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Silicon dioxide and titanium dioxide particles found in human tissues

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ABSTRACT

Silicon dioxide (silica, SiO₂, SAS) and titanium dioxide (TiO₂) are produced in high volumes and applied in many consumer and food products. As a consequence, there is a potential human exposure and subsequent systemic uptake of these particles. In this study we show the characterization and quantification of both total silicon (Si) and titanium (Ti), and particulate SiO₂ and TiO₂ in postmortem tissue samples from 15 deceased persons. Included tissues are liver, spleen, kidney and the intestinal tissues jejunum and ileum. Low-level analysis was enabled by the use of fully validated sample digestion methods combined with (single particle) inductively coupled plasma high resolution mass spectrometry techniques (spICP-HRMS). The results show a total-Si concentration ranging from <2 to 191 mg Si/kg (median values of 5.8 (liver), 9.5 (spleen), 7.7 (kidney), 6.8 (jejunum), 7.6 (ileum) mg Si/kg) while the particulate SiO₂ ranged from <0.2 to 25 mg Si/kg (median values of 0.4 (liver), 1.0 (spleen), 0.4 (kidney), 0.7 (jejunum), 0.6 (ileum) mg Si/kg), explaining about 10% of the total-Si concentration. Particle sizes ranged from 150 to 850 nm with a mode of 270 nm. For total-Ti the results show concentrations ranging from <0.01 to 2.0 mg Ti/kg (median values of 0.02 (liver), 0.04 (spleen), 0.05 (kidney), 0.13 (jejunum), 0.26 (ileum) mg Ti/kg) while particulate TiO₂ concentrations ranged from 0.01 to 1.8 mg Ti/kg (median values of 0.02 (liver), 0.02 (spleen), 0.03 (kidney), 0.08 (jejunum), 0.25 (ileum) mg Ti/kg). In general, the particulate TiO₂ explained 80% of the total-Ti concentration. This indicates that most Ti in these organ tissues is particulate material. The detected particles comprise primary particles, aggregates and agglomerates, and were in the range of 50–500 nm with a mode in the range of 100–160 nm. About 17% of the detected TiO₂ particles had a size <100 nm. The presence of SiO₂ and TiO₂ particles in liver tissue was confirmed by scanning electron microscopy with energy dispersive X-ray spectrometry.

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Introduction

Synthetic amorphous silica (SAS, SiO₂) and titanium dioxide (TiO₂), the latter as a white pigment, are industrially produced in high volumes. SAS is used as a food additive, is manufactured by several production processes, and consists mainly of nanosized primary particles that form small aggregates and larger agglomerates (Sepeur et al. 2008; Peters et al. 2012). TiO₂ as a white pigment is used as a food additive, in personal care products (e.g. toothpaste) and in many other consumer products. It contains a fraction of nanosized primary particles (<100 nm) (Peters, Van Bommel et al. 2014; Weir et al. 2012).

As a consequence, human exposure and subsequent systemic uptake of these particles becomes likely. However, only limited data are available on the presence of SiO₂ and TiO₂ particles in human organs. We reported only recently on the presence of TiO₂ particles in liver and spleen (Heringa et al. 2018). In this study, the focus was originally on the determination of SiO₂ particles in liver, spleen, kidney and intestinal samples, however, to strengthen the results of the previous study, TiO₂ particles were also measured in these new samples. Since the 1960s, SiO₂ as an anti-caking agent and TiO₂ as a white pigment are authorized food additives (i.e.

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 Supplemental data for this article can be accessed [here](#).

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in the EU as E171 and E551, respectively (EU Official Journal of the European Union 2008a; EU Official Journal of the European Union 2008b), and in the US as a food color additive (TiO_2) and food contact substance in food packaging) and also applied in consumer and medical products (Peters et al. 2012; Weir et al. 2012; Lim et al. 2015; European Commission 1994). Sodium, calcium, and magnesium silicates and hydrated silica, $\text{SiO}_2 \cdot n\text{H}_2\text{O}$, contain naturally present inorganic Si. The latter may form small particles in the size range of 1–5 nm and can be found in natural waters, including drinking and mineral waters (Bergna 2006; Mojsiewicz-Pienkowska and Lukasiak 2003). There are limited data on the presence of TiO_2 particles in the environment or in untreated food products such as raw milk, vegetables, and meat (Sugibayashi, Todo, and Kimura 2008; Peters, Van Bommel, et al. 2018).

During the life cycle of products, release of SiO_2 and TiO_2 particles occurs, resulting in direct (oral, lung, and dermal) and indirect (via the environment) human exposure (Mueller and Nowack 2008; Maynard et al. 2006; Bouwmeester et al. 2011). Although human tissue levels of the element Ti and particulate TiO_2 have been reported (Heringa et al. 2018; Frisken et al. 2002; Crosera et al. 2015), no data are available on human tissue concentrations of the element Si and particulate SiO_2 (Sarmiento-Gonzalez et al. 2009; National Research Council 2005; WHO 1974). While no human data on the systemic uptake of SiO_2 particles are available (Napierska et al. 2010; Fruijtier-Polloth 2012), a study with rodents implied limited oral uptake of silica at realistic consumer exposure levels (van der Zande et al. 2014). Uptake of TiO_2 particles by the gut has been studied in animals, but rarely in humans (Jovanovic 2015). The only human volunteer studies conducted with single dose administration suggest that the oral bioavailability of TiO_2 is low (Pele et al. 2015; Böckmann et al. 2000; Jones et al. 2015). It should be noted that low oral uptake of nanomaterials can still lead to high organ burdens when there is long-term, frequent exposure in combination with low excretion or high persistence.

In the current study, the presence of SiO_2 and TiO_2 particles in postmortem liver, spleen, kidney, jejunum, and ileum from 15 deceased persons was determined, enabled by the latest developments in analytical detection methods. Liver and spleen were

included in this study because nanomaterials are generally taken up by the mononuclear phagocyte system (MPS) and thereby typically distribute to the liver and spleen, as well as to the kidney (Hardy et al. 2018). Information on the presence of SiO_2 and TiO_2 particles in intestinal tissues is also considered relevant because of the reported uptake of particles by M-cells in Peyer's patches, which are mainly found in the jejunum and ileum (Hardy et al. 2018). Total-Si and total-Ti concentrations were measured using inductively coupled plasma high-resolution mass spectrometry (ICP-HRMS) while SiO_2 and TiO_2 particles were measured using single-particle ICP-MS (spICP-MS) on, respectively, a triple quadrupole ICP-MSMS and a ICP-HRMS and instrument. The tissues were further studied with high resolution scanning electron microscopy with energy dispersive X-ray spectrometry (SEM-EDX) to confirm the presence and size of SiO_2 and TiO_2 particles.

Methods

A full description of the applied methods can be found in the [Supplementary information](#).

Samples and sample preparation

Five types of post mortem human samples were analyzed: liver, spleen, kidney, and parts of the jejunum and ileum. These were obtained from 15 subjects (of which only 12 kidneys, jejunum and ileum were obtained). The complete and intact organs and tissue related to the beginning of the jejunum and the end of the ileum parts of the small intestine were obtained from bodies that were donated to the Department of Anatomy of the University Medical Center Utrecht for educational and research purposes. The bodies, 7 men and 8 women who died at the age of 64–98 years, had been fixed in 4% formaldehyde. During sample preparation, each organ was cut into small pieces and ground to a size range of 0.5–1 mm diameter. All sample handling and sample preparation were carried out in a laminar flow cabinet. To determine potential sample contamination, all materials that have been in contact with the organs were collected. The total-Si and -Ti concentrations in these materials or released by these materials were

determined. The sum of the blank contributions of each of these materials was equal to (total-Si) or lower than (total-Si) the limit of detection (LOD) of the chemical analysis method for the tissues.

Determination of total-Si and -Ti concentration

A subsample of the ground organ was digested in a microwave system using a mixture of nitric acid and hydrofluoric acid (Peters, Undas et al. 2018). The diluted digests were analyzed with ICP-HRMS as described in the next section. Quantification was based on Si and Ti standards diluted in the same acidic matrix as the samples. The method was validated over a period of several days. The repeatability and recovery of the total-Si and -Ti method was determined by spiking samples at 10 and 0.1 mg/kg with NM-202 and NM-104 (a SiO₂ and a TiO₂ nano-material, respectively) and analyzing these on different days. The repeatability for both parameters is <15% and the recovery >90%. The LOD of the methods is 2 mg total-Si/kg and 0.01 mg total-Ti/kg. Method blanks were determined by performing the complete procedure, however, without the addition of a sample. The total-Si and -Ti concentrations in the blanks were below the LOD.

Determination of SiO₂ and TiO₂ particles

For the determination of SiO₂ and TiO₂ particles, a two-step digestion procedure was followed to liberate the particles, as was previously analytically validated (Peters, Undas et al. 2018). In the first step, the tissue in the formaldehyde-fixed sample was depolymerized while in the second step a standard enzymatic digestion was performed. A subsample of 200 mg was collected from the grinded subsamples and the digestion buffer (containing glycine) was added, vortexed for 1 min and heated for 3 h at 100 °C to depolymerize the formaldehyde-fixed tissue. In the second step, and after cooling to room temperature, proteinase K, and digestion buffer were added and the tube was incubated for 16 h at 37 °C. After cooling to room temperature, the digest was diluted 1000 times with Milli-Q water and analyzed using spICP-MS.

Instrumental analysis with ICP-MS

spICP-MS is a method for the detection and characterization of (nano-)particles (Peters, Undas et al.

2018; Peters, Rivera, et al. 2014; Laborda, Bolea, and Jimenez-Lamana 2014; Peters et al. 2015). A triple quadrupole ICP-MSMS was used to measure SiO₂ particles in single-particle mode while a sector-field high-resolution ICP-HRMS was used to measure total-Si and total-Ti in standard mode, and TiO₂ particles in single-particle mode. To minimize any background resulting from Si emissions, all quartz parts in the ICP-MS instruments were replaced by other materials. In addition, the collision cell was used to remove polyatomic interferences from ¹⁴N¹⁴N and ¹²C¹⁶O. Isotopes monitored for Si were *m/z* 28 and 29 in standard mode and *m/z* 28 in single-particle mode. The ICP-HRMS was operated in medium resolution mode with Ti measured at *m/z* 46.95 to avoid interferences from ³⁶Ar¹²C, ³²S¹⁶O, and ⁴⁸Ca. Finally, single-particle data were processed in a dedicated spreadsheet for the calculation of particle sizes, particle size distributions, and particle number and mass concentrations (Peters et al. 2015). As for the total analysis methods, the particle analysis method was validated over a period of several days. The repeatability and the recovery of the SiO₂ and TiO₂ particles were determined by spiking samples at 1 and 0.1 mg/kg with NM-202 and NM-104 and analyzing these in sevenfold on three different days. The recovery was found to be 70 and 76%, respectively, and the repeatability was <35% for both parameters. This recovery of TiO₂ particles was much better than in the previous determination of these particles (Heringa et al. 2018; Peters, Undas et al. 2018). The LOD for SiO₂ and TiO₂ particles is 0.2 mg Si/kg and 0.01 mg Ti/kg, respectively. The size detection limits are 50 nm for TiO₂ particles using spICP-HRMS and 100 nm for SiO₂ using spICP-MSMS with H₂ as the collision gas. While upper size detection limits were not determined they are expected to be at least 1000 nm based on earlier research (Peters et al. 2015). The mass-based SiO₂ and TiO₂ particle concentrations in method blanks were below the LOD.

Confirmation of SiO₂ and TiO₂ particles with SEM-EDX

Samples were studied using scanning electron microscopy with energy dispersive X-ray detection (SEM-EDX) to confirm the presence of SiO₂ and TiO₂ particles in human liver. Typically, subsamples of >100 tissue

Table 1. Summary of total-Si and -Ti concentrations in the organs.

	All organs 69	Liver 15	Spleen 15	Kidney 15	Jejunum 12	Ileum 12
<i>n</i>						
Total-Si	mg Si/kg	mg Si/kg	mg Si/kg	mg Si/kg	mg Si/kg	mg Si/kg
Non-detects ^a	12	3	2	3	1	3
Minimum ^b	2.7	3.2	3.0	4.4	2.7	5.2
Maximum	191	26	47	21	23	191
Median ^c	7.2	5.8	9.5	7.7	6.8	7.6
Average ^c	12	7.8	12	8.2	8.9	23
Std. dev. ^c	23	7.7	12	6.1	6.4	53
Total-Ti	mg Ti/kg	mg Ti/kg	mg Ti/kg	mg Ti/kg	mg Ti/kg	mg Ti/kg
Non-detects ^a	8	4	2	1	1	0
Minimum ^b	0.01	0.01	0.02	0.01	0.02	0.06
Maximum	2.0	0.16	0.25	0.37	2.0	1.4
Median ^c	0.06	0.02	0.04	0.05	0.13	0.26
Average ^c	0.17	0.03	0.06	0.08	0.34	0.43
Std. dev. ^c	0.33	0.04	0.06	0.09	0.57	0.43

^aNumber of samples below the LOD of 2 mg total-Si/kg and 0.0 mg total-Ti/kg.

^bMinimum measured value.

^cFor calculation of median, average and std. dev. non-detects were set at half of the LOD value.

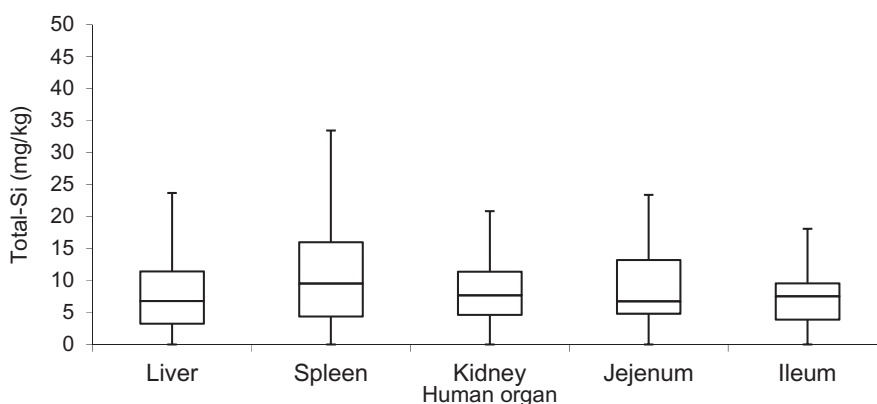


Figure 1. Box and whisker plots of total-Si concentration in human (post mortem) liver, spleen, kidney, jejunum, and ileum. The boxes represent the upper 25% quartile (Q3), median (Q2), and lower 25% quartile (Q1) concentrations, respectively. The whiskers indicate 1.5IQR (Q3–Q1).

grains were collected on a sampling stub and dried to remove water. These subsamples were analyzed with a high-resolution field emission gun scanning electron microscopy in combination with energy dispersive X-ray analysis (FEG-SEM/EDX). Approximately 500 images for each sample were viewed at different magnifications (5000–100,000 \times) and the surface of the sample grains was systematically searched for SiO₂ and TiO₂ particles using the backscattered electron imaging mode. After the detection of particles in a field of view, X-ray spectra from the detected particle and surrounding matrix were acquired to determine the identity.

Results

Total-Si and -Ti concentration in human organ tissues

Postmortem human liver, spleen, kidney, jejunum and ileum samples were analyzed from 15 human

subjects, 7 male and 8 female, who died at the age of 64–98 years. From these persons, written informed consent was obtained during life that allowed the use of their entire bodies for educational and research purposes. While there is no information about their diets, it is known that all persons involved are of Caucasian ethnicity. All have lived in the Netherlands and it is therefore expected that most followed a normal Dutch diet (Van Rossem et al. 2011). It is unknown whether any of the subjects received Ti implants during their lifetime. Individual data can be found in the [Supplementary Information \(Table S1\)](#). The total-Si concentration in the human post mortem livers ranged from 2.7 to 191 mg Si/kg tissue with an average value of 12 mg Si/kg tissue and a standard deviation of 23 mg Si/kg tissue indicating large differences between subjects. The total-Si concentrations in the individual organs are presented in [Table 1](#) while they are graphically

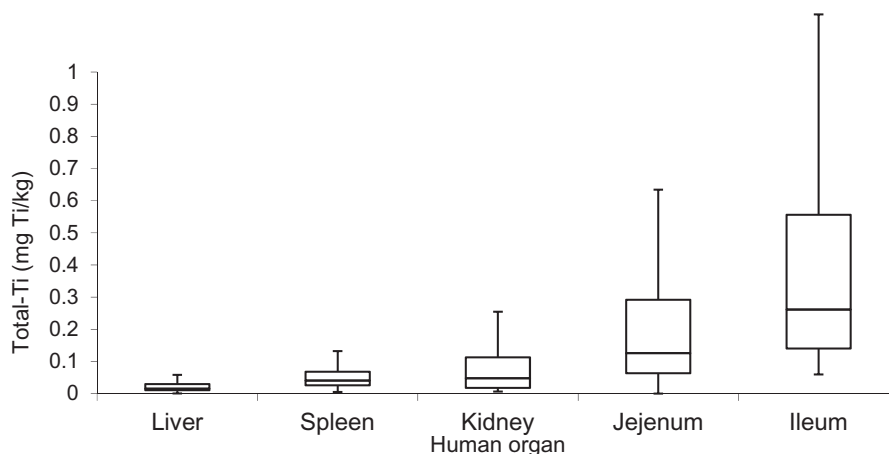


Figure 2. Box and whisker plots of total-Ti concentration in human (post mortem) liver, spleen, kidney, jejunum, and ileum. The boxes represent the upper 25% quartile (Q3), median (Q2), and lower 25% quartile (Q1) concentrations, respectively. The whiskers indicate 1.5IQR (Q3–Q1).

presented in the box plot in Figure 1. In general, total-Si concentrations are comparable between the organs and if the total-Si concentration is high in a subject it is high in all the organs of that subject. The total-Ti concentration in the organs ranged from 0.01 to 2.0 mg Ti/kg tissue with an average value of 0.17 mg Ti/kg tissue and a standard deviation of 0.33 mg Ti/kg tissue indicating large differences between subjects and organs. The total-Ti concentrations in the individual organs are presented in Table 1. Within a subject, there are also large differences between the concentrations in different organs. The box plots in Figure 2 show that the total-Ti concentration in the jejunum and ileum are substantially higher than in liver, spleen and kidney. Individual data are presented in the Supplementary Information (Table S1). Special care was taken to include blank samples of all materials involved in organ collection, tissue processing, and analyses. This includes the formaldehyde used to fix the body tissues, scalpels and knives, and materials and utensils to process and homogenize the organs. The sum of the contributions of these materials to the total-Ti concentration was lower than the LOD of the analytical method. Especially, the formaldehyde contributed to the total-Si concentration (about 2 times the LOD) and the total-Si results were corrected for this contribution.

SiO₂ and TiO₂ particles in human organ tissues

A combination of gentle chemical and enzymatic digestion combined with spICP-MS was used for the

detection and characterization of SiO₂ and TiO₂ particles. The sample preparation is known not to affect the presence and size of particles (Peters, Rivera et al. 2014). This was determined in an extensive validation of the method which showed a recovery >70% for particle concentration and >90% for particle size. We found that all tissue samples contained particles containing Si and Ti and, as confirmed later by electron microscopy analysis, these are mainly SiO₂ (and silicates) and TiO₂ particles. Their particle nature is evident from the characteristic spikes in the time scans of the spICP-MS analysis of the tissue samples as shown in Figure 3. The number of spikes in the time scan is directly proportional to the number of particles in the sample. The signal height of the peaks relates to the mass of the particle from which the particle size is calculated assuming a spherical particle shape and applying densities of 2.63 and 4.23 g/cm³ for SiO₂ and TiO₂. For SiO₂ and TiO₂ the size-limit of detection is 100 and 50 nm, respectively. The detected particles can consist of primary particles, aggregates or agglomerates. Confirmation analysis with SEM-EDX supported the presence of particles in postmortem human tissues. From the spICP-MS data, the particle size, particle size distribution, and particle concentrations were calculated (Peters et al. 2015).

The SiO₂ particle mass concentrations in the tissues ranged from 0.2 to 25 mg Si/kg tissue with an average of 1.2 ± 3.1 mg Si/kg tissue. The results for the individual organs are presented in Table 2 and graphically shown in the box plots in Figure 4.

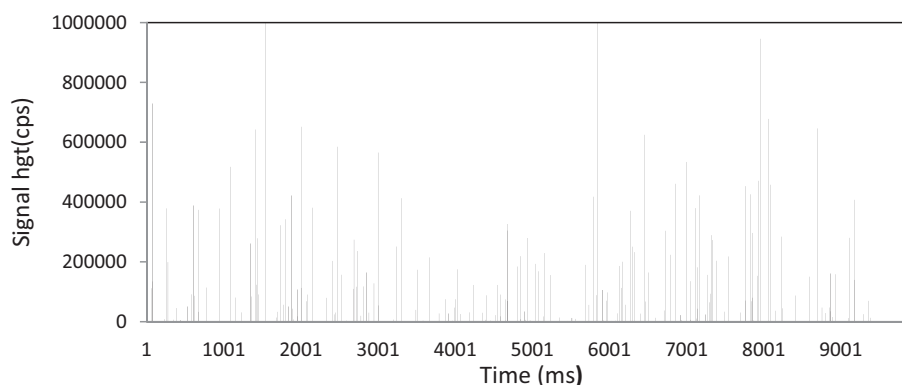


Figure 3. TiO₂ particles in human (post mortem) tissue. Time scan of the spICP-MS analyses of a human tissue sample.

Table 2. Summary of particle SiO₂ and TiO₂ concentrations in the organs.

<i>n</i>	All organs 69	Liver 15	Spleen 15	Kidney 15	Jejunum 12	Ileum 12
Particle SiO ₂	mg Si/kg	mg Si/kg	mg Si/kg	mg Si/kg	mg Si/kg	mg Si/kg
Non-detects ^a	14	3	3	2	3	3
Minimum ^b	0.2	0.3	0.4	0.3	0.2	0.3
Maximum	25	2.2	6.5	4.1	2.2	25
Median ^c	0.6	0.4	1.0	0.4	0.7	0.6
Average ^c	1.2	0.6	1.3	0.9	0.7	2.6
Std. dev. ^c	3.1	0.6	1.6	1.1	0.7	7.1
Particle TiO ₂	mg Ti/kg	mg Ti/kg	mg Ti/kg	mg Ti/kg	mg Ti/kg	mg Ti/kg
Non-detects ^a	0	0	0	0	0	0
Minimum ^b	0.01	0.01	0.01	0.01	0.01	0.01
Maximum	1.8	0.05	0.13	0.21	1.8	1.2
Median ^c	0.03	0.02	0.02	0.03	0.08	0.25
Average ^c	0.14	0.02	0.03	0.05	0.31	0.34
Std. dev. ^c	0.30	0.01	0.03	0.06	0.55	0.37

^aNumber of samples below the detection limit of 0.2 mg Si/kg and 0.01 mg Ti/kg.

^bMinimum measured value.

^cFor calculation of median, average and std. dev. non-detects were set at half of the LOD value.

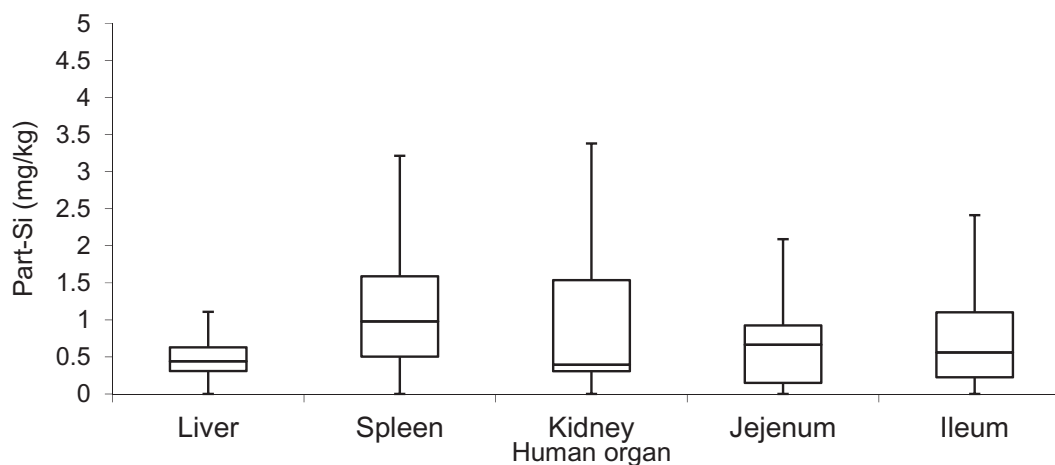


Figure 4. Box and whisker plots of the SiO₂ particle concentration in human (post mortem) organs. The boxes represent the upper 25% quartile (Q3), median (Q2), and lower 25% quartile (Q1) concentrations respectively. The whiskers indicate 1.5IQR (Q3–Q1).

Comparable with the total-Si concentration results, there are differences in the particulate Si mass concentration in the organs, although tissues containing high total-Si also contained high SiO₂ particle

concentrations. However, only 10% of the total-Si concentration is explained by the particle SiO₂ concentrations. For SiO₂ the number-based particle size distributions in the different tissue samples are also

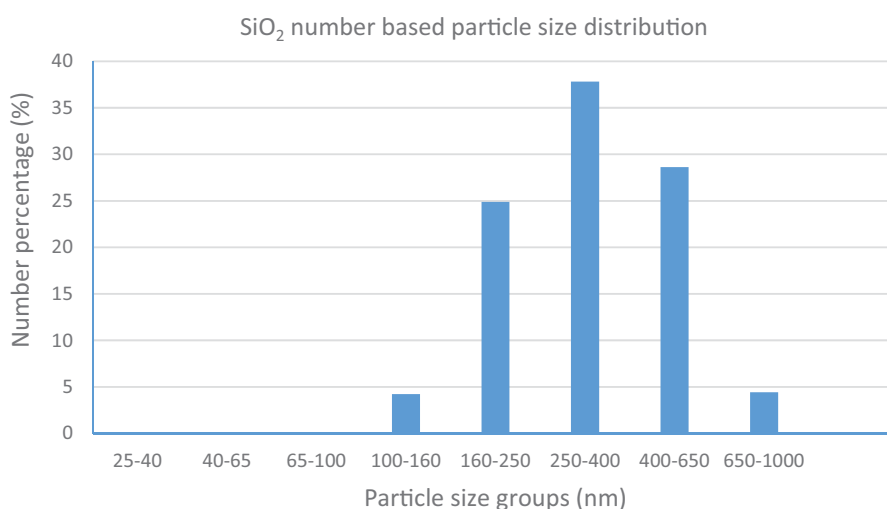


Figure 5. Number-based particle size distribution for SiO₂ particles found in tissues of human organs. Particles can consist of primary particles, aggregates or agglomerates.

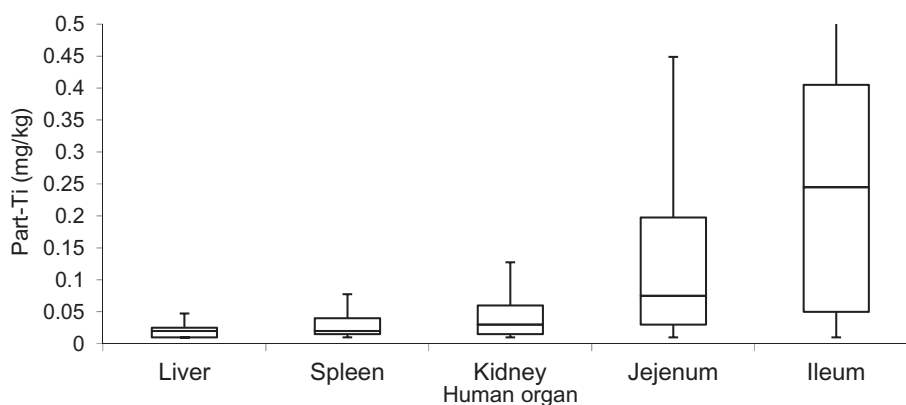


Figure 6. Box and whisker plots of the TiO₂ particle concentration in human (post mortem) organs. The boxes represent the upper 25% quartile (Q3), median (Q2), and lower 25% quartile (Q1) concentrations respectively. The whiskers indicate 1.5IQR (Q3–Q1).

comparable and have a size range of 150–850 nm with the mode of the observed particle sizes being in the range of 250–400 nm (Figure 5). Individual data for TiO₂ and SiO₂ particle mass concentrations are presented in the [Supplementary Information \(Table S1\)](#).

The TiO₂ particle mass concentration in the organs ranged from 0.01 to 1.8 mg Ti/kg tissue with an average of 0.14 ± 0.30 mg Ti/kg tissue indicating large differences between individual results. [Table 2](#) lists the results for the individual organs and [Figure 6](#) shows the box plot for TiO₂ particles in the different organs. While particle concentrations in the liver, spleen, and kidney are comparable between individuals, the concentrations in the jejunum and ileum samples show a higher variability among different samples (as was observed for total-Ti). This may be a result of the fact that only parts of the jejunum and ileum were

analyzed. In general, tissues with high total-Ti concentrations also contained high TiO₂ particle concentrations. The TiO₂ particle concentrations explain about 80% of the total-Ti concentrations showing that most of the Ti material in these organs consist of particulate material. This is in line with earlier findings (Heringa et al. 2018). The number-based TiO₂ particle size distributions in the different tissue samples are comparable and have a size range of 50 to 500 nm with a mode of in the size bin of 100–160 nm as shown in [Figure 7](#). Seventeen percent of the TiO₂ particles in the number-based size distribution is <100 nm.

Confirmation of the presence of SiO₂ and TiO₂ particles using SEM-EDX

We used SEM-EDX as an independent technique for the confirmation of the presence of SiO₂ and TiO₂

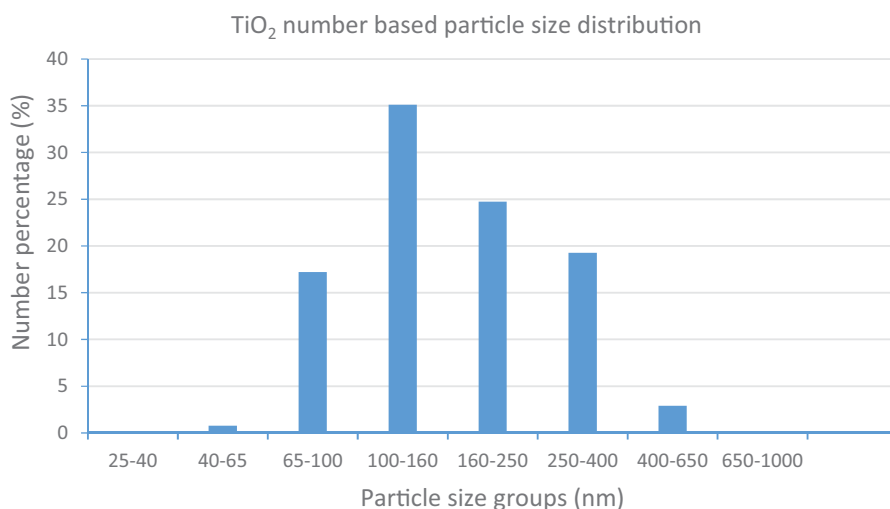


Figure 7. Number-based particle size distribution for TiO₂ particles found in tissues of human organs. Particles can consist of primary particles, aggregates or agglomerates.

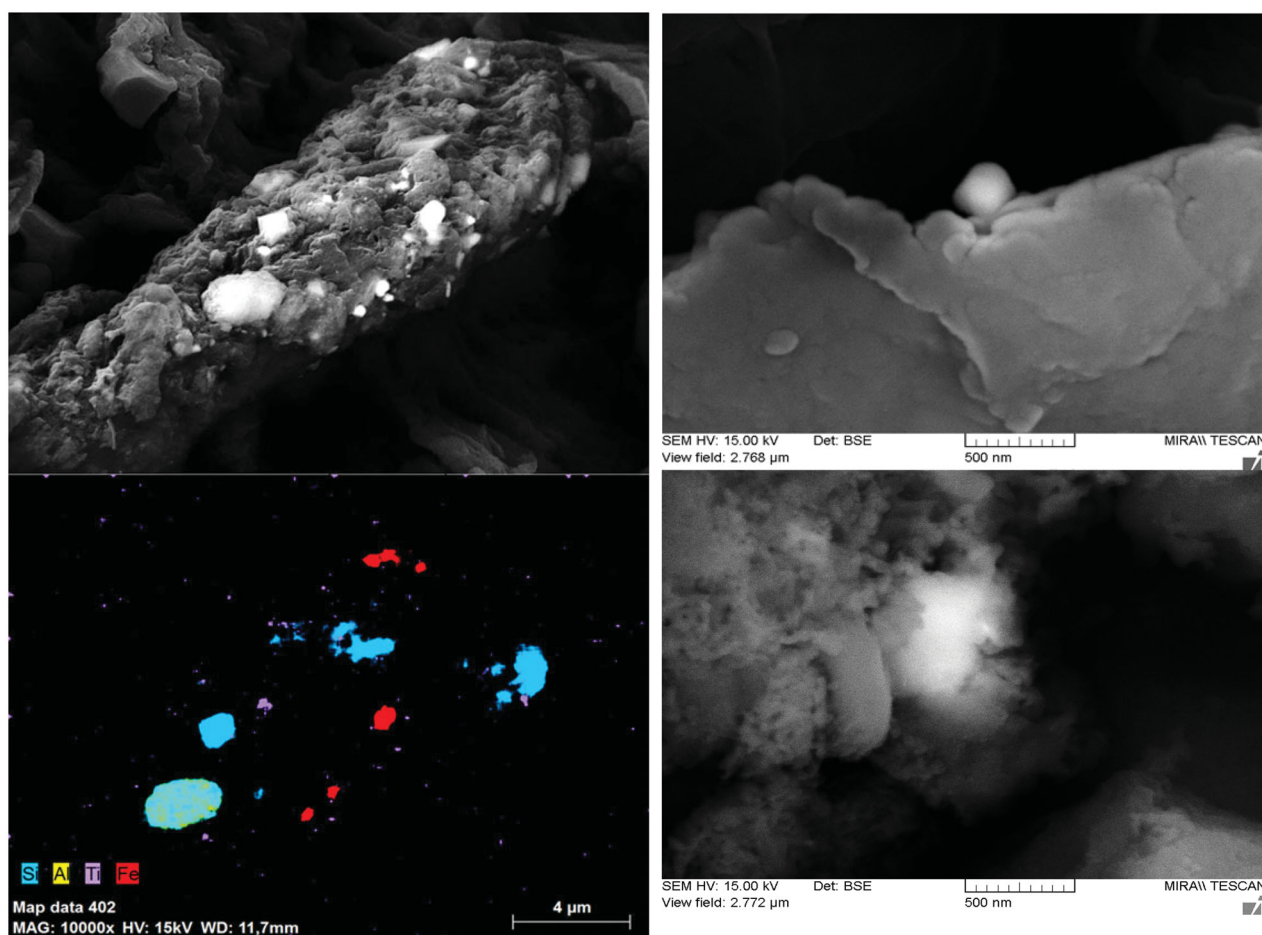


Figure 8. SEM characterization of detected particles detected in a dried liver sample. In addition to the elements indicated in the picture, oxygen was also measured but not shown in the picture. The SEM image on the left shows TiO₂ and SiO₂ particles as well as aluminum silicate and iron particles. The images on the right show a TiO₂ particle (top) and a SiO₂ particle (bottom).

particles in tissues. **Figure 8** is an image of a liver sample that shows the presence of (large) SiO₂ particles as well as smaller TiO₂ particles. Element

identification, including oxygen, was by EDX analysis (see color code for the other elements in **Figure 8**) which also revealed that the large

Table 3. Literature data of total-Si and -Ti concentrations in human liver and spleen.

	Liver (mg/kg)	Spleen (mg/kg)	Comments/reference
Total-Si	8 ± 8	12 ± 12	This study, ICP-MSMS, <i>n</i> = 15
	2 to 50	2 to 50	WHO, colorimetric method (WHO 1974)
	12	17	Austin et al., colorimetric method (Austin, Rinehart, and Ball 1972)
	33.6 ± 13.8	–	Carlisle et al., atomic absorption spectrometry (Carlisle and Mertz 1986)
	1.4 ± 0.5	3.3 ± 0.8	Evans et al., ICP-AES (<i>n</i> = 10, female) (Evans et al. 1996)
Total-Ti	0.03 ± 0.04	0.06 ± 0.06	This study, ICP-HRMS, <i>n</i> = 15
	0.04 ± 0.02	0.08 ± 0.11	Previous study, ICP-HRMS, <i>n</i> = 15 (Heringa et al. 2018)
	1.3	0.2 to 1.4	NRC, X-ray fluorescence analyses (National Research Council 2005)
	1.7 ± 3.4	1.9 ± 2.4	Yukawa et al., neutron activation analysis, <i>n</i> = 15 (liver) and <i>n</i> = 6 (spleen) (Yukawa et al. 1980)

Si-bearing particle in the image is, in fact, an aluminum silicate particle. Smaller SiO₂ particles are also present. In addition, a number of iron-containing particles are visible. It should be remarked that the image in Figure 8 with so many particles is a hot-spot, on the average only about 10 individual particles were detected in the 500 images. In general, TiO₂ particles were found with diameters in the range of 50–200 nm. Small SiO₂ particles were difficult to detect in backscattered electron mode because the density difference with the surrounding tissue is small, especially when particles are just below the surface. Therefore, tissue samples were plasma-ashed to remove the upper layer of the tissue resulting in a better contrast between particles and matrix. This revealed the presence of smaller SiO₂ and TiO₂ particles from which one of each is also shown in Figure 8. The presence of micro-sized silica particles (and also iron particles) in human tissues is in agreement with recent findings by Locci et al. (Locci et al. 2019). They studied liver and kidney samples and found particles in virtually all samples with Si and Fe as the main metallic constituents.

The presence of micro-sized Si particles may also help to understand why the SiO₂ particles detected in the spICP-MS analysis explain no more than 10% of the total-Si concentration. If the measured total-Si concentration would consist of micro-sized particles, the number of SiO₂ particles would be so few that it is unlikely that they will be observed in the spICP-MS scan resulting in an underestimation of the mass-based SiO₂ particle concentration. The sub-sample collected for spICP-MS analysis is simply too small to be representative of the larger micro-sized SiO₂ particles. Another explanation may be that in the plasma only a part of the micro-sized SiO₂ particles is atomized and ionized. However, in previous research, we reported that SiO₂ particles

up to 2.5 μm are completely vaporized and ionized in the ICP-MS plasma (Peters et al. 2015). Therefore, the former explanation is most likely in explaining where the missing part of the particulate SiO₂ is to be found, in the micro-sized SiO₂ particles.

Discussion

Although SiO₂ and TiO₂ are authorized ingredients in food, and used in consumer and medical products, knowledge about their tissue concentrations in humans is very limited, and virtually absent with regard to particle content. Therefore, the data from this study are unique and highly useful for risk assessment. The presented concentrations represent the total organ burden of SiO₂ and TiO₂ particles. While it is assumed that most human subjects followed a western European diet, no historical data on specific exposure conditions of the human subjects are available and therefore we cannot establish a definite relation with the source of the SiO₂ and TiO₂ particles found in the human tissues. Consumer intake of SiO₂ from food has been estimated at 9.4 mg Si/kg bw/day (Dekkers et al. 2011). Based on these and other data, Van Kesteren et al. developed a kinetic model and estimated steady state Si liver concentration in human tissues at 21–23 mg Si/kg (van Kesteren et al. 2015). Apart from that little is known about the total-Si concentrations in human tissues (Table 3) and quantitative information on the presence of particles is absent. While uptake of Si or SiO₂ in humans is unknown, recent studies with rodents imply limited oral uptake of silica at realistic consumer exposure levels, while also accumulation in time may occur (van der Zande et al. 2014; van Kesteren et al. 2015). Silica and silicic acid and its calcium, magnesium and aluminum salts occur ubiquitously in the environment and some have been used for many years

medically, for example, against osteoporosis. As a consequence, small amounts of silica are normally present in our bodies and according to the WHO the silica concentration in human tissues varies from 10 to 200 mg/100 g on a dry weight basis which translates to 2 to 50 mg Si/kg tissue (Yukawa et al. 1980). The total-Si liver concentrations found in this study are in the range of 8 ± 8 mg Si/kg tissue and are about 50% of the estimated steady state Si liver concentration by Van Kesteren et al. and in the range of the literature data in Table 3. Human exposure by inhalation of silica dust is very common in both working and living environments with SiO₂ air concentrations in the range of $0.1\text{--}0.5\text{ mg/m}^3$, resulting in an estimated maximum exposure of 5 mg/day (Morfeld et al. 2014; Chen et al. 2012), or 0.08 mg/kg bw/day for a person of 60 kg. While transport from the lungs into the circulatory system is possible and silica particles may also be cleared and transported from the lungs into the digestive tract, the contribution is limited. Dermal exposure to silica nanoparticles did not lead to any effects (Ryu et al. 2014) and, therefore, we conclude that penetration of SiO₂ through the skin does not contribute. As for TiO₂, oral exposure seems to be the most important source of SiO₂ for humans in non-occupational settings, but in contrast to TiO₂, other routes may also contribute substantially.

The number-based particle size distribution, as determined by spICP-MS, for the detected SiO₂ particles ranged from 150 to 850 nm. Particles consist of primary particles, aggregates and agglomerates. SEM-EDX analyses confirmed the presence of SiO₂ aggregates/agglomerates in the liver but only for particle sizes from around 500 nm to several micrometers due to lower density difference with the surrounding tissue. Oral exposure is assumed to be the major exposure route for SiO₂ in non-occupational situations.

Based on estimates from the Dutch food consumption surveys (Van Rossem et al. 2011), food may contribute 0.15–2.0 mg Ti/kg body weight/day and since TiO₂ is highly stable, it is not likely degraded (dissolved) in the intestine and therefore mostly defecated (Weir et al. 2012; Rompelberg et al. 2016; Sohal et al. 2018). Human volunteer studies indicate that the oral bioavailability of TiO₂ is low, but accumulation in tissues may occur in

time (Pele et al. 2015; Böckmann et al. 2000). While inhalation may be the primary route for TiO₂ exposure in the workplace, in non-occupational settings Ti concentrations in air are very low, in the range of 0.01–0.1 $\mu\text{g/m}^3$, resulting in a maximum exposure through inhalation of only 1 μg Ti/day (WHO 1982). Most dermal exposure studies, whether *in vitro*, *ex vivo* or *in vivo*, report that TiO₂ particles do not penetrate the (intact) skin (Crosera et al. 2015; Shi et al. 2013), or that only minor amounts of nano TiO₂ are absorbed in humans (Pelclova et al. 2019). Medical implants have a strongly bound oxide layer which makes them inert, however, due to corrosion a Ti implant surface can be a potential source for the release of micro- and nano-sized particles (Frissen et al. 2002; Sarmiento-Gonzalez et al. 2009). On the other hand, in the previous study, 2 of the 15 human subjects had Ti implants but did not show increased total-Ti or particle TiO₂ concentrations (Heringa et al. 2018). While it is unknown whether any of the human subjects in this study received Ti implants during their lifetime, TiO₂ particles were found in all tissue samples. In conclusion, oral exposure, e.g. from food, toothpaste and medicines, seems to be the most important source for TiO₂ in non-occupational settings (Rompelberg et al. 2016).

Total-Ti concentrations found in this study were in the range of 0.01–2.0 mg Ti/kg tissue. For liver and spleen, the total-Ti concentrations ranged from 0.01 to 0.25 mg Ti/kg which is comparable to the results in a previous study with tissues from 15 other deceased persons (Heringa et al. 2018). These concentrations are low compared to some other total-Ti concentrations reported in literature (Table 3). However, using gentle chemical and enzymatic digestion combined with spICP-MS analyses, a method completely independent from the total-element determination, comparable mass concentrations are found for the particle fraction of TiO₂. In the spICP-MS analyses of TiO₂ particles, primary particles, aggregates or agglomerates with an external size in the range of 50–500 nm are found with a mode in the range of 100–160 nm. SEM-EDX analysis confirmed the presence of partly spherical shaped TiO₂ particles in liver tissues with primary particle sizes in the range of 50–200 nm, which is in agreement with the spICP-MS results. White pigment TiO₂, as is among others used in food,

toothpaste and many consumer products, has primary particle sizes in the range of 50–500 nm diameter, with 10–50% of these TiO₂ primary particles having a size <100 nm³ (Weir et al. 2012; Younes et al. 2019). The TiO₂ particles found in the present study are of similar size. Given the similarities in the size distribution and the low exposure from other exposure routes, we deem it plausible that the detected TiO₂ particles in human organ tissues originate from oral exposure.

Conclusion

Methods were developed and successfully validated for the determination of total-Si and -Ti and particulate SiO₂ and TiO₂ in human tissues. The total-Si concentrations ranged from <2 to 191 mg Si/kg tissue while the particulate SiO₂ concentration in the tissue samples ranged from 0.2 to 25 mg Si/kg tissue. Particulate SiO₂ explained about 10% of the total-Si concentration, probably because micro-sized SiO₂ particles that were present in the samples were not detected by the spICP-MS method. Total-Ti concentration in samples of human liver, spleen, kidney, jejunum, and ileum ranged from 0.01 to 2.0 Ti mg/kg tissue while the particulate TiO₂ concentration ranged from 0.01 to 1.8 mg Ti/kg tissue, explaining 80% of the total-Ti concentration. These results confirm the findings of an earlier study of particulate TiO₂ in the liver and spleen (Heringa et al. 2018). The presence of SiO₂ and TiO₂ particles in the liver was confirmed by SEM/EDX analyses. The sizes of the SiO₂ and TiO₂ particles detected with spICP-MS are in the range of 150–850 nm for SiO₂ and 50–500 nm for TiO₂. Oral exposure is assumed to be the major source of SiO₂ and TiO₂ particles in non-occupational settings. While oral exposure may partly result from natural background sources, we deem it plausible that at least a part of the detected SiO₂ and TiO₂ particles in human liver and spleen originate from food, toothpaste and/or medicines that are consumed.

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Ethical approval

Written informed consent was obtained during life from the persons of which the organs were used in this study, that allowed the use of their entire bodies for educational and research purposes. All ethical regulations concerning the use of these organs were followed, and approval for this specific scientific use was obtained from the board of University Medical Center Utrecht.

Disclosure statement

The authors declare no competing financial interests.

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