



# Quantitative biokinetics of titanium dioxide nanoparticles after intravenous injection in rats (Part 1)

Journal:	<i>Nanotoxicology</i>
Manuscript ID	TNAN-2016-0176.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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Keywords:	Size-selected, radiolabeled titanium dioxide nanoparticles, intravenous injection, accumulation in organs and tissues, translocation across organ membranes, Hepato biliary nanoparticle clearance
Abstract:	<p>Submicrometer TiO<sub>2</sub> particles, including nanoparticulate fractions, are used in an increasing variety of consumer products, as food additives and drug delivery applications are envisaged. Beyond exposure of occupational groups this entails an exposure risk to the public. However, nanoparticle translocation from the organ of intake and potential accumulation in secondary organs is poorly understood and in many investigations excessive doses are applied.</p> <p>The present study investigates the biokinetics and clearance of a low single dose (typically 40-400 µg/kg BW) of 48V-radiolabeled, pure TiO<sub>2</sub> anatase nanoparticles ([48V]TiO<sub>2</sub>NP) with a median aggregate/agglomerate size of 70 nm in aqueous suspension after intravenous injection into female Wistar rats. Biokinetics and clearance were followed from 1-hour to 4-weeks. The use of radiolabeled nanoparticles allowed a quantitative [48V]TiO<sub>2</sub>NP balancing of all organs, tissues, carcass and excretions of each rat without having to account for chemical background levels possibly caused by dietary or environmental titanium exposure.</p> <p>Highest [48V]TiO<sub>2</sub>NP accumulations were found in liver (95.5%ID on day-1), followed by spleen (2.5%), carcass (1%), skeleton (0.7%) and blood (0.4%). Detectable nanoparticle levels were found in all other organs. The [48V]TiO<sub>2</sub>NP content in blood decreased rapidly after 24h while the distribution in other organs and tissues remained rather constant until day-28.</p> <p>The present biokinetics study is part 1 of a series of studies comparing biokinetics after three classical routes of intake (intravenous (IV) injection (part 1), ingestion (part 2), intratracheal instillation (part 3)) under identical laboratory conditions, in order to verify the common hypothesis that IV-injection is a suitable predictor for the biokinetics fate of nanoparticles administered by different routes. This hypothesis is disproved by this series of studies.</p>

# Quantitative biokinetics of titanium dioxide nanoparticles after intravenous injection in rats (Part 1)

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33    **Keywords**  
34    Size-selected, radiolabeled titanium dioxide nanoparticles; intravenous injection,  
35    accumulation in organs and tissues, translocation across organ membranes, Hepato biliary  
36    nanoparticle clearance  
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For Peer Review Only

**Abstract**

Submicrometer TiO<sub>2</sub> particles, including nanoparticulate fractions, are used in an increasing variety of consumer products, as food additives and drug delivery applications are envisaged. Beyond exposure of occupational groups this entails an exposure risk to the public. However, nanoparticle translocation from the organ of intake and potential accumulation in secondary organs is poorly understood and in many investigations excessive doses are applied. The present study investigates the biokinetics and clearance of a low single dose (typically 40-400 µg/kg BW) of <sup>48</sup>V-radiolabeled, pure TiO<sub>2</sub> anatase nanoparticles (<sup>48</sup>V]TiO<sub>2</sub>NP) with a median aggregate/agglomerate size of 70 nm in aqueous suspension after intravenous injection into female Wistar rats. Biokinetics and clearance were followed from 1-hour to 4-weeks. The use of radiolabeled nanoparticles allowed a quantitative [<sup>48</sup>V]TiO<sub>2</sub>NP balancing of all organs, tissues, carcass and excretions of each rat without having to account for chemical background levels possibly caused by dietary or environmental titanium exposure. Highest [<sup>48</sup>V]TiO<sub>2</sub>NP accumulations were found in liver (95.5%ID on day-1), followed by spleen (2.5%), carcass (1%), skeleton (0.7%) and blood (0.4%). Detectable nanoparticle levels were found in all other organs. The [<sup>48</sup>V]TiO<sub>2</sub>NP content in blood decreased rapidly after 24h while the distribution in other organs and tissues remained rather constant until day-28. The present biokinetics study is part 1 of a series of studies comparing biokinetics after three classical routes of intake (intravenous (IV) injection (part 1), ingestion (part 2), intratracheal instillation (part 3)) under identical laboratory conditions, in order to verify the common hypothesis that IV-injection is a suitable predictor for the biokinetics fate of nanoparticles administered by different routes. This hypothesis is disproved by this series of studies.

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61     **Introduction**

62     Submicron titanium dioxide particles are increasingly used in food additives, in cosmetics and  
63     personal care products such as tooth paste, as UV-absorbers in sunscreen (Jia, 2008), and in  
64     other products such as pigments or fillers in paints, inks, and ceramics (Christensen, 2011).  
65     Their antimicrobial and even antiviral effects make them advantageous in water and air  
66     disinfection (Li, 2008). An analysis of (Weir, 2012) showed that approximately one third of  
67     the number of TiO<sub>2</sub> particles in common food products are nano-sized (<100 nm). (Peters,  
68     2014) recently confirmed in 27 food items and personal care products that between 10% and  
69     25% of the TiO<sub>2</sub> particles exhibit dimensions below 100 nm. Despite the extraordinary growth  
70     of use and applications of titanium dioxide nanoparticles (TiO<sub>2</sub>NP) it is still unclear whether  
71     this entails health risks, especially for subjects occupationally exposed to inhalation of  
72     TiO<sub>2</sub>NP during manufacturing and handling (Christensen, 2011) and when considering their  
73     high biopersistence and long-term retention known for 2-3 decades as reviewed by Shi and  
74     co-workers (Shi, 2013).  
75     Recently medical applications of TiO<sub>2</sub>NP for drug delivery have also been envisaged  
76     (Carlander, 2016), e.g., for restenosis treatment (Gu, 2013), making use of their physical  
77     properties for light-controlled drug release (Wang, 2015) or ultrasound cancer treatment  
78     (Ninomiya, 2014). On the other hand the release and fate of nanosized fractions of wear  
79     corrosion debris from orthopedic and dental titanium implants has become a concern  
80     (Matusiewicz, 2014).  
81     Numerous *in vivo* and *in vitro* studies describe adverse effects in the mammalian organism but  
82     the results are not yet conclusive. One important issue is the dose of intravenously  
83     administered TiO<sub>2</sub>NP when studying the behaviour of nanoparticles that reach systemic  
84     circulation. Doses of 10 mg/kg body weight (BW) and more have been reported. However,  
85     the question arises as to whether the results achieved with such high doses are still  
86     representative for the biodistribution that can be expected for much smaller quantities that

may reach systemic circulation following realistic exposure scenarios. Given our concern about excessive doses we refer to several studies which report biodistribution data (Fabian, 2008) (Patri, 2009) (Xie, 2011) (Geraets, 2014) and a likelihood of pro-inflammatory (Fabian, 2008) (Setyawati, 2013), genotoxic (Louro, 2014), immunotoxic (Auttachoat, 2013) as well as fetotoxic (Yamashita, 2011) responses to IV-injected TiO<sub>2</sub>NP at high doses, with and without measurements during a recovery time.

Interestingly, an electron-microscopic study on the micro-biokinetics of 40 nm gold nanoparticles in the liver of mice after administration of 1.4 mg/kg BW (Sadauskas, 2009) found most of the nanoparticles in lysosomal / endosomal vacuoles of Kupffer cells, but the number of Kupffer cells containing nanoparticles decreased over time, while the nanoparticle load in the vacuoles increased since the overall nanoparticle clearance out of the liver was very low (Sadauskas, 2009). In our low-dose IV-injection study of monodisperse 18 nm gold nanoparticles (30 µg/kg BW) we confirmed the nanoparticle presence in Kupffer cells together with additional nanoparticles in sinusoidal endothelial cells and hepatocytes (Hirn, 2011). A recent comprehensive review addressed these issues in more detail (Shi, 2013). Furthermore, biokinetics data obtained from IV-injected engineered TiO<sub>2</sub>NP are controversial since some reports note accumulation in organs after IV-injection, whereas others only note liver retention, depending very much on the sensitivity of the detection methodology used (Shi, 2013).

In the present series of three biokinetics studies we performed quantitative biokinetics studies in female rats by applying radiolabeled, engineered, commercially available TiO<sub>2</sub> anatase agglomerated/aggregated nanoparticles. After size selection of a true nano-fraction with a hydrodynamic diameter of about 70nm single doses of aqueous [<sup>48</sup>V]TiO<sub>2</sub>NP suspensions were applied by three routes of intake: intratracheal instillation (Kreyling, submitted-a) , intra-oesophageal instillation (gavage) (Kreyling, submitted-b), and in the present first part by intravenous injection. By using nanoparticles radiolabeled with the gamma-emitting

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radionuclide  $^{48}\text{V}$ , a high sensitivity is achieved over five orders of magnitude which is not affected by chemical background levels that might be caused by dietary and environmental titanium exposure of the animals. Moreover, no chemical processing of the biological specimens is required for subsequent  $\gamma$ -spectrometry and a complete  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  balancing of all organs, tissues, carcass and excretions can be performed even for low administered doses. However, using the  $^{48}\text{V}$  radiolabel, which is chemically different from the element Ti, requires stable integration into the NP matrix and careful control of labeling stability *in vivo*. Therefore, we conducted additional auxiliary biokinetics studies to quantify any release of the label for each organ and corrected the biokinetics data accordingly.

For each of these routes of intake quantitative biokinetics studies were performed by serial biodistribution analyses at five different retention time points between one hour and 28 days after application in order to determine the accumulated and retained nanoparticle doses in different organs of interest, selected tissues and body fluids, and also to provide a complete overview of the fate of the applied  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  in the entire organism by additional evaluations of the carcass and the entire fecal and urinary excretion of each animal. The entire nanoparticle distribution is balanced in each animal and not normalized to a nominally administered nanoparticle dose loaded in a syringe, which (as reported below) may differ appreciably from the dose effectively delivered to an animal. Only such a quantitative approach can provide a detailed overview of the nanoparticle biokinetics and fate, whereas biokinetics studies focusing on a few organs of interest cannot provide sufficient information for a comprehensive understanding of the nanoparticle transport and accumulation processes within the organism.

The IV-injection study was carried out firstly to check the hypothesis that IV-injection may be a suitable surrogate approach for the biokinetics after oral or respiratory delivery of nanoparticles, and secondly to provide a quantitative biokinetics assay for a better understanding of targeted delivery of  $\text{TiO}_2\text{NP}$ -based drugs via the circulation. Since we knew



from previous biokinetics studies on a suite of monodisperse gold nanoparticles (AuNP) administered via the same three routes (Kreyling, 2011, Hirn, 2011, Kreyling, 2014, Schleh, 2012) and after inhalation of 20 nm iridium nanoparticles (IrNP) (Kreyling, 2002, Semmler, 2004, Semmler-Behnke, 2007) or 20 nm elemental carbon nanoparticles (ECNP) (Kreyling et al., 2011), that the accumulation dynamics occurs rather rapidly during the first 24-hours we chose three time points of investigations – 1h, 4h, 24h – in order to study the rapid accumulation dynamics observed previously, followed by two time points at 7d and 28d in order to assess possible slower processes of accumulation, redistribution and clearance.

## Materials and Methods

### Radiolabeling and size selection of TiO<sub>2</sub>NP

Two batches of 20 mg ST-01 TiO<sub>2</sub>NP were irradiated with a protons at a beam current of 5  $\mu$ A. One, with an activity concentration of 1.0 MBq/mg (<sup>48</sup>V-activity per TiO<sub>2</sub> mass), was used for the 1h, 4h and 24h retention experiments. The second one was irradiated on five consecutive days, yielded an activity concentration of 2.35 MBq/mg and was used for the 7d and 28d retention experiments. At these radioactivity concentrations the atomic ratio of <sup>48</sup>V:Ti in the nanoparticles is about  $2.6 \times 10^{-7}$  and  $6.2 \times 10^{-7}$ , respectively. Since proton bombardment and the chemical difference of the radiolabel, may result in a non-perfect integration of the <sup>48</sup>V in the TiO<sub>2</sub> matrix the [<sup>48</sup>V]TiO<sub>2</sub>NP were repeatedly washed to remove released <sup>48</sup>V-ions.

Size selection was performed in a repeated sequence of nanoparticle suspension, ultrasound homogenization, washing by centrifugation and re-suspension in order to remove excess sodium pyrophosphate, to eliminate larger aggregates/agglomerates and to minimize the content of free, ionic <sup>48</sup>V (see Supplementary Materials (SM-IV)). The final size selected and

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radiolabeled, nano-sized aggregates or agglomerates of  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  were suspended and dispersed in water.

For each of the retention time points to be studied a new batch of size-selected  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  was prepared, characterized and immediately applied intravenously, by gavage or intratracheal instillation to groups of four rats each, which improves the comparability between the exposure routes as the studies were started with the same nanoparticle properties.

**Characterization of  $[^{48}\text{V}]\text{TiO}_2\text{NP}$**

The hydrodynamic diameter of the size-selected  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  and the zeta potential were measured in triplicates several times during the size selection process for control purposes and prior to IV-injection using a Malvern Zetasizer (Malvern, Herrenberg, Germany). Samples for transmission electron microscopy were prepared from the aqueous suspension ready for administration on glow discharged 300 mesh Formvar<sup>®</sup>-coated copper grids and investigated with a Philips 300 TEM at 60 kV acceleration voltage.

**Study design – Main study with  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  and auxiliary study with soluble  $^{48}\text{V}$**

After a single IV-injection dose of typically 10-20  $\mu\text{g}$  (1h, 4h, 24h) nano-sized  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  suspended in 60  $\mu\text{L}$  water into the tail vein over 20-30 seconds, the biokinetics was followed in five groups of four rats each up to five time points (1h, 4h, 24h, 7d and 28d) as sketched:

Study	IV-injection, 0h	dissection time-points for biodistribution analyses				
MAIN	$[^{48}\text{V}]\text{TiO}_2\text{NP}$	1h	4h	24h	7d	28d
AUX	$^{48}\text{V}$ ions			24h	7d	

The time points at 7d and 28d were studied with higher doses (see Table 1) in order to ensure sufficient sensitivity in spite of radioactive decay and to detect also minor redistribution and clearing processes.

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3 188 In addition to the study with [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP, an auxiliary study was performed to investigate the  
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5 189 absorption and biodistribution of soluble, ionic  $^{48}\text{V}$  at 24h and 7d after IV-injection. These  
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7 190 data were used for correction of  $^{48}\text{V}$  release from the [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP. In order to mimic  $^{48}\text{V}$   
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9 191 released by [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP we added 0.33  $\mu\text{g}/\mu\text{L}$  ionic Ti(NO<sub>3</sub>)<sub>4</sub> to the carrier-free, ionic  $^{48}\text{V}$   
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11 192 isotope, thus obtaining a nitrate solution of sufficient ionic strength to stably maintain the ions  
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13 193 in solution, and adjusted the pH value to 5. For the experiments 60  $\mu\text{L}$  of solution containing  
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15 194 27 kBq ionic  $^{48}\text{V}$  and 20  $\mu\text{g}$  of ionic Ti were IV-injected into the tail vein of each rat.  
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## 20 196 **Animals**

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23 197 Healthy, female Wistar-Kyoto (WKY) rats (Janvier, Le Genest Saint Isle, France), 8–10  
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25 198 weeks of age ( $263 \pm 10$  g mean ( $\pm$  STD) body weight) were housed in pairs in relative-  
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27 199 humidity and temperature controlled ventilated cages on a 12-hr day/night cycle. Rodent diet  
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29 200 and water were provided ad libitum. After purchase, the rats were adapted for at least two  
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31 201 weeks and then randomly attributed to the experimental groups. All experiments were  
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33 202 conducted under German federal guidelines for the use and care of laboratory animals and  
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35 203 were approved by the Regierung von Oberbayern (Government of District of Upper Bavaria,  
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37 204 Approval No. 211-2531-94/04) and by the Institutional Animal Care and Use Committee of  
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## 45 207 **[ $^{48}\text{V}$ ]TiO<sub>2</sub>NP IV-injection and animal maintenance in metabolic cages**

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47 208 Using minimal-dead-space, 1-mL-insulin-syringes (Omnican<sup>®</sup> 100, Braun, Melsungen,  
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49 209 Germany, specified dead space <0.4 $\mu\text{L}$ ), aqueous [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP suspensions (60  $\mu\text{L}$ ) were  
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51 210 intravenously injected into the tail vein of non-fasted animals early in the morning. The  
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53 211 syringes and cannulas used for intravenous injection were collected for measurements of the  
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55 212 residual [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP content, which was motivated by the discovery of losses of  
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57 213 nanoparticles due to adherence on the polymer syringe material. After IV-injection of the  
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[<sup>48</sup>V]TiO<sub>2</sub>NP suspensions, rats of the first four groups (up to 7-day retention time) were kept individually in metabolism cages for separate daily collection of urine and feces. Rats of the 28-day group were maintained individually on cotton cloths in normal cages. The cloth was replaced by a new one every 3-4 days and fecal droppings were separated from the collected cloth; after separation the dried cloth contained only non-particulate <sup>48</sup>V originating from urine.

**Sample preparation and <sup>48</sup>V radioanalysis**

At 1h, 4h, 24h, 7d and 28d after IV-injection, rats were anesthetized (by 5% isoflurane inhalation) and euthanized by exsanguination *via* the abdominal aorta. For  $\gamma$ -spectrometry, blood, all organs, tissues and excretions were collected and <sup>48</sup>V-radioactivities were measured without any further physico-chemical processing, as detailed in the SM-IV and in earlier works (Kreyling, 2011, Hirn, 2011, Kreyling, 2014, Schleh, 2012). Since by exsanguination only about 60-70% of the blood volume could be recovered the residual blood contents of organs and tissues after exsanguination were calculated according to the findings of (Oeff, 1955) and the <sup>48</sup>V-radioactivities of the organs were corrected for these contributions.

Throughout this report nanoparticle quantities are given as percentages of the total intravenously injected [<sup>48</sup>V]TiO<sub>2</sub>NP radioactivity in each animal. The total injected activity was calculated as the sum of all samples of each entire animal, including its total fecal and urinary excretion, corrected for background and radioactive decay during the experiments using detectors calibrated in  $\gamma$ -ray energy and detection efficiency for <sup>48</sup>V. The percentages are averaged over the group of four rats per each retention time point and are given with the standard error of the mean (SEM). Samples yielding background-corrected counts in the 511 keV region-of-interest of the <sup>48</sup>V  $\gamma$ -spectrum were defined to be below the detection limit (<DL; 0.2 Bq) when the number of counts was less than three standard deviations of the background counts.

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3 240 The data compiled in Table 2 below are presented (i) as raw data of the  $^{48}\text{V}$ -activity directly  
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5 241 determined from the retrieved samples, (ii) as data corrected for the residual blood content in  
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7 242 the organs or tissues and (iii) additionally corrected for free  $^{48}\text{V}$ -ions. The detailed execution  
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9 243 of these corrections is presented in the SM-IV. All calculated significances are based on the  
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11 244 One-Way-ANOVA test and the post-hoc Tukey test. In case of direct two-groups comparison,  
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14 245 the unpaired t-test was used.  $p \leq 0.05$  was considered significant.  
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**Results**

**Physicochemical properties of [<sup>48</sup>V]TiO<sub>2</sub>NP**

The size distributions of the size-selected [<sup>48</sup>V]TiO<sub>2</sub>NP determined by DLS are presented in Figure 1. These were prepared for each of the five retention time points prior to intravenous injection. They indicate a good reproducibility of the size selection procedure. The Z-averages (see Table 1) are in a narrow range of  $88 \pm 11$  nm, and the PDI values  $0.18 \pm 0.04$  indicate that the size distributions have a rather narrow size distribution. Only the suspension for the 4h time point appeared to have a particle size somewhat smaller than the others. These conclusions are supported by TEM investigations after the size selection and dispersion process (see Figure 2) which revealed approximately spherical aggregated/agglomerated entities of roughly 50 nm in diameter, made up of smaller primary particles.

From the known activity concentration (1 MBq/mg (1h, 4h, 24h) and 2.35 MBq/mg (7d, 28d)) after proton irradiation and the determined <sup>48</sup>V-activity of the applied [<sup>48</sup>V]TiO<sub>2</sub>NP, the applied nanoparticle mass was calculated for each IV-injection as reported in Table 1. The effectively injected dose (activity) takes into account that a fraction of the activity loaded into the syringes was retained there after injection.

**Biokinetics of [<sup>48</sup>V]TiO<sub>2</sub>NP in blood, whole organs and tissues**

Table 2 gives a comprehensive summary of the biodistribution of intravenously injected [<sup>48</sup>V]TiO<sub>2</sub>NP at the five retention time points. For each organ or tissue the [<sup>48</sup>V]TiO<sub>2</sub>NP content is given in percent of the injected dose (ID) based on the measured <sup>48</sup>V-activity balance, referred to as *raw data*. As described earlier and elaborated in mathematical detail in the SM-IV the data were corrected for the residual blood retained in organs and tissues after exsanguination. These data are referred to as *w/o residual blood content*. In a next step the contribution of free <sup>48</sup>V-ions was also corrected for, referred to as *w/o free <sup>48</sup>V ions*, making use of the auxiliary study with ionic <sup>48</sup>V (see SM-IV for the mathematical correction

procedure). This step is advisable because  $^{48}\text{V}$  could be released from  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  even after careful washing during suspension preparation when diffusion processes bring radiolabels close to the surface of the nanoparticles or by a slow dissolution process of the nanoparticles (Vogelsberger, 2008). This correction effect would be most prominent if  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  and free  $^{48}\text{V}$ -ions had distinctly different biodistribution patterns. The fully corrected data are visualized in Figure 3 (panels A-C).

Table 2 shows that during the first hour after IV-injection more than 99% of  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  were very rapidly removed from the blood. After that the  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  concentration decreased slowly over the following week and then it remained approximately constant until day 28. This implies that the corrections for retained blood become rather small already after 1h.

The data show that the  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  rapidly cleared from the blood were retained mainly in the liver (95.5% of ID after 4h) with only slow clearance from there over the entire observation period (88.9% of ID after 28d). Retention in the spleen was between 2.5% and 4% of ID over the entire observation period, while retention was only about 0.1% in the lungs. Accumulation in the kidneys increased slightly over the four-week period (from 0.05% to about 0.2% of ID) while retention in all other secondary organs, such as brain, heart and uterus was rather low, which is also reflected in the scatter of the data. No trend can be identified over the four-week period showing virtually constant values and no net clearance from those organs. The lowest, but still detectable,  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  retention of 0.0005% was observed in the brain. The corrections for free  $^{48}\text{V}$ -ions, which contribute well below 1% of to the total retained activity, may lead to significant reductions of the values for  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  retention. However, since all input data are very small and subjected to large scatter, these corrections are also subject to large uncertainty. Nevertheless, the corrections are conservative enough to attribute a measureable radioactivity to the presence of a tiny amount of  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  after 24h and 28d. Remarkably, the skeleton and to a lesser extend the soft

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tissue (non-osseous tissues of the carcass including muscles, fat, skin, connective tissue, paws) exhibit a persistent  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  content that amounts to nearly 1% and 0.7% of ID at 28d, respectively. The relatively high  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  retention in the skeleton may be explained by reported experimental evidence (Rinderknecht, 2008) that  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  are retained in the bone marrow following blood translocation into the bones and probable uptake by phagocytes and other cells like pluripotent stem cells.

**$[^{48}\text{V}]\text{TiO}_2\text{NP}$  concentrations per weight of organ or tissue**

Due to their importance for toxicological comparisons, in Table 2 the percentages of injected activity assigned to  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  after all corrections for residual blood content and presence of free  $^{48}\text{V}$ , are converted into mass (ng) of nanoparticles per gram of organ or tissue. Since these data allow a straightforward comparison only for the same injected dose, the data are additionally presented as percentages of the injected dose per organ mass (%ID/g) and shown in Figure 3 (panel D-F). The effectively injected mass doses varied because a highly variable fraction of the  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  loaded into the syringes for intravenous injection was retained there after application. Additionally, the study design has foreseen higher doses for the 7d and 28d studies, in order to preserve high detection sensitivity in spite of the radioactive decay during the prolonged retention times.

The highest concentrations of about 10-11 %ID•g<sup>-1</sup> are determined in the liver and are about 2.5-4 %ID•g<sup>-1</sup> in the spleen. Both of these stayed rather constant during the entire time period. In the lungs the concentrations were much lower at about 0.05 %ID•g<sup>-1</sup> and remained rather constant over time. The concentrations in kidneys increased from 0.02 to 0.08 %ID•g<sup>-1</sup> during the 28-days observation period. Fractional concentrations in the heart and uterus were below 0.01 %ID•g<sup>-1</sup> throughout the observation period. No  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  were detected in the brain at 1h, 4h and 7d (< DL) but a detectable concentration of 0.0006 %ID•g<sup>-1</sup> was reached after



28d. This very low concentration is however notable since it is already corrected for nanoparticles retained in the residual blood of the brain and for free  $^{48}\text{V}$ .

### Urinary excretion

Figure 4 shows the fraction of  $^{48}\text{V}$ -activity excreted daily in urine. The data sets obtained from the 7-days and the 28-days retention experiments were used. The data show that there is rapid decline of daily urinary excretion from 0.34% to 0.18% of ID during the first three days after IV-injection followed by a slower decrease towards 0.12%ID after 2 weeks before a plateau below 0.1%ID of daily urinary excretion is reached after about 20 days. For the applied estimates on  $^{48}\text{V}$ -ion release we assumed no nanoparticulate urinary excretion as a conservative (upper) estimate of ionic  $^{48}\text{V}$ -release, although excretion of smaller nanoparticles cannot be totally excluded. This assumption is in agreement with the work of Choi and co-workers (Choi, 2007) who suggest that renal glomerular filtration does not allow urinary nanoparticle excretion of nanoparticles larger than 8 nm.

### Hepato-biliary [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP clearance (HBC)

[ $^{48}\text{V}$ ]TiO<sub>2</sub>NP observed in the gastro-intestinal tract (GIT) and fecal excretions resulted from their clearance from the liver *via* bile into the small intestine. The cumulative cleared fraction of [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP is shown in Figure 5. Over four weeks there was a steady increase of clearance up to about 3% of the applied dose *via* this pathway.

### Discussion

In order to estimate relevant dose levels for nanoparticle toxicology studies we should consider the main routes of intake which are either *via* inhalation or ingestion, since there is growing evidence that dermal uptake is usually so low that it is not detectable (Gontier, 2008). For inhalation the New Energy and Industrial Technology Development Organization

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(NEDO) in Japan has recently estimated an acceptable workplace airborne particulate concentration to be 1.2 mg/m<sup>3</sup> TiO<sub>2</sub>NP as a time weighted average for an 8h working day and a 40h working week (Morimoto, 2010). This may lead to a daily deposited TiO<sub>2</sub>NP dose in the lungs of 2.4 mg per day (assuming an inhaled volume of 20 m<sup>3</sup> per day and a deposition fraction of 0.3 averaged over a size range of 20-100 nm (MPPD (Multiple Path Particle Dosimetry); ((A.R.A.), 2009) corresponding to a daily dose of 34 µg/kg BW for a normal 70-kg person. No human translocation data across the air-blood barrier (ABB) are available but based on animal data the daily translocated TiO<sub>2</sub>NP fraction should be 1% or less (Kreyling, 2013). Therefore, a relevant daily dose to the circulation resulting from inhalation should not exceed 0.34 µg/kg BW.

A similar estimate can be made for ingested TiO<sub>2</sub>NP: Based on a survey of the British population (Lomer, 2004) the average daily intake of submicron and nano-sized TiO<sub>2</sub> particles is 2.5 mg/d by an average consumer corresponding to a daily dose of about 35 µg/kg BW of a normal 70-kg person. Also for absorbed TiO<sub>2</sub>NP across the human gut no consolidated data are available but based on animal data the daily absorbed TiO<sub>2</sub>NP fraction should be 5% or less (Jani, 1990). Therefore, a realistic daily dose to the circulation resulting from ingested and absorbed TiO<sub>2</sub>NP should not exceed 2 µg/kg BW. Taking together the daily TiO<sub>2</sub>NP absorbed through the gut epithelium into the circulation, a relevant daily dose would be a few tenths of µg/kg BW. With respect to this value, IV-injected TiO<sub>2</sub>NP doses of 1 mg/kg BW are 100-fold higher or more and usually applied over about 10 seconds corresponding to instantaneous dose rates about a million times higher than in realistic exposure scenarios. For the identification of potential organs at risk the extrapolation from results obtained from such high and even higher doses are not straightforward. In light of these considerations *in vivo* biokinetics studies using TiO<sub>2</sub>NP intravenous doses beyond tenths of µg/kg BW need solid justification.

For the present study a commercially available, engineered pure titanium dioxide material with (aggregated/agglomerated) primary particles of 7-10 nm in size has been used.. In contrast to many other studies our study aim was to quantify the biokinetics fate of nanoparticles (<100nm) in the entire organism, including total excretion, by making use of the high sensitivity of radiotracer studies which are not susceptible to matrix and background effects or artefacts introduced by specimen preparation. Hence, a truly nano-sized fraction was separated and prepared for simultaneous IV-injection, gavage and intratracheal instillation. The preparation was repeated five times to study a single retention time point (1h, 4h, 24, 7 days and 28 days) by all three exposure routes with the same [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP suspension. Quantitative biokinetics studies analyzing the entire organism with similar precision are presently not available in literature. However, several papers have also reported highest particle accumulations in the liver, followed by spleen, and then by the other organs studied (Fabian, 2008, Geraets, 2014, Louro, 2014, Patri, 2009, Shi, 2013, Yamashita, 2011) (Xie, 2011). In addition, there is a recent review on the toxicology of titanium dioxide nanoparticle including a discussion of biokinetics (Shi, 2013), but no data are reported concerning nanoparticle translocation to the skeleton and soft tissues.

Using  $^{48}\text{V}$ -labeled pure anatase TiO<sub>2</sub>NP allowed us to perform rather precise determinations of the biokinetics of IV-injected [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP over a dynamic dose range of five orders of magnitude between the applied dose and the content in individual organs and tissues up to 28 days after IV-injection. Since we found that blood contained circulating [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP at any retention time, we estimated the [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP content in the residual blood volume of each organ and tissue after exsanguination by applying the results of Oeff and Konig (Oeff, 1955), and subtracted this amount from the measured organ activity to determine with greater accuracy the parenchymal [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP organ/tissue content.

Additionally, we aimed to use rather low [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP doses of about 10  $\mu\text{g}/\text{rat}$  for the biokinetics studies up to 24 hours and of about 100  $\mu\text{g}/\text{rat}$  for the 7-day and 28-day studies (to

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3 400 compensate for radioactive  $^{48}\text{V}$  decay and  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  elimination from the body), which is  
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5 401 a compromise between physiologically reasonable daily doses and preserving high detection  
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7 402 sensitivity. The combination of applied low doses and high detection sensitivity ensures that,  
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9 403 neither the rather low  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  mass used in our study nor its radioactivity is likely to  
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11 404 cause any detectable detrimental effect. Additionally, the  $^{48}\text{V}$ -radioactivity concentration  
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13 405 chosen corresponded to an atomic ratio of  $^{48}\text{V}:\text{Ti}$  in the order of  $4 \times 10^{-7}$  which represents a  
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15 406 negligible mass-impurity of the  $^{48}\text{V}$  in the  $\text{TiO}_2\text{NP}$  matrix, unlikely to affect its lattice stability  
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17 407 or any physico-chemical property.  
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19 408 However, the study design is also associated with some shortcomings. This study remains at  
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21 409 the level of macroscopic biokinetics and does not provide any microscopic details, such as  
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23 410 any cell-type interactions with the  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  in any of the secondary organs or tissues,  
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25 411 which of course would have been highly desirable. It should be noted that we never directly  
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27 412 observed actual  $\text{TiO}_2$  particles in our *in vivo* studies and relied on  $\gamma$ -spectrometric  
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29 413 determination of  $^{48}\text{V}$ -activity. At the low activity levels detected in some organs the  
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31 414 calculated amounts of  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  are more sensitive to errors especially when subtracting  
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33 415 the estimated contribution of free  $^{48}\text{V}$ -ions. Therefore, further independent studies with  
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35 416 similarly high sensitivity are desirable. Although we corrected for the  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  content in  
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37 417 the residual blood of all organs and tissues, we could not distinguish between  $[^{48}\text{V}]\text{TiO}_2\text{NP}$   
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39 418 content translocated to the parenchyma and that eventually trapped in the walls of minor  
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41 419 blood vessels. Yet, at the doses chosen it would have been impossible to identify and quantify  
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43 420  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  in biological specimens using electron microscopy because of their very sparse  
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45 421 distribution in any of the secondary organs and tissues probably with exception of the liver.  
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47 422 However, in a previous inhalation study on WKY rats using freshly generated  $\text{TiO}_2$  anatase  
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49 423 nanoparticles (median size 20 nm) the lung distribution of  $\text{TiO}_2\text{NP}$  had been  
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51 424 morphometrically quantified by TEM analysis (Geiser, 2008, Geiser, 2005). Furthermore, in  
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53 425 an earlier study, we have identified 18 nm gold nanoparticles in electron-micrographs of  
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3 426 Kupffer cells, hepatocytes and endothelial cells of the rat liver 24h after IV-injection (Hirn,  
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5 427 2011), indicating that nanoparticles do indeed translocate into the organ tissues.  
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7 428 Intravenous injection of suspended [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP provides a high dose rate to blood.  
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9 429 Therefore, it is likely that only very few nanoparticles will be taken up by monocytes and/or  
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11 430 thrombocytes of the blood and, hence, most will initially interact and bind to blood proteins  
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13 431 and biomolecules (called opsonization or more recently protein-corona) which subsequently  
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15 432 will affect uptake in organs and tissues. Most organs and tissues have only a relatively low  
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17 433 capacity for acute particle uptake via their mononucleated-phagocytic-system (MPS) which  
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19 434 differs considerably between organs and tissues (Hume, 2008). In contrast, the liver has a high  
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21 435 capacity which causes rapid and predominant accumulation in the liver for many  
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23 436 nanoparticles (Almeida, 2011, Zarschler, 2016). This uptake is likely be affected by the  
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25 437 protein-corona in blood. However, it remains unclear which biomolecules lead to rapid  
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27 438 receptor recognition and phagocytosis by Kupffer cells, and, likewise, how and by which  
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29 439 biomolecule mediation the uptake occurs in MPS cells of the other organs and tissues. After  
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31 440 only 1h the [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP concentration in blood decreases 200-fold so that circulating  
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33 441 [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP may well be phagocytized/endocytosed, and subsequently the composition of  
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35 442 the dynamic protein corona may change and/or blood monocytes and thrombocytes may  
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37 443 modify their further fate in the body.  
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39 444 It is quite remarkable how constant the [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP retention is in most of the organs,  
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41 445 skeleton and the tissue after the correction for  $^{48}\text{V}$  release from the nanoparticle matrix (see  
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43 446 Table 2 and Figure 3). It underlines the stability of the nano-fraction of the commercial ST-01  
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45 447 TiO<sub>2</sub> powder and its radiolabel  $^{48}\text{V}$ . However, the increasing hepato-biliary clearance (HBC)  
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47 448 over time (see Figure 5) highlights that minor biokinetic [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP exchanges and/or  
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49 449 clearance occurs in the liver and probably in the entire organism over time. The cumulative  
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51 450 HBC steadily increases up to 3% over 28 days. In our previous IV-injection study we could  
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53 451 only determine 24-hour data because of the short half-life of the  $^{198}\text{Au}$  radiotracer used, but  
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we showed that HBC is linearly inversely related to the Au nanoparticles diameter between 2.8 nm to 80 nm (Hirn, 2011). For 80-nm-size Au nanoparticles we obtained 0.5% HBC after 24h. This corresponds reasonably well with the clearance level of 70 nm  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  (0.4%) at 24h found in this study. Differences may be related to the differences in nanoparticle materials and/or their morphologies.

In the SM-IV (Figure S7) we derive a small release rate (less than 0.1% per day) of  $^{48}\text{V}$  from the  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  which appears to be effective during the whole study period of 28d. This might be interpreted either as loss of imperfectly fixed labels in the  $\text{TiO}_2$  matrix or as a very slow dissolution and shrinking of the nanoparticles (Vogelsberger, 2008) setting free less than 0.1% of the nanoparticle mass per day. If the latter would be the case (or even a combination of the two) such a process may contribute to nanoparticle clearance from organs and from the organism.

**Conclusion**

The quantitatively balanced biokinetics assay used for retention times up to 28d after IV-injection of  $^{48}\text{V}$  radiolabeled  $\text{TiO}_2\text{NP}$  provides a sensitive methodology with a dynamic dose range over five orders of magnitude and allows quantitative  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  distribution balancing at each retention time point in the entire organism, including excretions. The  $^{48}\text{V}$  release rate from the  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  matrix was less than 0.1% per day and the  $^{48}\text{V}$ -activity related to free  $^{48}\text{V}$ -ions was corrected for according to the auxiliary biokinetics study on ionic  $^{48}\text{V}$ .  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  were detected in most organs and tissues most likely retained in their MPS. Highest  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  accumulations were found in liver (95.5% ID during day-1), followed by spleen (2.3%), skeleton (0.7%), blood (0.5%) and, with detectable nanoparticle burdens in all other organs. It is remarkable that nanoparticles were retained in organs and tissues that are usually not considered in biodistribution studies. The  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  content in blood decreased 200-fold within one hour while the distribution in other organs and tissues remained roughly

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3 477 constant over 28 days. Hepato-biliary clearance of [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP from the liver continued over  
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5 478 the entire 28-days period.  
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**Acknowledgements**

We would like to thank Sebastian Kaidel, Paula Mayer and Nadine Senger from the Helmholtz Center Munich for their excellent technical assistance, as well as Antonio Bulgheroni, Kamel Abbas, Federica Simonelli, Izabela Cydzik and Giulio Cotogno from the EU-Joint Research Center who strongly supported the nanoparticle radiolabeling activities. We also express our sincere gratitude to Barbara Rothen-Rutishauser and David Raemy from the University of Fribourg, Switzerland, who performed the TEM analysis of the TiO<sub>2</sub>NP.

**Declaration of Interest**

The authors declare that they have no financial, consulting, and personal relationships with other people or organizations that could influence (bias) the author's work. This work was partially supported by the German Research Foundation SPP 1313, the EU-FP6 project Particle-Risk (012912 (NEST)), and the EU FP7 projects NeuroNano (NMP4-SL-2008-214547), ENPRA (NMP4-SL-2009-228789) and InLiveTox (NMP-2008-1.3-2 CP-FP 228625-2).

**Supplementary Material available online.**

- Radiolabeling of titanium dioxide (TiO<sub>2</sub>) nanoparticles
- Nanoparticle preparation for application and characterization
- Animals and animal housing
- Nanoparticle application and animal maintenance in metabolic cages
- Sample preparation for radiometric analysis
- Radiometric and statistical analysis
- Blood correction and total blood volume



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3 504 •  $^{48}\text{V}$ -activity determination of skeleton and soft tissue  
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5 505 • Biokinetics of soluble  $^{48}\text{V}$  in ionic form after IV-injection  
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7 506 • Correction of the biokinetics assigned to  $[^{48}\text{V}]\text{TiO}_2\text{NP}$  for the effect of free  $^{48}\text{V}$ -ions  
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10 507 • Evaluation of the auxiliary and main study by pharmacokinetic modeling  
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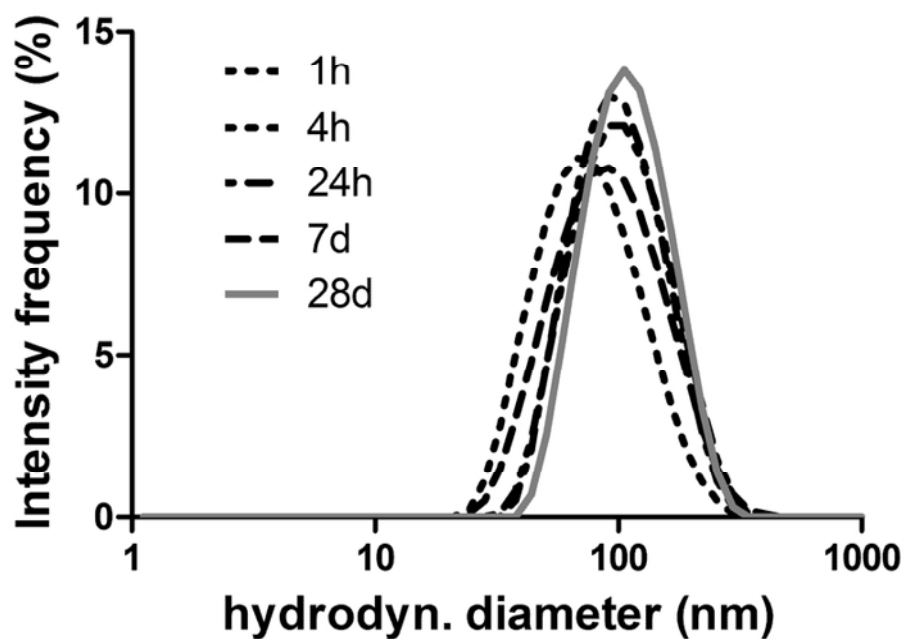


Figure 1: Hydrodynamic diameter of the five separately prepared [48V]TiO<sub>2</sub>NP suspensions used to study the five retention times of 1h, 4h, 24h, 7d and 28d measured directly before IV-injection.

73x52mm (300 x 300 DPI)



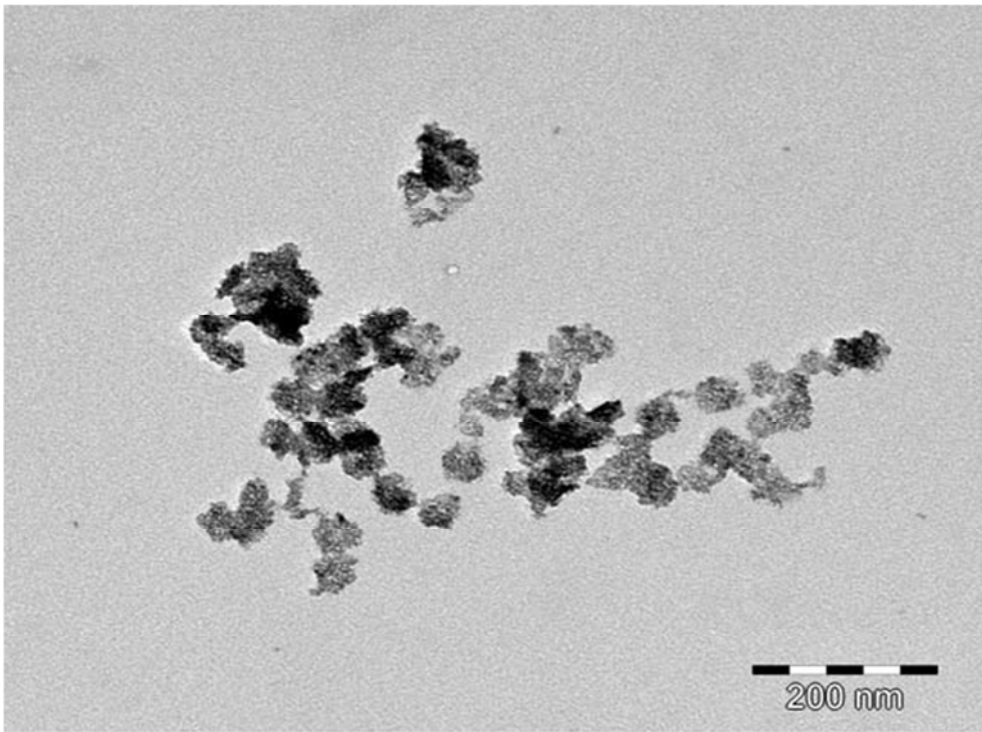


Figure 2: Transmission electron micrograph of size-selected TiO<sub>2</sub>NP sampled immediately after the size-selection procedure. TEM sample preparation leads to 'clumping' together of aggregates/agglomerates on the support grid.

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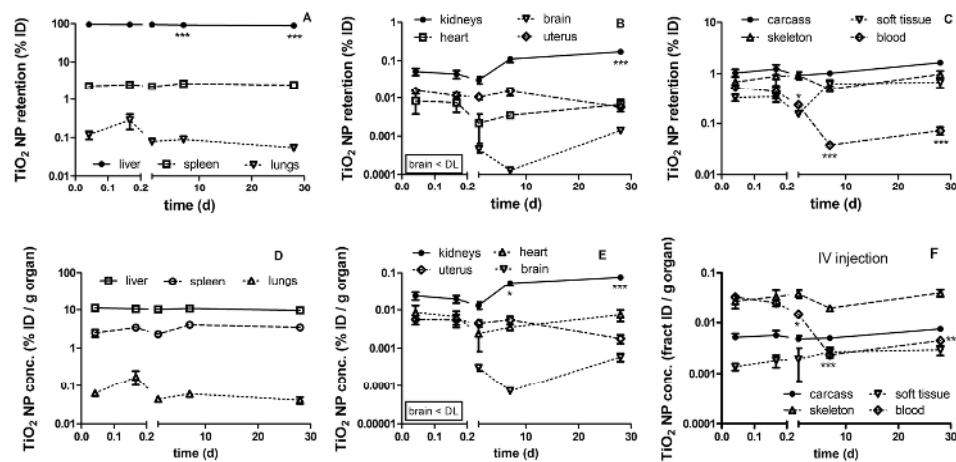


Figure 3: Graphical presentation of the biokinetics of IV-injected [48V]TiO<sub>2</sub>NP. The [48V]TiO<sub>2</sub>NP retention is expressed in terms of the retained percentage of the effectively injected nanoparticle dose which is equivalent to the percentage of injected 48V-activity corrected for the effect of free 48V-ions (% ID) (panels A-C). In panels D-F the values normalized to the organ weight are presented in %ID•g<sup>-1</sup>. Mean ± SEM of n=4 rats at each time point. Compared to 1h data levels of significance are p<0.05 (\*), p<0.01 (\*\*), p<0.001 (\*\*\*).

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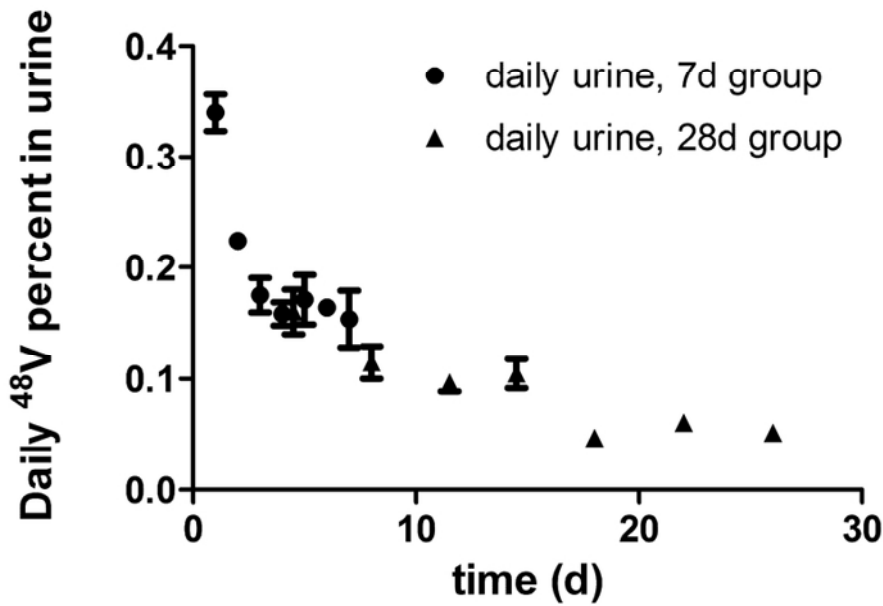


Figure 4: Daily urinary <sup>48</sup>V-activity excretion presented as percent-rates of the total IV-injected radioactivity (%ID) over four weeks. Data from 24h to 7d after IV-injection are daily averages of the 24h group and the 7d group (n = 4). Data of the 28d group were determined as integral samples over 3-4 days and are plotted as daily urinary excretion at the mean day of the sampling period. Mean ± SEM of n=4 rats at each time point.

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Figure 5, IV, 13-12-2016

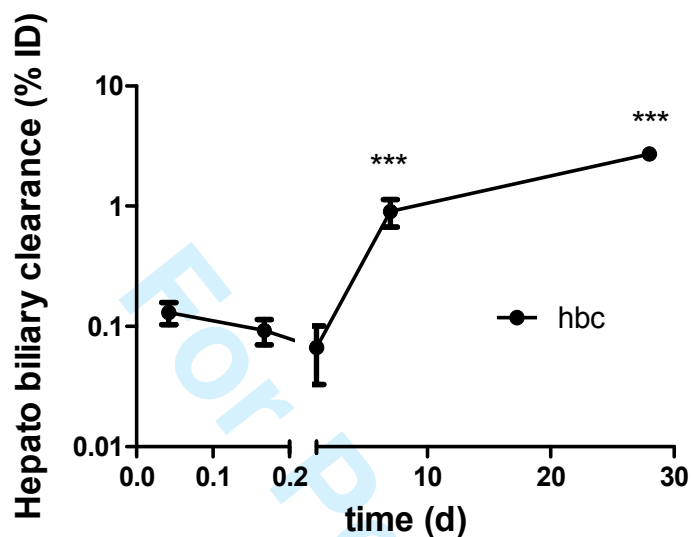


Figure 5: Cumulative Hepato-Biliary Clearance (HBC) of [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP from the liver into the GIT and fecal excretions as percent of the total IV-injected [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP radioactivity (%ID) over four weeks. Mean  $\pm$  SEM of n=4 rats at each time point. Compared to 1h data levels of significance are  $p < 0.001$  (\*\*\*).

**Table 1:** Physicochemical characteristics of the [<sup>48</sup>V]TiO<sub>2</sub>NP suspensions used for IV-injection studies at five different retention times and the mean values of the applied <sup>48</sup>V activity and mass of [<sup>48</sup>V]TiO<sub>2</sub>NP effectively received by the rats. The mean dose in µg/kg BW is also given. Additionally, [<sup>48</sup>V]TiO<sub>2</sub>NP losses in the syringe and/or cannula are provided as detailed in SI-IV.

Retention time		1h	4h	24h	7d	28d
Zeta Potential	[mV]	-38.9 ± 4.2	-33.2 ± 2.4	-29.9 ± 8.1	-42.7 ± 9.2	-35.2 ± 7.6
Z-average	[nm]	93	72	93	82	101
PDI		0.157	0.228	0.160	0.197	0.135
Effective <sup>48</sup> V radioactivity received by rats	[kBq]	18.15 ± 3.37	11.29 ± 3.69	16.53 ± 3.69	253.99 ± 54.23	110.27 ± 5.76
applied [ <sup>48</sup> V]TiO <sub>2</sub> NP mass	[µg]	18.15 ± 3.37	11.29 ± 3.69	16.53 ± 3.69	108.08 ± 54.23	23.08 ± 46.92
Mean applied dose	[µg/g BW]	69.62 ± 69.7	40.13 ± 13.29	60.24 ± 13.29	392.54 ± 73.52	127.44 ± 10.17
Percentage of [ <sup>48</sup> V]TiO <sub>2</sub> NP retained in the syringe after application	[%]	62.8 ± 6.2	71.3 ± 1.7	32.6 ± 4.5	n.d.	n.d.

**Table 2:** [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP retention in organs and tissues at five time points 1h, 4 h, 24h, 7d and 28d after intravenous injection. The data are presented as retained percentage of the total intravenously injected [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP dose (*raw data*). The raw data were corrected for the [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP content in the residual blood present in organs and tissues after exsanguination (*w/o residual blood content*) and additionally for the contributions of free  $^{48}\text{V}$ -ions to the biodistribution (*w/o free  $^{48}\text{V}$* ). After these corrections the  $^{48}\text{V}$ -activity data were converted into [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP concentrations per mass of organ or tissue, given in  $\text{ng}\cdot\text{g}^{-1}$ , and as  $\% \text{ID}\cdot\text{g}^{-1}$ . Since the effectively applied [ $^{48}\text{V}$ ]TiO<sub>2</sub>NP doses varied due to nanoparticle retention in the syringes and were intentionally increased for the 7d and 28d groups most mass concentrations in  $\text{ng}\cdot\text{g}^{-1}$  exhibit an increase from 24h to 7d. The values in  $\% \text{ID}\cdot\text{g}^{-1}$  are independent of the applied doses. (< DL = below detection limit).

organ	retention time (d)	1h	4h	24h	7d	28d
	percent (%)	mean $\pm$ SEM	mean $\pm$ SEM	mean $\pm$ SEM	mean $\pm$ SEM	mean $\pm$ SEM
liver	raw data (% ID)	95.56 $\pm$ 0.42	94.77 $\pm$ 0.50	94.61 $\pm$ 0.23	92.55 $\pm$ 0.50	88.97 $\pm$ 0.17
liver	w/o resid. blood cont.	95.52 $\pm$ 0.42	94.74 $\pm$ 0.51	94.59 $\pm$ 0.23	92.54 $\pm$ 0.50	88.96 $\pm$ 0.17
liver	w/o free $^{48}\text{V}$	95.50 $\pm$ 0.42	94.70 $\pm$ 0.51	94.48 $\pm$ 0.23	92.44 $\pm$ 0.45	88.92 $\pm$ 0.17
liver	TiO <sub>2</sub> conc. (% ID $\cdot\text{g}^{-1}$ tiss.)	11.14 $\pm$ 0.19	10.62 $\pm$ 0.23	10.16 $\pm$ 0.18	10.74 $\pm$ 0.32	9.67 $\pm$ 0.62
liver	TiO <sub>2</sub> conc. ( $\text{ng}\cdot\text{g}^{-1}$ tiss.)	2008 $\pm$ 222	1208 $\pm$ 213	1682 $\pm$ 38	11564 $\pm$ 1243	4551 $\pm$ 364
spleen	raw data (% ID)	2.35 $\pm$ 0.26	2.57 $\pm$ 0.18	2.27 $\pm$ 0.07	2.77 $\pm$ 0.20	2.48 $\pm$ 0.27
spleen	w/o resid. blood cont.	2.35 $\pm$ 0.2576	2.57 $\pm$ 0.18	2.27 $\pm$ 0.07	2.77 $\pm$ 0.20	2.48 $\pm$ 0.27
spleen	w/o free $^{48}\text{V}$	2.34 $\pm$ 0.26	2.57 $\pm$ 0.18	2.26 $\pm$ 0.07	2.75 $\pm$ 0.20	2.48 $\pm$ 0.27
spleen	TiO <sub>2</sub> conc. (% ID $\cdot\text{g}^{-1}$ tiss.)	2.51 $\pm$ 0.55	3.43 $\pm$ 0.31	2.32 $\pm$ 0.21	4.06 $\pm$ 0.44	3.49 $\pm$ 0.32
spleen	TiO <sub>2</sub> conc. ( $\text{ng}\cdot\text{g}^{-1}$ tiss.)	454 $\pm$ 122	402 $\pm$ 90	384 $\pm$ 35	4299 $\pm$ 389	1635 $\pm$ 145
kidneys	raw data (% ID)	0.078 $\pm$ 0.011	0.078 $\pm$ 0.018	0.100 $\pm$ 0.007	0.169 $\pm$ 0.018	0.193 $\pm$ 0.012
kidneys	w/o resid. blood cont.	0.062 $\pm$ 0.010	0.065 $\pm$ 0.015	0.090 $\pm$ 0.007	0.167 $\pm$ 0.018	0.191 $\pm$ 0.012
kidneys	w/o free $^{48}\text{V}$	0.0523 $\pm$ 0.0111	0.045 $\pm$ 0.0107	0.032 $\pm$ 0.007	0.112 $\pm$ 0.021	0.172 $\pm$ 0.011
kidneys	TiO <sub>2</sub> conc. (% ID $\cdot\text{g}^{-1}$ tiss.)	0.023 $\pm$ 0.005	0.019 $\pm$ 0.004	0.0131 $\pm$ 0.003	0.053 $\pm$ 0.010	0.076 $\pm$ 0.007
kidneys	TiO <sub>2</sub> conc. ( $\text{ng}\cdot\text{g}^{-1}$ tiss.)	3.89 $\pm$ 0.43	1.93 $\pm$ 0.15	2.16 $\pm$ 0.44	54.10 $\pm$ 6.05	35.60 $\pm$ 2.39
lungs	raw data (% ID)	0.134 $\pm$ 0.032	0.297 $\pm$ 0.125	0.092 $\pm$ 0.010	0.095 $\pm$ 0.013	0.057 $\pm$ 0.008
lungs	w/o resid. blood cont.	0.119 $\pm$ 0.028	0.286 $\pm$ 0.123	0.083 $\pm$ 0.009	0.094 $\pm$ 0.013	0.055 $\pm$ 0.008
lungs	w/o free $^{48}\text{V}$	0.118 $\pm$ 0.029	0.285 $\pm$ 0.123	0.079 $\pm$ 0.009	0.089 $\pm$ 0.013	0.054 $\pm$ 0.008
lungs	TiO <sub>2</sub> conc. (% ID $\cdot\text{g}^{-1}$ tiss.)	0.063 $\pm$ 0.008	0.178 $\pm$ 0.0742	0.044 $\pm$ 0.005	0.060 $\pm$ 0.009	0.041 $\pm$ 0.008
lungs	TiO <sub>2</sub> conc. ( $\text{ng}\cdot\text{g}^{-1}$ tiss.)	10.83 $\pm$ 0.43	16.21 $\pm$ 3.71	7.24 $\pm$ 16.46	67.02 $\pm$ 16.46	19.51 $\pm$ 4.31
heart	raw data (% ID)	0.013 $\pm$ 0.005	0.012 $\pm$ 0.005	0.006 $\pm$ 0.001	0.005 $\pm$ 0.0002	0.008 $\pm$ 0.003
heart	w/o resid. blood cont.	0.009 $\pm$ 0.005	0.008 $\pm$ 0.003	0.004 $\pm$ 0.002	0.005 $\pm$ 0.0002	0.007 $\pm$ 0.002

heart	w/o free 48V	0.008 ± 0.005	0.007 ± 0.003	0.002 ± 0.002	0.004 ± 0.0003	0.007 ± 0.002
heart	TiO <sub>2</sub> conc. (% ID·g <sup>-1</sup> tiss.)	0.009 ± 0.004	0.007 ± 0.003	0.002 ± 0.002	0.004 ± 0.0002	0.008 ± 0.003
heart	TiO <sub>2</sub> conc. (ng·g <sup>-1</sup> tiss.)	1.29 ± 0.51	0.614 ± 0.111	0.40 ± 0.27	3.76 ± 0.36	3.52 ± 1.18
brain	raw data (% ID)	< DL	< DL	0.0015 ± 0.0001	0.0005 ± 0.00005	0.0015 ± 0.0002
brain	w/o resid. blood cont.	< DL	< DL	0.0009 ± 0.0001	0.0005 ± 0.00004	0.0013 ± 0.0002
brain	w/o free 48V	< DL	< DL	0.0005 ± 0.0001	< DL	0.0011 ± 0.0002
brain	TiO <sub>2</sub> conc. (% ID·g <sup>-1</sup> tiss.)	< DL	< DL	0.0003 ± 0.0001	< DL	0.0006 ± 0.0001
brain	TiO <sub>2</sub> conc. (ng·g <sup>-1</sup> tiss.)	< DL	< DL	0.051 ± 0.009	< DL	0.273 ± 0.059
uterus	raw data (% ID)	0.018 ± 0.001	0.015 ± 0.003	0.016 ± 0.002	0.026 ± 0.005	0.009 ± 0.0006
uterus	w/o resid. blood cont.	0.016 ± 0.001	0.013 ± 0.003	0.015 ± 0.002	0.026 ± 0.005	0.009 ± 0.0006
uterus	w/o free 48V	0.016 ± 0.001	0.011 ± 0.002	0.011 ± 0.002	0.015 ± 0.004	0.006 ± 0.0008
uterus	TiO <sub>2</sub> conc. (% ID·g <sup>-1</sup> tiss.)	0.006 ± 0.001	0.006 ± 0.002	0.005 ± 0.001	0.006 ± 0.001	0.002 ± 0.0005
uterus	TiO <sub>2</sub> conc. (ng·g <sup>-1</sup> tiss.)	1.00 ± 0.15	0.66 ± 0.28	0.74 ± 0.15	5.51 ± 1.13	1.01 ± 0.15
blood	raw data (% ID)	0.524 ± 0.060	0.443 ± 0.115	0.300 ± 0.018	0.047 ± 0.004	0.075 ± 0.013
blood	w/o resid. blood cont.	0.524 ± 0.060	0.443 ± 0.115	0.300 ± 0.018	0.047 ± 0.004	0.075 ± 0.013
blood	w/o free 48V	0.512 ± 0.062	0.419 ± 0.111	0.230 ± 0.018	0.037 ± 0.003	0.072 ± 0.013
blood	TiO <sub>2</sub> conc. (% ID·g <sup>-1</sup> tiss.)	0.031 ± 0.004	0.024 ± 0.006	0.015 ± 0.001	0.002 ± 0.0002	0.004 ± 0.0007
blood	TiO <sub>2</sub> conc. (ng·g <sup>-1</sup> tiss.)	5.42 ± 0.10	3.60 ± 0.41	2.4006 0.19	2.48 ± 0.24	2.09 ± 0.36
carcass	raw data (% ID)	1.2 ± 0.16	1.53 ± 0.31	1.63 ± 0.12	1.75 ± 0.09	1.90 ± 0.02
carcass	w/o resid. blood cont.	1.14 ± 0.15	1.44 ± 0.23	1.57 ± 0.12	1.74 ± 0.09	1.89 ± 0.021
carcass	w/o free 48V	1.03 ± 0.18	1.22 ± 0.27	0.92 ± 0.13	1.01 ± 0.12	1.63 ± 0.03
carcass	TiO <sub>2</sub> conc. (% ID·g <sup>-1</sup> tiss.)	0.005 ± 0.001	0.006 ± 0.001	0.005 ± 0.001	0.005 ± 0.0006	0.008 ± 0.0003
carcass	TiO <sub>2</sub> conc. (ng·g <sup>-1</sup> tiss.)	0.89 ± 0.10	0.58 ± 0.08	0.13 ± 0.13	5.22 ± 0.23	3.54 ± 0.18
skeleton	raw data (% ID)	0.81 ± 0.13	1.02 ± 0.34	1.28 ± 0.17	1.04 ± 0.12	1.22 ± 0.16
skeleton	w/o resid. blood cont.	0.79 ± 0.13	1.00 ± 0.33	1.27 ± 0.17	1.04 ± 0.12	1.22 ± 0.16
skeleton	w/o free 48V	0.68 ± 0.16	0.88 ± 0.32	0.92 ± 0.16	0.49 ± 0.06	0.96 ± 0.15
skeleton	TiO <sub>2</sub> conc. (% ID·g <sup>-1</sup> tiss.)	0.026 ± 0.006	0.032 ± 0.011	0.036 ± 0.007	0.019 ± 0.002	0.038 ± 0.006
skeleton	TiO <sub>2</sub> conc. (ng·g <sup>-1</sup> tiss.)	4.18 ± 0.53	3.02 ± 0.57	6.02 ± 1.05	18.43 ± 2.50	17.71 ± 2.90
soft tissue	raw data (% ID)	0.44 ± 0.07	0.51 ± 0.05	0.352 ± 0.07	0.71 ± 0.13	0.68 ± 0.14
soft tissue	w/o resid. blood cont.	0.34 ± 0.05	0.43 ± 0.05	0.30 ± 0.07	0.70 ± 0.13	0.67 ± 0.14
soft tissue	w/o free 48V	0.32 ± 0.05	0.33 ± 0.07	0.15 ± 0.01	0.61 ± 0.13	0.67 ± 0.14
soft tissue	TiO <sub>2</sub> conc. (% ID·g <sup>-1</sup> tiss.)	0.0014 ± 0.0002	0.0013 ± 0.0003	0.0007 ± 0.0001	0.0027 ± 0.0005	0.0029 ± 0.0006
soft tissue	TiO <sub>2</sub> conc. (ng·g <sup>-1</sup> tiss.)	0.24 ± 0.02	0.16 ± 0.05	0.11 ± 0.01	2.54 ± 0.46	1.38 ± 0.32