rods) and 5 (long rods) aspect ratio; standard or PEGylated | Laboratory synthesis | Mice | 20 mg/kg bw | 2 h, 24 h and 7 days | Blood | Blood cell count | Hematocytometer | No effect | Huang et al. (2011) |
|             |                |                       |        |                    |               |             |       | Organ damage biomarkers | Serum analyzer | Increase of TBIL, CREA and BUN | Gross tissue damage in kidneys | PEGylation reduced distribution in liver and spleen |
|             |                |                       |        |                    |               |             |       | Histopathology | DAPI | | |
|             |                |                       |        |                    |               |             |       | Tissue distribution | ICP-OES | | |
|             |                |                       |        |                    |               |             |       | | | |
|             |                |                       |        |                    |               |             |       | Urine and feces | Excretion kinetics | TEM/EDX | | |
|             |                |                       |        |                    |               |             |       | Short rods cleared rapidly than long rods | | | |
Table 2 continued

| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|---------------|----------------|-----------------------|--------|--------------------|---------------|------------------|-------------------|------------------|------------------|---------|------------|
| M-SiNPs       | Intraperitoneal | ~198 nm               | Laboratory synthesis | Mice; BALB/C | 150, 300, and 600 mg/kg bw | 2 and 12 days | Blood | Organ damage biomarkers | Serum analyzer | Increase of AST, ALT, BUN and CREA | | Chen et al. (2015) |
|               |                |                       |         |                    |               |                  | Kidneys | Histopathology | H & E staining | Detection of renal interstitial fibrosis | | |
|               |                |                       |         |                    |               |                  | Tissue damage | Masson’s trichrome staining | | Dose and time-dependent increase in kidney injury | | |
| Pr-SiNPs      | Oral gavage    | 18–24 nm              | JRC repository | Rat; SD (male) | 5, 10, and 20 mg/kg bw | 0, 24, and 45 h | Blood | Oxidative stress | LPO assay | No effect | Tarantini et al. (2015a, b) |
| (NM 200 & 201) |                |                       |         |                    |               |                  | Liver, kidneys, spleen, intestine, blood and bone marrow | Histopathology | Genotoxicity | No effect | |
| Py-SiNPs      | Oral gavage    | 10–15 nm              | JRC repository | Rat; Wistar | 100, 300, or 1000 mg/kg bw at a dose volume of 10 mL/kg bw | Daily for 14 days (from gestation period 6–19) | Animal | Clinical signs | Observation | No animal death | Hofmann et al. (2015) |
| (NM 202 & 203) |                |                       |         |                    |               |                  | Gravid uterus | Body weight necropsy | Cesarean | No effect | |
| Pr-SiNPs      | Oral gavage    | 10–15 nm              | JRC repository | Rat; Wistar | 100, 300, or 1000 mg/kg bw at a dose volume of 10 mL/kg bw | Daily for 14 days (from gestation period 6–19) | Animal | Clinical signs | Observation | No animal death | Hofmann et al. (2015) |
| (NM 200)      |                |                       |         |                    |               |                  | Gravid uterus | Body weight necropsy | Cesarean | No effect | |
Table 2 continued

| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|---------------|----------------|-----------------------|--------|--------------------|---------------|------------------|-------------------|----------------------|---------------------|---------|------------|
| Py-SiNPs      | Exposure via food | 7 nm and NM 202 (10–25 nm) | JRC repository | Rat; SD | Sub-acute: 100, 1000, 2500 mg/kg bw | Daily for 28 days | Blood | Organ damage biomarkers | Serum analyzer | No effect | Zande et al. (2014) |
|               |                |                       |        |                    | Sub-acute: daily for 84 days |                |                   | Plasma IgG and IgM and cytokine analysis | ELISA | No effect |
|               |                |                       |        |                    |               |                  |                   | Serum analyzer | Increase of ALP at 7 d | No change | Zhuravskii et al. (2016) |
|               |                |                       |        |                    |               |                  |                   | Histopathology | Si detected in liver, lungs and spleen | Induction of fibrosis |

| Py-SiNPs | Intravenous | 13 ± 5 nm | Vekton Ltd | Rat; Wistar | 7 mg/kg bw | 7, 30 and 60 days | Blood | Hemodynamics | Blood pressure measurement and heart rate | Hematoanalyser | No effect |
|          |            |          |           |             |           |                  |       |             | Blood cell count | No change | Zhuravskii et al. (2016) |
|          |            |          |           |             |           |                  |       |             | Organ damage biomarkers | Serum analyser | Increase of ALP at 7 d |
|          |            |          |           |             |           |                  |       |             | Tissue distribution | Atomic absorption spectrometry | Si detected in liver, lungs and spleen |
|          |            |          |           |             |           |                  |       |             | NP localization | SEM | NPs detected in hepatocytes |
|          |            |          |           |             |           |                  |       |             | Histopathology | H & E staining | Induction of fibrosis |
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|--------------|----------------|-----------------------|--------|--------------------|---------------|------------------|-------------------|---------------------|---------------------|---------|------------|
| SiNPs        | Oral gavage    | 70 nm, 300 and 1000 nm with or without carboxyl or amine groups | Micromod Partikeltechnologie | Mice; BALB/c | 2.5 mg/mouse | Daily for 28 days | Blood | Blood cell count | Hematocytometer | No effect | Yoshida et al. (2014) |
|              |                |                       |        |                    |               |                  |                   | Organ damage bio-markers | Serum analyzer | No effect |           |
| SiNPs        | Oral gavage    | 10–15 nm              | TECNAN | Rat; Wistar (male) | 333.3 mg/kg bw | Daily for 5 days | Animal | Clinical signs | Observation | Symptoms of vomiting and severe lethargy | Hassankhani et al. (2014) |
|              |                |                       |        |                    |               |                  |                   | Organ damage biomarkers | Serum analyzer | Increase |           |
| SiNPs        | Intratracheal  | Three SiNPs (30, 60, and 90 nm) and one fine-sized silica (600 nm) | Laboratory synthesis | Rat; Wistar (male) | 2.5 and 10 mg/kg bw | Daily for 16 days | Blood and Heart | Blood cell count | Hematocytometer | Increase of WBCs & platelets and decrease of hemoglobin & RBCs | Du et al. (2013) |
|              |                |                       |        |                    |               |                  |                   | Oxidative stress | LPO, GSH, SOD and GSH-Px assay | Increase of MDA formation |                     |           |
|              |                |                       |        |                    |               |                  |                   | Oxidative stress | NO/NOS | Increase of NO and decrease of NOS |                     |                     |           |
|              |                |                       |        |                    |               |                  |                   | Pro-Inflammatory response(s) | ELISA | Increase of TNF-α, IL-1β and IL-6 |                     |                     |           |
|              |                |                       |        |                    |               |                  |                   | Tissue distribution | ICP-OES | NPs detected in heart |                     |                     |           |
Table 2 continued

| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay (s)/method(s) | Results | References |
|---------------|----------------|-----------------------|--------|--------------------|---------------|------------------|-------------------|----------------------|----------------------|---------|------------|
| SiNPs in paints | Oropharyngeal | 19 nm | SiNPs | Mice; BALB/c mice | 20 µg/aspiration | Once a week for 5 w | Lungs | Inflammation | BALF Cell count | Increase of macrophages and neutrophils | Smulders et al. (2014) |
| | | | | | | | | Pro-inflammatory response(s) | ELISA | No effect | |
| | | | | | | | Tissue distribution | ICP-MS | NPs detected in lungs | |
| SiNPs | Intranasal | 10 and 80 nm | Nanoamor SiNPs | Rat; Wistar (male) | 150 µg/50 µl PBS/rat | Daily for 30 days | Brain | Oxidative stress | LPO assay | Increase of MDA formation | Parveen et al. (2015) |
| | | | | | | | | Pro-Inflammatory response(s) | Xylenol orange assay | Increase of H2O2 levels | |
| | | | | | | | | | GSH depletion | Increase | |
| | | | | | | | | | SOD, CAT and GPx levels | Increase | |
| | | | | | | | | | RT-PCR and ELISA | Increase TNF-α, IL-1β and MCP-1 | |
| | | | | | | | | | Nuclear binding activity | Immuno blot analysis | Increase | |
| | | | | | | | | | Tissue distribution | ICP-OES | Si detected in frontal cortex, corpus striatum and hippocampus | |
| Type of SiNPs | Exposure route | Primary particle size | Source | Species and Strain | Exposure dose | Exposure duration | Organ(s)/sample(s) | Effect(s)/endpoint(s) | Assay(s)/method(s) | Results | References |
|--------------|----------------|-----------------------|--------|--------------------|---------------|------------------|-------------------|----------------------|-------------------|---------|------------|
| SiNPs        | Topical        | 70 nm, 300 and 1,000 nm | Micromod Partikeltechnologie | Mice; BALB/c | 250 mg/ear | Daily for 28 days | Skin               | Apoptosis            | TUNEL staining | Increase | Nabeshi et al. (2011b) |
|              | Intravenous    |                       |        |                    | 30 mg/kg bw  | 24 h             | Animal            | Tissue distribution | In vivo Imaging | NP internalization | 70 nm SiNPs detected around the liver |
|              |                |                       |        |                    |               |                  |                   | 70 nm SiNPs detected in cytoplasm and nucleus of the parenchymal hepatocytes (liver) | |
| SiNPs        | Intravenous    | 15 nm                 | Sigma-Aldrich | Rat; SD (male)     | 50 mg/kg bw  | 48 h             | Blood             | Blood cell count   | Hematoanalyzer    | Increase of WBC, lymphocytes, monocytes and neutrophils | Chen et al. (2015) |
|              |                |                       |        |                    |               |                  |                   | Liver               | CD-68 positive cells | Increase | |
|              |                |                       |        |                    |               |                  |                   | Oxidative stress   | CD-68 staining | Increase | |
|              |                |                       |        |                    |               |                  |                   | Injury biomarkers   | GSH and SOD assay | Increase | |
|              |                |                       |        |                    |               |                  |                   |                     | Proton-NMR spectroscopic analysis | Increase | |

*JRC Joint research commission, TEM Transmission electron microscopy, WBCs White blood cells, ICP-MS Inductively coupled plasma mass spectrometry, H & E Hematoxylin and eosin, EDX Energy dispersive X-ray spectroscopy, ELISA Enzyme linked immuno sorbent Assay, CD Cluster of differentiation, ICP-OES Inductively coupled plasma optical emission spectroscopy, BALF Bronchoalveolar lavage fluid, VEGFR Vascular endothelial growth factor receptor, MMP Mitochondrial membrane potential, DAPI-4',6-diamidino-2-phenylindole, dilactate, SEM Scanning electron microscopy, TUNEL Terminal deoxynucleotidyl transferase dUTP nick end labeling, NMR Nuclear magnetic resonance.*
including SiNPs (Manke et al. 2013). Nevertheless, Py- and S-SiNPs caused cytotoxicity without measurable levels of ROS production. It was demonstrated that the disturbance of membrane integrity due to direct cell-membrane interaction might be another possible mechanism of NP cytotoxicity (Fröhlich et al. 2009; Thomassen et al. 2011). However, neither of these studies did substantiate these observations and, therefore, SiNPs cytotoxic effects in the absence of oxidative stress remain poorly understood.

Furthermore, some authors used very high concentrations that may cause “overloading” of cells and modify the nature of NP–cell interactions (Wittmaack 2011). In these cases, it is difficult to evaluate whether the observed effects are physiologically relevant. Although it is challenging, we consider a dose of 384 µg/cm² or higher as irrelevant to human inhalation exposure for amorphous silica, based on the estimation that can be derived from the occupational exposure levels (OELs) (Fig. 2).

### Table 3 Comparison of toxic effects induced by different types of SiNPs (in vitro)

| SiNP type         | Cell type         | Cytotoxicity | Apoptosis | Genotoxicity | Oxidative stress | Pro-inflammation | References               |
|-------------------|-------------------|--------------|-----------|--------------|------------------|------------------|------------------------|
| Colloidal         | Caco2             | ✓            | ✓         | ✓            | ✓                | ✓                | Tarantini et al. (2015a, b) |
| Colloidal         | HepG2             | ✓            | ✓         | ✓            | ✓                | ✓                | Li et al. (2011)        |
| Colloidal         | V79 and A549      | ✓            | ✓         | ✓            | ✓                | ✓                | Maser et al. (2015)    |
| Colloidal         | J744.1            | ✓            | ✓         | ✓            | ✓                | ✓                | Lee et al. (2011)      |
| Colloidal         | PBMC              | ✓            | ✓         | ✓            | ✓                | ✓                | Mendoza et al. (2014)  |
| Stöber            | Huvecs            | ✓            | ✓         | n/a          | ✓                | ✓                | Duan et al. (2013a)    |
| Stöber            | HepG2             | ✓            | ✓         | n/a          | ✓                | n/a              | Sun et al. (2011)      |
| Stöber            | HepG2             | ✓            | ✓         | n/a          | ✓                | n/a              | Wang et al. (2013)     |
| Stöber            | HaCaT             | ✓            | ✓         | n/a          | ✓                | n/a              | Liang et al. (2014)    |
| Stöber            | EA.hy926          | ✓            | n/a       | ✓            | ✓                | n/a              | Napierska et al. (2012a, b) |
| Precipitated      | V79               | ✓            | ✓         | ✓            | ✓                | ✓                | Guichard et al. (2016) |
| Precipitated      | Mouse fibroblast  | ✓            | ✓         | ✓            | ✓                | ✓                | Uboldi et al. (2012)   |
| Precipitated      | GES-1 and caco2   | ✓            | ✓         | ✓            | ✓                | ✓                | Yang et al. (2014a, b) |
| Precipitated      | HepG2             | ✓            | ✓         | n/a          | ✓                | n/a              | Ahmad et al. (2012)    |
| Precipitated      | A549 and A431     | ✓            | ✓         | n/a          | ✓                | n/a              | Ahamed (2013)          |
| Precipitated      | M-HS              | ✓            | n/a       | n/a          | ✓                | ✓                | Di Cristo et al. (2016) |
| Precipitated      | RAW.264.7         | ✓            | n/a       | n/a          | ✓                | ✓                | Di Cristo et al. (2016) |
| Pyrogenic         | V79               | ✓            | ✓         | ✓            | ✓                | ✓                | Guichard et al. (2016) |
| Pyrogenic         | GES-1 and caco2   | ✓            | ✓         | ✓            | ✓                | ✓                | Yang et al. (2014a, b) |
| Pyrogenic         | HT-9              | ✓            | ✓         | ✓            | ✓                | ✓                | Gehrke et al. (2013)   |
| Pyrogenic         | RAW.264.7         | ✓            | n/a       | n/a          | ✓                | ✓                | Di Cristo et al. (2016) |
| Pyrogenic         | M-HS              | ✓            | n/a       | n/a          | ✓                | ✓                | Di Cristo et al. (2016) |

n/a, not investigated; ✓, positive; ✘, negative

### Genotoxicity

In this section, we presented studies on genotoxic effects of SiNPs as it is used as another major endpoint to characterize hazard of NMs. Direct interaction with DNA, oxidative DNA damage, depletion of anti-oxidants, cell cycle arrest, and abnormal expression of genes have been identified as potential mechanisms of NP mediated (geno)toxicity (Donaldson et al. 2010).

#### DNA damage associated with oxidative stress

Exposure to SiNPs (15, 30, and 100 nm) resulted in a size- and dose-dependent increase in 8-hydroxy-2′-deoxyguanosine levels (8-OHdG), phosphorylation of histone on serine-139 (γH2AX), and DNA strand breaks (comet) in human keratinocytes (HaCaT) (Gong et al. 2012). Nabeshi et al. (2011a) also...
demonstrated that exposure to SiNPs (70 nm; 10–90 µg/ml for 24 h) resulted in the increase of oxidative DNA damage (8-OH-dG levels) in HaCaT cells. SiNPs were taken up via actin-mediated endocytosis. Micron-sized particles used in these studies showed no or little effects.

The viability of human Caucasian colon adenocarcinoma (Caco-2) cells dropped to 40% when exposed to 15 nm C-SiNPs (64 µg/ml for 24 h), and, at this same concentration, nearly a threefold increase in micronuclei formation, fivefold increase in histone phosphorylation (γH2AX), and a significant increase in DCF fluorescence were observed. The particles were localized within lysosomes and endocytic compartments, but not in the nucleus. 55 nm C-SiNPs did not induce any of these effects at the same concentration (Tarantini et al. 2015b).

A non-significant increase in % tail DNA (comet assay) and no chromosomal aberrations were induced by SiNPs in human peripheral lymphocytes treated with 100 µg/ml, while a dose-dependent (50–100 µg/ml for 24 h) ROS production (DCFH-DA) and GSH depletion were observed (Rajiv et al. 2015).

**Cell cycle arrest associated with oxidative stress**

S-SiNPs (62 nm) induced increase in DCF fluorescence and decrease in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in HUVECs in a dose-dependent manner (25–100 µg/ml for 24 h). Oxidative stress was linked to cell cycle arrest at G2/M checkpoint (upregulation of chk 1 and downregulation of Cdc25c, Cyclin B1, and Cdc2) and increase in apoptosis (Duan et al. 2013a). In the study by Li et al. (2011), a size-dependent (19, 43, and 68 nm) increase in oxidative stress (DCF fluorescence) and cell cycle arrest in S and G2/M was observed in HepG2 cells exposed to 100 µg/ml of C-SiNPs. Cell cycle arrest in G2/M phase along with the increase in ROS was also noticed in human hepatic cell line (LC-02) treated with S-SiNPs (50 nm) in a dose-dependent manner (50–200 µg/ml for 24 h) (Wang et al. 2013).

**DNA damage not associated with oxidative stress**

Genotoxicity of Py-SNP (20 and 25–70 nm), Pr-SNP (20 nm), and C-SNP (15 and 40–80 nm) SiNPs were studied in Chinese hamster lung fibroblasts. Py-SiNPs (20 nm) induced a significant increase in DNA strand breaks at 66 µg/ml (24 h), while C-SiNPs (15 nm) showed a similar effect only at 252 µg/ml. Neither of these SiNPs did induce ROS. SiNPs in the size range 25–80 nm exerted no or little genotoxicity (Guichard et al. 2016).

**Genotoxicity reports without the assessment of oxidative stress**

M-SiNPs (100 nm) induced a significant increase in phosphorylated-γH2AX-foci in HT-29 cells treated with a dose of 10 µg/ml for 24 h (Sergent et al. 2012). In the human embryonic kidney cell line (HEK293), 579 genes were upregulated and 1263 genes were downregulated after 24 h of exposure (100 µg/ml) to 100 nm M-SiNPs (Zhang et al. 2015). In another study, 15-nm C-SiNPs induced a significant increase in DNA strand breaks (comet assay) in Chinese hamster cells (V79) and A549 cells at 100 µg/ml (24 h), but, for 55 nm, this effect was observed only in A549 cells (Maser et al. 2015). A significant increase in DNA tail length (comet assay) was observed in HaCaT cells treated with 30 µg/ml (24 h) of 70 nm SiNPs (Nabeshi et al. 2011b).

Exposure to C-SiNPs (~7 nm) resulted in positive genotoxic effects (Lymphoma assay) in mouse lymphoma cells treated with 100 and 150 µg/ml for 4 h (Demir and Casstranova 2016). SiNPs (12 nm) induced DNA strand breaks in RAW 264.7 at 200 and 400 µg/ml, but the induction of micronuclei was noticed only at 400 µg/ml. The particles were internalized in vesicles and in the nucleus (Hashimoto and Imazato 2015).

At any tested concentrations (1–100 µg/ml for 72 h), SiNPs (10–25, 30, 15, 80, and 90 nm) neither induced cytotoxicity nor micronuclei in immortalized Balb/3T3 fibroblasts (Uboldi et al. 2012). In another study, Pr-SiNPs (NM-200 and NM-201) and Py-SiNPs (NM-202 and NM-203) with primary size between 14.5 and 16 nm did not induce any micronuclei (cytokinesis block micronucleus assay) in human peripheral lymphocytes exposed to different concentrations (200–1250 µg/ml) over 24 h (Tavares et al. 2014). It is also worthy to note that, in the latter study, the positive control used did not differ from control conditions.

**Conclusion: genotoxicity**

C-SiNPs and S-SiNPs induced genotoxicity in human tumor cell lines (lung, kidney, skin, and gastro-intestinal systems) and the amplitude of the effect negatively correlated with the size of the NPs. DNA strand breaks were observed at low concentrations (2.5–10 µg/ml), particularly in skin-derived cell lines. The genotoxic effects of C- and S-SiNPs were mainly associated with the induction of oxidative stress, while such information is very limited for other types (Py- and Pr-SiNPs). One study indicated that Py-SiNPs induce DNA damage without the generation of ROS, suggesting that other mechanisms such as direct DNA damage might be involved (Magdolenova et al. 2014). However, it is very difficult to judge whether
such genotoxic effect is direct or indirect, since the cellular uptake and subcellular localization of SiNPs are not often reported. Furthermore, several factors such as SiNP properties, cell type, and exposure scenarios (such as concentrations, assays, and endpoints) may influence the outcomes (Magdolenova et al. 2014), making the comparison difficult between studies and indicating an urgent need for the standardization of genotoxicity studies.

Immunotoxicity

NPs entering the body will most probably interact with immune cells, as they are the first line of defence in human body. In this section, we presented the immune responses induced by SiNPs in different cell lines.

Immunotoxicity associated with oxidative stress

Hara et al. (2014) exposed THP-1-derived macrophages to 100 µg/ml of SiNPs (30 nm) for 6 h and found a significant increase in interleukin-1-beta (IL-1β), ROS production, and SiNP uptake via phagocytosis. In the study of Choi et al. (2010), larger sized SiNPs (150–200 nm) were effectively phagocytosed by primary rat microglial cells after 24 h of exposure to different concentrations (0.0728–7.28 µg/ml). A significant increase in ROS, reactive nitrogen species (RNS) and IL-1β was detected at all concentrations.

Immunotoxicity not associated with oxidative stress

At 10 and 20 µg/ml, Di Cristo et al. (2016) found that Py-SiNPs (~14 nm) induced a stronger increase of tumor necrosis factor-alpha (TNF-α), interleukin(IL)-6, and IL-1β in RAW.264.7 macrophages compared to similar sized Pr-SiNPs; Notably, no SiNPs induced ROS in RAW.264.7 macrophages.

Immunotoxicity reports without the assessment of oxidative stress

A significant increase in TNF-α, IL-6, and IL-1α, mitogen activated protein kinases (MAPKs), and nuclear factor (NF)-κB were observed only for C-SiNPs (100 nm) in J774A.1 macrophages exposed to 100 µg/ml of same sized C-SiNPs or M-SiNPs (Lee et al. 2011). Uemura et al. (2016) showed that SiNPs (10 and 50 nm) caused dose-dependent (6.25–100 µg/ml) increase in the production of TNF-α and decrease of IL-6 in RAW.264.7 macrophages, while their amine surface-modified counterparts did not. Furthermore, 300 and 1000 nm micron-sized particles (both bare and amine modified) also showed a dose-dependent decrease of IL-6. Notably, the effects were stronger for 50 nm compared to other particles. The same cell line was utilized by Yu et al. (2011) to investigate phagocytosis using inductively coupled plasma mass spectroscopy (ICP-MS), and they found that S-SiNPs (25 nm) were phagocytosed at least ten times more than M-SiNPs of same size and high aspect ratio SiNPs (AR 2, 4, and 8). In the study by Napier ska et al. (2012b), THP-1 cells dosed with 5 µg/cm² S-SiNPs (2 nm) showed a significant increase of IL-8, TNF-α, and macrophage inflammatory protein (MIP)-1α, while only a non-significant increase in MIP-1α expression was observed for 16 and 104 nm S-SiNPs.

Conclusion: immunotoxicity

The main cells used to study immune responses to SiNPs were ‘innate’ cells such as monocytes and macrophages. Therefore, the identified in vitro studies only address a very limited part of the immune system, essentially pro-inflammatory responses and potential phagocytosis. Furthermore, the data on immune responses and oxidative stress are very limited and, therefore, no firm conclusions can be made. SiNPs, not only induced stronger pro-inflammatory responses compared to sub-micron and micron sized particles but also size-specific effects within the nano-range in immune cells are observed. Besides size, shape and porosity seem to influence the phagocytosis of SiNPs.

Autophagy

Recently, a growing body of evidence identified autophagy as a cellular defence mechanism against NP toxicity, since it plays a key role in removing misfolded proteins and clearing damaged organelles (Glick et al. 2010). Hence, we present here studies that show induction of autophagy upon exposure to SiNPs.

Autophagy associated with oxidative stress

The same S-SiNPs (62 nm) were used in three studies to investigate the induction of autophagy. Along with the dose-dependent (25–100 µg/ml) increase in ROS production, increase in autophagy bio-marker-microtubule-associated protein 1A/1B-light chain 3 (LC3) and monodansylcadaverine (MDC) labelled autophagic vacuoles were detected in HepG2 cells treated with 62 nm S-SiNPs. In addition, transmission electron microscopy (TEM) images revealed that autophagosomes and autolysosomes induced in the presence of SiNPs (Yu et al. 2014). The same S-SiNPs (62 nm) induced increase of LC3-II/LC3-I ratio and decrease of p-mTOR/mTOR, p-P13 K/P13 K and p-Akt/Akt in HUVEC cells in a dose-dependent (25–100 µg/ml) manner (Duan et al. 2013b, 2014a, b). The results of Guo et al. (2016) also suggest that 50 µg/ml
of S-SiNPs (58 nm) could induce autophagy via MAPK/Bcl-2 and PI3K/Akt/mTOR signaling in HUVECs. After 4 h of exposure to 200 µg/ml of S-SiNPs (50 nm), autophagosomes and ROS production was observed in HaCaT cells. The TEM images revealed that SiNPs were in the cytoplasm and lysosomes, but not in the nucleus. (Liang et al. 2014).

SiNPs (4–13 nm, 62.5 µg/ml) induced a time-dependent (24, 48, and 72 h) reduction in cell viability and increase in oxidative stress (DCF fluorescence and GSH depletion) in the lung fibroblast cell line MRC-5. Compared to control, a significant increase of autophagic vacuoles and LC-3 II/LC3-I ratio was also observed in a time-dependent manner (Voicu et al. 2015). A549 cells, when exposed to 100 and 1000 µg/ml of 20-nm SiNPs, showed threefold and fivefold increase in MDC fluorescence, respectively. In addition, autophagy genes such as ATG-12 and BECN were significantly upregulated (30- and 50-fold, respectively) along with increased production of ROS in cells dosed with 1000 µg/ml (Nowak et al. 2014).

Conclusion: autophagy

SiNPs, particularly S-SiNPs induced autophagy mainly via oxidative stress-mediated upregulation of autophagy-related genes and differential regulation of Akt/mTOR signaling. Similar to cytotoxicity, 25 µg/ml appeared to be the lowest exposure concentration at which SiNPs exhibited significant effects. Furthermore, induced autophagy is correlated to cytotoxicity, suggesting that exposure to SiNPs caused irreversible (serious) cellular damage and resulted in autophagic cell death. Besides autophagy induction, lysosomal and autophagy dysfunction could be a potential mechanism of NPs toxicity (Stern et al. 2012), which, however, had not been investigated for SiNPs.

Toxic effects on blood cells and endothelial dysfunction

Several studies suggest that NPs, when inhaled or ingested, can translocate across barriers (such as air–blood) of the body, enter the circulation, and interact with the cardiovascular system. In this section, we summarized the studies that report the effects of SiNPs on blood and endothelial cells.

Toxic effects on blood cells

Nemmar et al. (2015) showed that mouse blood platelets could aggregate after 3 min of incubation with 5 or 25 µg/ml of 50-nm C-SiNPs, while in the study of Jose Corbalan et al. (2012), such aggregation was observed in 15 min (10 µg/ml of 10 nm C-SiNPs). The latter study also showed fourfold reduction of the nitric oxide (NO)/peroxynitrite (ONOO−) ratio compared to non-treated platelets.

Maurer-jones et al. (2010) investigated the role of porosity of SiNPs on blood cell toxicity. M-SiNPs (25 nm) reduced the cell viability of red blood cells (RBCs) to 50% at the concentration of 270 µg/ml, while non-porous S-SiNPs of similar size required only 20 µg/ml to reach this level of cytotoxicity. In another study, 10% hemolysis (LC10) of RBCs was observed at 36 µg/ml for S-SiNPs (115 nm), while M-SiNPs required 154 µg/ml to induce the same effects. For amine-coated counterparts, LC10 were 97 and 30 µg/ml for S- and M-SiNPs, respectively (Yu et al. 2011).

Endothelium dysfunction

Exposure to different concentrations (12.5–100 µg/ml) of S-SiNPs (58 nm) resulted in a dose-dependent increase in inflammatory mediators such as IL-1β, IL-8, and TNFα, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1) in HUVEC cells (Guo et al. 2015). In the study of Corbalan et al. (2011), 10 µg/ml of 10-nm C-SiNPs induced extremely low NO/NOO− ratio (~0.1) in HUVEC cells. Furthermore, free radical production, pro-inflammatory cytokines (IL-6 and IL-8), and NF-κB-binding activity were significantly increased in treated cells. In these two studies, increase in ROS was observed at all tested concentrations.

S-SiNPs (62 nm) induced an imbalance in the ratio NO/nitric oxide synthase (NOS) enzyme in HUVEC cells and such imbalance resulted in a significant increase of pro-inflammatory response (c-reactive protein CRP, IL-1β, IL-6, and TNFα) in a dose- (50–100 µg/ml) dependent manner (Duan et al. 2014b).

Conclusion: Toxic effects on blood cells and endothelial dysfunction

Endothelial cells and platelets together play a key role in maintaining the vascular homeostasis (Rajendran et al. 2013). C-SiNPs induced oxidative stress and disturbed NO/NOO− ratio, which resulted in the aggregation of platelets and endothelial dysfunction. This information is not available for other types of SiNPs. Furthermore, C-SiNPs mediated endothelial dysfunction resulted in pro-inflammatory signals via the secretion of cytokines and adhesion molecules. Together, these results suggest the potential of SiNPs to cause vascular thrombosis and atherosclerosis (Radomski et al. 2005). Furthermore, SiNPs caused hemolysis of RBCs in a size-, charge-, and porosity-dependent manner.
Neurotoxicity

NPs of very small size are capable of translocating across the blood–brain barrier (Hu and Gao 2010). Therefore, studies investigating effects on cells relevant for neurotoxicity are presented here.

Rat medulla tumor cells (PC12 cell line) incubated with the supernatant of 20 nm SiNPs-treated-microglial cells (250 µg/ml and 500 µg/ml for 24 h) did not show any effects compared to the control. Earlier in this study, no secretion of bio-mediators was observed in SiNPs-treated-microglial cells (Xue et al. 2012). In contrast to this study, PC12 cells exposed directly to SiNPs (25 nm; 25–200 µg/ml for 24 h) showed increased uptake and a dose-dependent increase in the induction of autophagy (increase in LC-II and Beclin 1) and inhibition of PI3 K-Akt-mTOR signaling (Xie and Wu 2016). Yang et al. (2014a) showed that exposure to SiNPs (15 nm; 10 µg/ml for 24 h) induced pathological signs of Alzheimer’s disease such as altered expression of amyloid precursor protein (APP) and neprilysin, enhanced phosphorylation of tau at Ser262 and Ser396, and activation of glycogen synthases kinase (GSK)-3β in human SK-N-SH and mouse neuro2a neuroblastoma cells.

Conclusion: neurotoxicity

In vitro studies used cell lines of CNS-based cells, mainly neuron like cells. Although the data on the neurotoxic effects of SiNPs are very limited, studies above suggest that SiNPs can induce adverse effects including the markers of Alzheimer’s disease, when in direct contact with neuroblastoma cells.

Miscellaneous issues

Influence of cell lines on SiNPs cytotoxicity

A dose- (80–640 µg/ml) dependent decrease in the viability and increase of apoptosis were observed in HepG2 cells in presence of SiNPs (7 and 20 nm), but a significant reduction was observed in normal human liver cells (LC-02) only at the unrealistic dose level of 640 µg/ml (Lu et al. 2011). In another study, SiNPs (10–50 nm) induced a dose-dependent (100–600 µg/ml) increase in LDH release in Caco-2 cells, but a significant release of LDH was observed only at the unrealistic dose of 600 µg/ml in human gastric epithelial cells (GES-1). Furthermore, exposure to these SiNPs (200 µg/ml for 48 h) also induced cell cycle arrest in S phase for GES cells and G2/M in Caco-2 cells (Yang et al. 2014b). In a porcine kidney cell line (LLC PK1) exposed to 20-nm SiNPs, a dose- (5–50 µg/ml) dependent increase in DCF fluorescence and MDA formation was observed, whereas human kidney cells (HK-2) showed little effects at 50 µg/ml (Passagne et al. 2012).

Conclusion: miscellaneous issues

No firm conclusions can be drawn from these cases; however, the cytotoxicity of SiNPs appears to vary with species and cell line.

Physiologically relevant cultures

Lung co-culture models  Co-cultures of lung cells are usually made with epithelial cells on the apical and endothelial cells on the basal compartment of a transwell membrane, with or without monocytes on top of the epithelial cells. In a co-culture (A 549 at the apical and ISO-HAS-1 at the basolateral compartment) exposed to 100-µg/ml 30-nm C-SiNPs (coated with or without surfactant), nearly a fivefold increase of IL-8 release for both forms of SiNPs was observed in both compartments (Kasper et al. 2015). When other epithelial cells were used—H441 cells—at the apical together with ISO-HAS-1 cells at the basolateral compartment, these C-SiNPs induced IL-8 were expressed in both compartments, while SiCAM-1 (6–600 µg/ml) and IL-6 (at 6 and 60 µg/ml) were observed only in the apical part (Kasper et al. 2011). The same co-culture model was exposed to 100 µg/ml of S-SiNPs (15, 35, and 80 nm) and the authors noticed an increase of IL-8, TNF-α, and surfactant protein (SP-A1 and SP-A2) expression compared to the control. In addition, less IL-8 and surfactant protein expression, and more TNF-α were observed in the co-culture added with THP-1, notably the effect was the highest for 35 nm (Farcal et al. 2012).

Napierska et al. (2012b) tested SiNPs with primary size 2, 16, 60, and 104 nm (dosed at 10 µg/cm²) in a co-culture (A549 at the apical and EA. hy926 at the basolateral compartment) and observed increase in cytokines such as IL-6, IL-8, TNF-α, and MIP-1α only for 60 and 2 nm (except IL-8). When THP-1 were added to the co-culture, a significant increase in IL-8 and decrease in TNF-α were observed only for 2 nm. The expression of cytokines was also differentially regulated for 16 and 104 nm before and after THP-1 added, but the effects were stronger for 60 and 2 nm, particularly 60-nm NPs.

Air–liquid interface  At the air–liquid interface (ALI), aerosolized and deposited 12-nm Py-SiNPs (52 µg/cm²) and 50-nm S-SiNPs (117 µg/cm²) induced significantly less biological effects (LDH leakage, IL-8 release, COX-2 expression, and p38 phosphorylation) in A549 cells compared to A549 exposed to 15.6 µg/cm² under sub-merged conditions (Panas et al. 2014).
Conclusion: physiologically relevant cultures

Sub-merged (co) cultures and ALI systems (Lenz et al. 2013; Panas et al. 2014) have been claimed to more closely mimicking the in vivo exposure scenarios compared to monocultures. In these systems, the toxicity and pro-inflammatory responses are significantly modulated by SiNPs, which represent the complexity of in vivo systems and need for the establishment of physiologically relevant in vitro cultures. However, at this moment, it is difficult to know whether these biological responses were influenced by SiNPs physico-chemical properties.

Chronic in vitro studies

In vitro chronic Py-SiNPs (12 nm) exposure of intestinal epithelial cell line (C2BBe1) was examined in a recent study. The cells were exposed to 10-µg/cm² SiNPs for 24 h. After 24 h, the medium was replaced (without NPs) and cells were allowed to grow for 4–6 days. At the end of incubation, cells were passaged and again exposed for 24 h and grown for 4–6 days; this cycle was repeated for 29 passages (total life span). The cells and supernatants were collected at the end of each passage for analysis. Though the particles were internalized (only in a fraction of cells), no significant induction of necrosis, apoptosis, and LDH release and decrease in cell viability was observed for any of these conditions (McCracken et al. 2013).

In vivo studies

Ingestion exposure

Single exposure

Lee et al. (2014) investigated the tissue distribution and excretion profiles in Sprague–Dawley (SD) rats orally administered with a very high dose of 500 or 1000-mg/kg bw of C-SiNPs (20 and 100 nm). Silicon (Si) levels were significantly elevated in liver, kidney, lung, and spleen at 6-h post-administration, while no such increase was noticed in brain, ovaries or testes, esophagus, stomach, and intestine even after 7 days of post-administration. Nearly 75–80% of administered SiNPs were excreted via urine and 7–8% via feces. The author also noticed that 20 nm SiNPs excreted faster than the 100 nm SiNPs. In another study, C-SiNPs (12 nm) did not induce any toxicity after 14 days in SD-rats administered with a single oral dose (1959 or 2061 mg/kg bw) (Yun et al. 2015).

In the study of Li et al. (2015), M-SiNPs with different aspect ratios (AR, spherical 83 nm with AR 1, short rods with AR 1.75 and long rods with AR 5) were administered in mice (40 mg/kg bw via gavage). After 7 days, a very high amount of Si was detected only in the liver of spherical SiNPs exposed mice. Urinary excretion, intestinal absorption, and organ distribution of SiNPs were decreased with increasing aspect ratio. Furthermore, increase in renal damage such as hemorrhage, vascular congestion, and renal tubular necrosis with increasing aspect ratio was observed after 14 days of administration.

Kim et al. (2014b) exposed C57BL/6 mice (oral gavage; 750 mg/kg bw) to C-SiNPs (20 and 100 nm) that had been modified with or without l-arginine to determine the influence of surface charge on immunotoxicity in vivo. After 14 days, the author noticed a size-dependent decrease in WBCs cell count and cytokines (in blood), and reduced proliferation of B cells and T cells (from spleen) only for uncoated SiNPs.

Repeated exposure

C-SiNPs (12 nm) neither induce abnormal changes in blood biochemical and hematological parameters nor accumulated in any organs of the acutely (489.8, 979.5, or 1959 mg/kg bw/day, during 14 days) or sub-chronically (244.9, 489.8, or 975.9 mg/kg bw/day, during 13 weeks) exposed (via gavage) SD rats (Yun et al. 2015). In the study by Kim et al. (2014c), C-SiNPs (20 and 100 nm) did not induce any significant changes (compared to control) in clinical signs, blood biochemical, hematological, and histopathological analysis in sub-chronically exposed Crl: CD (SD) specific pathogen-free rats (500, 1000, or 2000 mg/kg bw/day for 90 days).

SD rats were administered (via oral gavage) with low doses (5, 10 or 20 mg/kgbw) of Pr-SiNPs (NM 200 and 201) and Py-SiNPs (NM 202 and 203) at 0, 24 and 45 h and sacrificed at 3 h after the last administration. No significant genotoxicity was observed in cells extracted from different organs (duodenum, colon, blood, kidney, liver, and spleen), and no pathological conditions were recorded in any of these organs (Tarantini et al. 2015a).

Hofmann et al. (2015) investigated the prenatal toxicity by exposing pregnant Wistar rats to Pr-SiNPs (NM 200) from gestation day 6–19. At doses of 100, 300, or 1000 mg/kgbw/day, administered SiNPs did not induce malformations in fetuses or death of the rats.

Zande et al. (2014) investigated the sub-acute and sub-chronic toxicity of Py-SiNPs (7 nm) and NM-202 (10–25 nm). In the sub-acute part, male SD rats were orally (via food) exposed to 100, 1000, or 2500 mg Py-SiNPs/kg bw/day for 28 days, while in the sub-chronic part, the rats were exposed only to the highest dose (2500 mg/kg bw/day) repeatedly for 84 days. ICP-MS analysis of target
organs showed that NM-202 was significantly distributed in the lung, kidney, and spleen. Although no Si was detected in the liver, histopathological analysis and gene expression studies revealed that fibrosis was induced in the liver after 84 days of exposure. Si was found in the spleen of Py-SiNPs exposed rats only at the end of chronic exposure. The examined parameters such as blood biochemistry, antibody levels (IgG and IgM), and cytokines indicated no systemic toxicity in any of the Py- or NM-202 treated rats.

Yoshida et al. (2014) found an increased intestinal absorption of carboxyl (twofold) and amine-coated SiNPs (1.5-fold) compared to uncoated SiNPs (70 nm) in orally exposed BALB/c mice (2.5 mg/mouse/day for 28 days). In this study, the whole small intestine was processed using the everted gut sac method and a significant amount of Si was detected by ICP-MS. However, no signs of systemic toxicity were observed for any of these SiNPs.

In male Wistar rats that exposed to 10–15 nm SiNPs (oral gavage; 333.3 mg/kg bw/day for 5 days), histopathological analysis revealed gross tissue damage in kidney (cell swelling and necrosis), lung (interstitial pneumonia and bronchopneumonia), and in the testis (congestion, reduction of spermatogenesis and edema). In addition, blood biochemical parameters such as albumin, cholesterol, triglycerides, total proteins, urea, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), as well as alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities were significantly increased in treated mice (Hassankhani et al. 2014).

Inhalation exposure

Single exposure

In the study of Morris et al. (2016), C57BL/6 mice were intratracheally instilled with 4- or 20-mg S-SiNPs/kg bw and significant effects were observed only at the dose of 20 mg/kg. Twenty hours after instillation, approximately 20- and 10-fold higher cell number was observed in the bronchoalveolar lavage (BAL) of mice treated with the bare and amine-coated SiNPs, respectively, compared to control mice; neutrophils were also increased about 30- and 20-fold, respectively.

In another study, 15- and 55-nm C-SiNPs neither induced DNA damage nor micronuclei in lung and bone marrow (erythrocytes) cells of Wistar rats at 72 h of post-instillation (360 µg) (Maser et al. 2015). However, the distribution of administered SiNPs to these organs was not investigated.

Repeated exposure

A size- and dose-dependent increase in the distribution of SiNPs was observed in the serum and heart of male Wistar rats intratracheally instilled (2, 5, or 10 mg/kg bw/day for 16 days) with SiNPs (30, 60, 90, and 300 nm). Blood parameters (WBCs and platelets), inflammatory bio-markers (TNF-α, IL-1β, and IL-6), and oxidative stress bio-markers (ROS and MDA formation) were significant increased, while NO, NOS, and eNOS were significantly decreased in the serum of the treated mice (Du et al. 2013).

A study compared the toxicity and biodistribution of pristine SiNPs (19 nm) and aged paints containing SiNPs in BALB/c mice. The suspensions (20 µg/aspiration) were oropharyngeally aspirated once a week for 5 weeks, and mice were sacrificed either at 2- or at 28-day post-final aspiration treatment. Pristine SiNPs were significantly distributed in the lungs and liver, while only a low amount of Si was detected in the liver of paint-exposed mice. No signs of toxicity were observed, except a slight inflammation (a slight increase in macrophages and neutrophils together with an increase of IL-1β) with pristine SiNPs (Smulders et al. 2014).

Male Wistar rats were intranasally instilled with 150 µg SiNPs (10 and 80 nm) repeatedly for 30 days. After 30 days, a size-dependent increase in the levels of hydrogen peroxide (H2O2), MDA formation, TNF-α, IL-1β, MCP-1, and NF-κβ was observed in the frontal cortex, corpus striatum, and hippocampus of the brain. A similar quantity of Si was detected in all these three regions. In addition, a significant decrease in GSH levels was observed in these tissues (Parveen et al. 2015). The latter study shows the potential translocation of (mainly small) SiNP from the nose to the brain, but it has to be noted that the mice received a high dose during 30 days.

Intratracheally administered (2 mg/kg bw; 15 times, once every 3 days) S-SiNPs (58 nm) resulted in reproductive toxicity in C57 mice via enhanced ROS production and cell cycle arrest (G0/G1 phase) in the testicular tissues, and by decreasing the number of mature sperms and primary spermocytes (Zhang et al. 2016). In another study, repeated intracheal instillation of S-SiNPs (58 nm; 0, 7, 21, and 35 mg/kg bw; 5 times, once every 3 days) induced local (macrophage activation in lung, liver, and spleen) and systemic inflammation (Increase in serum IL-8, TNF-α, and IL-6) in BALB/c mice. In addition, SiNPs were also detected in the lysosomes of macrophages in lung, liver, and heart tissues (Yang et al. 2016).

Dermal exposure

Repeated exposure

The skin of SD rats was repeatedly exposed to different doses (500, 1000 mg, or 2000 mg/kg bw) of l-arginine coated C-SiNPs (20 nm) for 90 days (6-h exposure/day). The repeated exposure neither induced gross changes in
the skin nor in any organs. In addition, hematological and blood biochemical parameters did not change in SiNPs-treated mice compared to controls (Ryu et al. 2014). In another study, Shim et al. (2014) showed that C-SiNPs (20 and 100 nm) did not induce toxicity in the right and left brain or distribution in the cerebellum, hippocampus, or striatum of the dermally exposed rats (1000 or 2000 mg/kg bw daily for 90 days). In contrast to these studies, a significant increase in apoptosis (TUNEL positive cells) was observed in the skin of the BALB/c mice topically exposed to 70-nm SiNPs (250 mg/ear/day for 28 days). The author reported that the SiNPs were found not only in the skin but also in the regional lymph nodes, cerebral cortex, hippocampus, and in the liver (Nabeshi et al. 2011b).

**Parenteral exposure**

### Single exposure

In a 48-h study, exposure to 15-nm SiNPs (dose of 50 mg/kg bw by intravenous injection) resulted in a significant increase of CD68-positive Kupffer cells (KCs), WBCs, lymphocytes, monocytes, neutrophils, and TNF-α in the serum of male SD rats. There was a decrease in GSH activity and elevation in MDA levels in the liver of treated mice. Furthermore, bio-markers of liver dysfunction such as lactate, phosphorylcholine, sn-glycero-3-phosphocholine, tyrosine, phenylalanine, and lysine were increased in blood, while the levels of succinate, glucose, and glycine were significantly decreased in mice exposed to SiNPs (Chen et al. 2013).

In another study, 50 nm C-SiNPs (0.25 mg/kg bw) induced a significant increase in blood parameters such as leukocyte number, creatinine kinase (CK), ALT, AST, and LDH release in male tuck ordinary mice at 24-h post-intraperitoneal injection. Oxidative stress bio-markers such as MDA formation, SOD, and catalase were significantly increased in the lung and differentially expressed in other organs. Furthermore, all organs showed a significant DNA damage compared to saline treated mice and the extent of damage was in the order heart > kidney > lung > liver > brain (Nemmar et al. 2016).

In the study of Duan et al. (2014a, b), ICR mice were intravenously injected with 29.5, 103.5, or 177.5 mg/kg bw, and the expression of ICAM-1 and VCAM-1 was significantly decreased at 177.5 mg/kg. Huang et al. (2011) injected the mice intravenously with mesoporous rods (pure or PEGylated) at a dose of 20 mg/kg bw and found a dysfunction in biliary excretion and glomerular filtration. Blood analysis showed that there was a significant increase in total bilirubin (TBIL), blood urea nitrogen (BUN), and creatinine (CREA). The mesoporous rods mainly distributed in the lung, liver, and spleen, but PEGylation significantly reduced their distribution in these organs. In addition, short rods cleared via urine and feces more rapidly than long rods.

Yu et al. (2013) estimated the i.v. median lethal dose (LD_{50}) for 64 nm C-SiNPs as 262 mg/kg bw in ICR mice using the Dixon’s Up and Down method. After 14-day post-administration, intravenously injected C-SiNPs (64 nm, 177.5 mg/kg bw) induced a significant increase in liver injury bio-markers (LDH, AST, and ALT). Histopathological analysis of target organs showed that the number of megakaryocytes in the spleen was significantly increased, and pulmonary hyperemia and interstitial thickening were observed in the lungs. Nearly 35% of the injected SiNPs were distributed in the spleen, 12.5% in the liver, and 2% in the lung of SNP-treated mice.

At 24 h of post-intravenous administration (30 mg/kg bw), TEM imaging of the BALB/c mice tissues showed that 70-nm SiNPs were localized in the regional lymph nodes, cerebral cortex, hippocampus, and the cytoplasm, and nucleus of hepatocytes (liver). Using in vivo imaging, the authors also found that the SiNPs were distributed near the liver immediately after the administration (20 min) and moved near the intestinal tract over time (6 h). Micron-sized silica particles (300 and 1000 nm) used in these studies were accumulated mainly around the gall bladder (Nabeshi et al. 2011b).

A significant increase in the levels of kidney injury bio-markers such as BUN and CREA and morphological changes associated with renal interstitial fibrosis was noticed in BALB/c mice intraperitoneally treated with 198-nm M-SiNPs (150, 300, or 600 mg/kg bw for 2 or 12 days). There was also a significant increase in the levels of fibronectin (FN), TGF-β and ICAM-1, and nuclear translocation of p65 in 300- or 600-mg/kg treated mice (Chen et al. 2015). Zhuravskii et al. (2016) demonstrated that intravenously injected (70 mg/kg bw) Py-SiNPs (13 ± 5 nm) distributed and persisted in the liver of Wistar rats at 60-day post-administration. The authors also indicated that the administered SiNPs induced fibrosis and liver tissue remodeling by noticing the increase in blood ALT, and presence of mast cells, connective tissues, and foreign body-type granulomas in the liver.

**Conclusion: in vivo**

The in vivo toxicity studies have been carried out using rats and mice, and exposure through various routes of
administered. In general, short-term exposure to SiNPs induced adverse effects in the lungs, kidneys, liver, and brain. SiNPs administered mainly via inhalation, ingestion, and intravenous routes were majorly distributed in the liver, lungs, spleen, and kidneys, and in the brain of the intranasally exposed rats. Most of the administered SiNPs were excreted via feces and to a lesser extent via urine, in a size- and shape-dependent manner. However, in most cases, the administered doses were very high compared to relevant human inhalation (Barsan 2007) and ingestion (Winkler et al. 2016; Dekkers et al. 2011) exposures to amorphous silica. Some studies showed accumulation of SiNPs in organs such as the liver, but such accumulation was not associated with any major effects. However, long-term effects of accumulated SiNPs were not studied. It is surprising that in contrast to acute studies, no toxicity (local or systemic) was observed in chronic oral and dermal exposure studies, regardless of the size of SiNPs and the high doses used. Moreover, surface-modified SiNPs showed a significant increase in absorption by the GI tract compared to bare SiNPs. Dosing SiNPs via i.v. showed in all studies some sign of damage/toxicity. No clear type- or size-dependent effect can be identified from the set of reviewed studies. Furthermore, where genotoxicity was studied, it was not clear whether SiNPs were taken up by the target cells or were able to reach the tissue examined, which is obviously needed to exert a direct genotoxic effect.

Discussion

Growing production and use of SiNPs increase the risk of human exposures. Available toxicity studies mainly focused on effects after exposure via inhalation or skin (occupational exposure), ingestion (food additive), or nano-therapeutics (parenteral exposure). In vitro and in vivo studies demonstrated that SiNPs can induce adverse effects, but multiple ‘inconsistencies’ were found in the collected toxicity data set. As small differences in the physico-chemical properties of SiNPs could contribute to significant variation in the toxicity (Napierska et al. 2010), it is critical to discriminate how these variations influence toxicity. It is also worthy to note that the choice of cell type, culture system, assay conditions (Fede et al. 2012; Geys et al. 2010; Hayashi et al. 2017), and exposure route influence the toxic responses to SiNPs. However, the nature of these variations remains unclear, which continues to limit our understanding of SiNPs toxicity and hampers their hazard assessment.

Physico-chemical characterization

In this review, a basic set of physico-chemical characteristics of primary SiNPs such as primary size, shape, crystallinity, and chemical composition (or) purity was set as inclusion criteria. Most studies associated the toxic endpoints to SiNPs size, while only a few related the toxicity to SiNPs porosity, shape, surface charge, and surface chemistry. Furthermore, hydrodynamic diameter is another widely reported property in these studies and the results suggest that, in most cases, SiNPs are often aggregated/agglomerated (AA) in cell culture media. Conversely, AA formation is also very likely in real-world matrices such as in air (Kim et al. 2014a), water, and in commercial products such as food (Dekkers et al. 2011). Such AA formation not only substantially alters the overall characteristics (such as size, shape and surface topology) but also potentially influences the biological outcomes (Luys et al. 2013; Drescher et al. 2011). Although some progress has been made in the characterization of SiNPs AA in recent years (De Temmerman et al. 2012), their biological effects remain poorly understood.

Toxicity of different types of SiNPs

Synthetic amorphous SiNPs are produced via different methods such as thermal (pyrogenic) or wet route (colloidal, precipitated and gel). The physico-chemical characteristics of SiNPs produced by these methods differ (Frujtier-Pölloth 2012; Napierska et al. 2010) and may influence the biological outcomes. In this review, nearly 70% of the papers clearly reported the synthesis method. Among those 70%, about 80% reported the toxicity of wet method based SiNPs and only approximately 20% on the other types (such as pyrogenic and mesoporous). A general overview of toxic effects induced by different types of SiNPs is presented in Table 3. Cytotoxicity and genotoxicity induced by S- and C-SiNPs are strongly correlated with the induction of oxidative stress. For Pr-SiNPs, oxidative stress was associated with cytotoxicity but not genotoxicity. Interestingly, Py-SiNPs caused cytotoxicity, mostly without the generation of oxidative stress. In addition, recent studies showed that Py-SiNPs are biologically more reactive than C-SiNPs (Zhang et al. 2012) and Pr-SiNPs (Di Cristo et al. 2016) of same composition and size. It is known that C-, S-, and Pr-SiNPs are hydrophilic in nature, while Py-SiNPs are hydrophobic due to the de-hydroxylation of surface OH groups during the production process (Napierska et al. 2010),
and such difference in surface chemistries might contribute to different biological activities. However, more systematic studies are required to verify these differences depending on production processes.

Toxicity mechanisms of amorphous SiNPs and crystalline silica

A fundamental question was raised by Napierska et al. (2010): do amorphous SiNPs induce biological responses similar to crystalline silica? Oxidative DNA and membrane damage have been reported as the major toxic mechanisms involved in the health effects of micron-sized crystalline silica, which is also observed for C- and Pr-SiNPs. The latter two possess silanols on the surface while Py-SiNPs mostly contain siloxanes (Napierska et al. 2010). In addition, recent studies showed that the presence of surface moieties such as silanols is more correlated with crystalline silica toxicity than crystallinity (Zhang et al. 2012; Turci et al. 2016). Thus, silanols appear to be a common surface feature in C-SiNPs, Pr-SiNPs, and crystalline silica, which might contribute to a similar toxic activity. Furthermore, toxicity elicited by Py-SiNPs appears to be oxidative stress-independent, indicating that Py-SiNPs induce adverse effects via other mechanisms (Gehrke et al. 2013; Napierska et al. 2012a). More studies are required to verify these hypotheses.

Influence of exposure routes in vivo

The exposure route obviously influences the in vivo absorption, biodistribution, and toxicity of SiNPs. For instance, after oropharyngeal aspiration or intratracheal instillation, the lungs are clearly the main target (Smulders et al. 2014), while after ingestion (Zande et al. 2014) or iv injection (Yu et al. 2013), the liver and/or the spleen were the targets. SiNPs accumulated in the brain of intranasally exposed rats (Parveen et al. 2015) but did not after oral exposure (Shim et al. 2014). In addition to the exposure route, physico-chemical properties such as size and shape clearly influence the clearance, distribution, and toxicity (Huang et al. 2011; Li et al. 2015).

Adverse effects of chronic exposure in vivo

In repeated (oral and dermal) dose studies (≥28 days), SiNPs did not induce any local or systemic toxicity even in (very) highly dosed rats. A recent study demonstrated that mild and highly dosed rats excreted most of the orally administered SiNPs via the feces (Yun et al. 2015). In addition, Van der Zande et al. (2014) demonstrated the in vitro gelation of SiNPs with increasing concentrations, which might reduce the gastro-intestinal absorption in highly dosed animals and increase excretion via feces. Therefore, the use of (low) realistic exposure doses appears more appropriate, since the toxicokinetics may depend on the level of exposure (Paek et al. 2014). Moreover, information on the physico-chemical properties of ingested and digested SiNPs is lacking, representing a huge knowledge gap in the risk assessment of SiNPs in food (Dekkers et al. 2012).

Correlation between in vitro and in vivo studies

Correlation between in vitro and in vivo effects is an indication that specific cells or tissues are potential targets for SiNPs toxicity. The results of in vitro and in vivo experiments (included within the same study) suggest that exposure to SiNPs could induce Kupffer cell mediated liver injury (Chen et al. 2013), kidney injury via the activation of NF-kB signaling pathways (Chen et al. 2015), and endothelial dysfunction via autophagy (Duan et al. 2014a). Several in vitro studies showed that SiNPs caused DNA double-strand breaks in a wide range of immortalized cell lines at low doses, but no such genotoxic effects were observed, even in animals at high doses. In addition, SiNPs did not induce micronuclei either in vitro or in vivo.

Dosimetry in vitro

Accurate in vitro dosimetry is an important yet complex aspect of nanotoxicology (Lison et al. 2014). In many studies reviewed here, authors often tested high in vitro exposure doses. Considering the exposure dose as nominal dose may be appropriate only for well dispersed and stable SiNP suspensions (Lison et al. 2008). However, Py-SiNPs, for instance, often re-agglomerate in culture media with an effective density lower than the material density (Deloid et al. 2014), thereby potentially affecting the dose reaching the cells (Cohen et al. 2014). Thus, a realistic estimation of the delivered dose is necessary to compare the biological effects between studies and to establish good in vitro and in vivo correlations (Pal et al. 2015).

Physico-chemical properties for the safer design of SiNPs

Immune responses after the administration of SiNPs are very crucial, as they can induce cascades of events by the secretion of cytokines, which may be harmful or beneficial. The data collected in this review show that the smaller the size, the stronger the pro-inflammatory effect. In addition to the size, the surface charge appears to play a role, since less negative charges seem to suppress the immune response. Porosity is another crucial factor influencing
blood biocompatibility, i.e., the more porous the SiNPs, the less hemolysis of RBCs (Maurer-Jones et al. 2010). Furthermore, SiNPs aspect ratio was shown to closely relate to in vivo organ retention and clearance (Huang et al. 2011). Therefore, the size, surface area, porosity, and geometry (shape) appear to be the key parameters for designing less-toxic and less-inflammagenic SiNPs, for bio-medical applications for instance.

In conclusion, SiNPs generally exhibit acute toxic effects in vitro and in vivo. The data on chronic effects of SiNPs exposure are rather conflicting with the acute effects and are still insufficient to draw firm conclusions. No concrete data were found to conclude whether amorphous SiNPs induce fibrosis like micrometric crystalline silica. Moreover, translation to human health effects is impossible at this moment due to the lack of realistic exposure and epidemiological data. Surface moieties (such as silanols, silanolates, and siloxanes) of SiNPs are found to be production-process-specific and seem to be the key determinants of SiNPs toxicity. AA formation for some SiNPs is very dynamic in physiological media, but it is still unclear how it contributes to the hazard characterization. Therefore, the design of safe(r) SiNPs for food, medical, and other applications will only be possible when physico-chemical characteristics can be unambiguously linked to toxicity. Furthermore, detailed investigations on the SNP bioaccumulation/bioavailability and their long-term consequences in vivo are required for a safer use.

Acknowledgements This work was funded by Belgian Science Policy (BELSPO) program “Belgian Research Action through Interdisciplinary Network (BRAIN-be)” for the project “Towards a toxicologically relevant definition of nanomaterials (To2DeNano)”.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Ahamed M (2013) Silica nanoparticles-induced cytotoxicity, oxidative stress and apoptosis in cultured A431 and A549 cells. Hum Exp Toxicol 32:186–195. doi:10.1177/0960327112459206

Ahmad J, Ahamed M, Akhtar MJ et al (2012) Apoptosis induction by silica nanoparticles mediated through reactive oxygen species in human liver cell line HepG2. Toxicol Appl Pharmacol 259:160–168. doi:10.1016/j.taap.2011.12.020

Arts JHE, Muijser H, Duistermaat E et al (2007) Five-day inhalation toxicity study of three types of synthetic amorphous silicas in Wistar rats and post-exposure evaluations for up to 3 months. Food Chem Toxicol 45:1856–1867. doi:10.1016/j.fct.2007.04.001

Athinarayanan J, Periasamy VS, Alsaif MA et al (2014) Presence of nanosilica (E551) in commercial food products: TNF-mediated oxidative stress and altered cell cycle progression in human lung fibroblast cells. Cell Biol Toxicol 30:89–100. doi:10.1007/s10565-014-9271-8

Barsan ME (2007) NIOSH pocket guide to chemical hazards. Department of Health and Human Services, Center for Disease Control and Prevention, DHHS (NIOSH). Publication No. 2005-149. NIOSH Publications, US

Bitar A, Ahmad NM, Fessi H, Elaissari A (2012) Silica-based nanoparticles for biomedical applications. Drug Discov Today 17:1147–1154. doi:10.1016/j.drudis.2012.06.014

Brinch A, Hansen S, Hartmann N et al (2016) EU regulation of nanobiocides: challenges in implementing the biocidal product regulation (BPR). Nanomaterials 6:33. doi:10.3390/nano6020033

Chen Q, Xue Y, Sun J (2013) Kupffer cell-mediated hepatic injury induced by silica nanoparticles in vitro and in vivo. Int J Nanomed 8:1129–1140. doi:10.2147/IJN.S42242

Chen X, Zhouhua W, Jie Z et al (2015) Renal interstitial fibrosis induced by high-dose mesoporous silica nanoparticles via the NF-kappab signaling pathway. Int J Nanomedicine 10:1–22. doi:10.2147/IJN.S73588

Choi J, Zheng Q, Katz HE, Guilate TR (2010) Silica-based nanoparticle uptake and cellular response by primary microglia. Environ Health Perspect 118:589–595. doi:10.1289/ehp.0901534

Chu Z, Huang Y, Li L et al (2012) Physiological pathway of human cell damage induced by genotoxic crystalline silica nanoparticles. Biomaterials 33:7540–7546. doi:10.1016/j.biomaterials.2012.06.073

Cohen JM, Teeguarden JG, Demokritou P (2014) An integrated approach for the in vitro dosimetry of engineered nanomaterials. Part Fibre Toxicol 11:20. doi:10.1186/1743-8977-11-20

Corbalan JJ, Medina C, Jacoby A (2011) Amorphous silica nanoparticles trigger nitric oxide/peroxynitrite imbalance in human endothelial cells : inflammatory and cytotoxic effects. Int J Nanomed. doi:10.2147/IJN.S25071

Croissant JG, Fatieiev Y, Khashab NM (2017) Degradeability and clearance of silicon, organosilica, silsesquioxane, silica mixed oxide, and mesoporous silica nanoparticles. Adv Mater. doi:10.1002/adma.201604634

De Temmerman P-J, Van Doren E, Verleyen E et al (2012) Quantitative characterization of agglomerates and aggregates of pyrogenic and precipitated amorphous silica nanoparticles by transmission electron microscopy. J Nanobiotechnol 10:24. doi:10.1186/1477-3155-10-24

Dekkers S, Krystek P, Peters RJB et al (2011) Presence and risks of nanosilica in food products. Nanotoxicology 5:393–405. doi:10.3109/17435590.2010.519836

Dekkers S, Bouwmeester H, Bos PMJ et al (2012) Knowledge gaps in risk assessment of nanosilica in food : evaluation of the dissolution and toxicity of different forms of silica. Nanotoxicology. doi:10.3109/17435590.2012.662250

Deloid G, Cohen JM, Darrah T et al (2014) Estimating the effective density of engineered nanomaterials for in vitro dosimetry. Nat Commun 5:3514. doi:10.1038/ncomms4514

Demir E, Castranova V (2016) Genotoxic effects of synthetic amorphous silica nanoparticles in the mouse lymphoma assay.
Toxicol Rep 3:807–815. doi:10.1016/j.toxrep.2016.10.006
Di Cristo L, Movia D, Bianchi MG et al (2016) Proinflammatory effects of pyrogenic and precipitated amorphous silica nanoparticles in innate immunity cells. Toxicol Sci 150:40–53. doi:10.1093/toxsci/kfv258
Donaldson K, Poland CA, Schins RPF (2010) Possible genotoxic mechanisms of nanoparticles: criteria for improved test strategies. Nanotoxicology 4:414–420. doi:10.3109/17435390.2010.482751
Drescher D, Orts-Gil G, Laube G et al (2011) Toxicity of amorphous silica nanoparticles on eukaryotic cell model is determined by particle agglomeration and serum protein adsorption effects. Anal Bioanal Chem 400:1367–1373. doi:10.1007/s00216-011-4893-2
Hu YL, Gao JQ (2010) Potential neurotoxicity of nanoparticles. Int J Nanomed 5:1229–1236. doi:10.2147/IJN.S28293
Fede C, Selvestrel F, Compagnin C et al (2012) The toxicity outcome of silica nanoparticles (Ludox??) is influenced by testing techniques for vascular homeostasis. Int J Nanomed 7:631–639. doi:10.2147/IJN.S28293
Kasai MR (2015) Nanosized particles of silica and its derivatives for applications in various branches of food and nutrition sectors. J Nanotechnol. doi:10.1155/2015/852394
Kasper J, Hermanns MI, Baniz C et al (2011) Inflammatory and cytotoxic responses of an alveolar-capillary coculture model to silica nanoparticles: comparison with conventional monocultures. Part Fibre Toxicol 8:6. doi:10.1186/1743-8977-8-6
Kasai MR (2015) Nanosized particles of silica and its derivatives for applications in various branches of food and nutrition sectors. J Nanotechnol. doi:10.1155/2015/852394
Federico C, Andriollo GD, Lazzarino E et al (2017) Autophagy: cellular and molecular mechanisms. J Pathol 231:3–12. doi:10.1002/path.2697
Glick D, Barth S, Macleod KF (2010) Autophagy: cellular and molecular mechanisms. J Pathol 221:3–12. doi:10.1002/path.2697
of nanoparticles leaking from a vacuum cleaner. Ind Heal 52:152–162. doi:10.2486/indhealth.2013-0087

Kim J-H, Kim C-S, Ignacio RMC et al (2014b) Immunotoxicity of silicon dioxide nanoparticles with different sizes and electrostatic charge. Int J Nanomed 9(Suppl 2):183–193. doi:10.2147/IJNN.S57934

Kim YR, Lee SY, Lee EJ et al (2014c) Toxicity of colloidal silica nanoparticles administered orally for 90 days in rats. Int J Nanomed 9:67–78. doi:10.2147/IJNN.S57925

Lee S, Yun HS, Kim SH (2011) The comparative effects of mesoporous silica nanoparticles and colloidal silica on inflammation and apoptosis. Biomaterials 32:9434–9443. doi:10.1016/j.biomaterials.2011.08.042

Lee JA, Kim MK, Paek HJ et al (2014) Tissue distribution and excretion kinetics of orally administered silica nanoparticles in rats. Int J Nanomed 9:251–260. doi:10.2147/IJNN.S57939

Lenz A-G, Karg E, Brendel E et al (2013) Inflammatory and oxidative stress responses of an alveolar epithelial cell line to airborne zinc oxide nanoparticles at the air-liquid interface: a comparison with conventional, submerged cell-culture conditions. Biomed Res Int 1:12

Leung CC, Yu ITS, Chen W (2012) Silicosis. Lancet 379:2008–2018. doi:10.1016/S0140-6736(12)60237c

Li Y, Sun L, Jin M et al (2011) Size-dependent cytotoxicity of amorphous silica nanoparticles in human hepatoma HepG2 cells. Toxicol Vitr 25:1343–1352. doi:10.1016/j.tiv.2011.05.003

Li L, Liu T, Fu C et al (2015) Biodistribution, excretion, and toxicity of mesoporous silica nanoparticles after oral administration depend on their shape. Nanomed Nanotechnol Biol Med 11:1915–1924. doi:10.1016/j.nano.2015.07.004

Liang H, Jin C, Tang Y et al (2014) Cytotoxicity of silica nanoparticles on HaCaT cells. J Appl Toxicol 34:367–372. doi:10.1002/jat.2953

Liljenström C, Lazarevic D, Finnveden G (2013) Silicon-based nanomaterials in a life-cycle perspective, including a case study on self-cleaning coatings. ISBN 978-91-7501-942-0

Lison D, Thomassen LCJ, Rabolli V et al (2008) Nominal and effective dosimetry of silica nanoparticles in cytotoxicity assays. Toxicol Sci 104:155–162. doi:10.1093/toxsci/kfn072

Lison D, Vietti G, Van Den Brule S (2014) Paracelsus in nanotoxicology. Part Fibre Toxicol. doi:10.1186/1743-8977-7-39

Lison D, Thomassen LCJ, Lison D et al (2010) The nanosilica hazard: another variable entity. Part Fibre Toxicol 7:39. doi:10.1186/1743-8977-7-39

Lobovikov E, Schulz M, Sauer UG et al (2015) In vitro dosimetry on toxicological ranking of low aspect ratio engineered nanoparticles. Biomaterials 32:2713–2724. doi:10.1016/j.biomaterials.2010.12.042

Lobovikov E, Schulz M, Sauer UG et al (2015) In vitro dosimetry on toxicological ranking of low aspect ratio engineered nanoparticles. Biomaterials 32:2713–2724. doi:10.1016/j.biomaterials.2010.12.042

Lusby R, Sorsa M, Urban N et al (2015) Silica nanoparticles induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. Part Fibre Toxicol 8:1. doi:10.1186/1743-8977-8-1

Lusby R, Sorsa M, Urban N et al (2015) Silica nanoparticles induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. Part Fibre Toxicol 8:1. doi:10.1186/1743-8977-8-1

Lusby R, Sorsa M, Urban N et al (2015) Silica nanoparticles induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. Part Fibre Toxicol 8:1. doi:10.1186/1743-8977-8-1

Macedo A, Torres-Hernandez JA, Ault JG et al (2014) Silica nanoparticles induce oxidative stress and inflammation of human peripheral blood mononuclear cells. Cell Stress Chaperones 19:777–790. doi:10.1007/s12192-014-0502-y

Michael Berg J, Romoser AA, Figueroa DE et al (2013) Comparative cytological responses of lung epithelial and pleural mesothelial cells following in vitro exposure to nanoscale SiO2. Toxicol Vitr 27:24–33. doi:10.1016/j.tiv.2012.09.002

Morris AS, Adamcakova-Dodd A, Lehman SE et al (2016) Amine modification of nonporous silica nanoparticles reduces inflammatory response following intratracheal instillation in murine lungs. Toxicol Lett 241:207–215. doi:10.1016/j.toxlet.2015.11.006

Nabeshi H, Yoshikawa T, Matsuyama K et al (2011a) Amorphous nanosilica induce endocytosis-dependent ROS generation and DNA damage in human keratinocytes. Part Fibre Toxicol 8:1. doi:10.1186/1743-8977-8-1

Nabeshi H, Yoshikawa T, Matsuyama K et al (2011b) Systemic distribution, nuclear entry and cytotoxicity of amorphous nanosilica following topical application. Biomaterials 32:2713–2724. doi:10.1016/j.biomaterials.2010.12.042

Napieriska D, Thomassen LCJ, Lison D et al (2010) The nanosilica hazard: another variable entity. Part Fibre Toxicol 7:39. doi:10.1186/1743-8977-7-39

Napieriska D, Rabolli V, Thomassen LCJ et al (2012a) Oxidative stress induced by pure and iron-doped amorphous silica nanoparticles in subtoxic conditions. Chem Res Toxicol 25:828–837. doi:10.1021/tx200361v

Napieriska D, Thomassen LCJ, Vanaudenaerde B et al (2012b) Cytokine production by co-cultures exposed to monodisperse amorphous silica nanoparticles: the role of size and surface area. Toxicol Lett 211:98–104. doi:10.1016/j.toxlet.2012.03.002

Nemmar A, Yuvara P, Beegam S et al (2015) In vitro platelet aggregation and oxidative stress caused by amorphous silica nanoparticles. Int J Physiol Pathophysiol Pharmacol 7:27–33

Nemmar A, Yuvara P, Beegam S et al (2016) Oxidative stress, inflammation, and DNA damage in multiple organs of mice acutely exposed to amorphous silica nanoparticles. Int J Nanomed. doi:10.2147/IJN.S92278

Nowak JS, Mehn D, Nativo P et al (2014) Silica nanoparticle uptake induces survival mechanism in A549 cells by the activation of autophagy but not apoptosis. Toxicol Lett 224:84–92. doi:10.1016/j.toxlet.2013.10.003

Oberdörster G (2010) Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. J Intern Med 267:89–105. doi:10.1111/j.1365-2796.2009.02187.x

Oh S, Kim B, Kim H (2014) Comparison of nanoparticle exposures between fumed and sol-gel nano-silica manufacturing facilities. Ind Health 52:190–198. doi:10.2486/indhealth.2013-0117

Paek H-J, Chung H-E, Lee J-A et al (2014) In vitro dosimetry on toxicological ranking of low aspect ratio engineered nanoparticles. Biomaterials 35:1021–1030. doi:10.1016/j.biomaterials.2014.01.034

Pal AK, Bello D, Cohen J, Demokritou P (2015) Implications of in vitro dosimetry on toxicological ranking of low aspect ratio engineered nanomaterials. Nanotoxicology 9:871–885. doi:10.3109/17435390.2014.986670

Panas A, Comouth A, Saathoff H et al (2014) Silica nanoparticles are less toxic to human lung cells when deposited at the air-liquid interface compared to conventional submersed exposure. Beilstein J Nanotechnol 5:1590–1602. doi:10.3762/bjnano.5.171
hepatic cell line. Toxicol Vitr 24:751–758. doi:10.1016/j.
tiv.2010.01.001
Yoshida T, Yoshioka Y, Takahashi H et al (2014) Intestinal absorption
and biological effects of orally administered amorphous silica
particles. Nanoscale Res Lett. doi:10.1186/1556-276X-9-532
Yu T, Malugin A, Ghandehari H (2011) Impact of silica nanoparticle
design on cellular toxicity and hemolytic activity. ACS Nano
5:5717–5728. doi:10.1021/nn2013904
Yu Y, Li Y, Wang W et al (2013) Acute toxicity of amorphous silica
nanoparticles in intravenously exposed ICR mice. PLoS One.
doi:10.1371/journal.pone.0061346
Yu Y, Duan J, Yu Y et al (2014) Silica nanoparticles induce autophagy
and autophagic cell death in HepG2 cells triggered by reactive
oxygen species. J Hazard Mater 270:176–186. doi:10.1016/j.
jhazmat.2014.01.028
Yun J-W, Kim S-H, You J-R et al (2015) Comparative toxicity of silici-
don dioxide, silver and iron oxide nanoparticles after repeated
oral administration to rats. J Appl Toxicol 35:681–693.
doi:10.1002/jat.3125
Zhang XQ, Yin LH, Tang M, Pu YP (2011) ZnO, TiO(2), SiO(2),
and Al(2)O(3) nanoparticles-induced toxic effects on
human fetal lung fibroblasts. Biomed Environ Sci 24:661–669.
doi:10.3967/0895-3988.2011.06.011
Zhang H, Dunphy DR, Jiang X et al (2012) Processing pathway
dependence of amorphous silica nanoparticle toxicity: colloidal
vs pyrolytic. J Am Chem Soc 134:15790–15804. doi:10.1021/
jacs.120134907c
Zhang Q, Xu H, Zheng S et al (2015) Genotoxicity of mesoporous
silica nanoparticles in human embryonic kidney 293 cells. Drug
Test Anal 7:787–796. doi:10.1002/dta.1773
Zhang J, Ren L, Zou Y et al (2016) Silica nanoparticles induce
start inhibition of meiosis and cell cycle arrest via down-regulat-
ing meiotic relevant factors. Toxicol Res 5:1453–1464.
doi:10.1039/C6TX00236F
Zhuravskii S, Yukina G, Kulikova O et al (2016) Mast cell accumu-
lation precedes tissue fibrosis induced by intravenously admin-
istered amorphous silica nanoparticles. Toxicol Mech Methods
26:260–269. doi:10.3109/15376516.2016.1169341