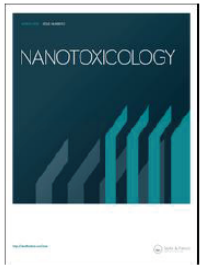
National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Risk assessment of TiO_2 nanoparticles via oral exposure, including toxicokinetic considerations

This presentation

- Overview main findings
- Relevance to safety assessment of E171



Nanotoxicology

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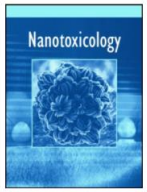
Risk assessment of titanium dioxide nanoparticles via oral exposure, including toxicokinetic considerations

Minne B. Heringa, Liesbeth Geraets, Jan C. H. van Eijkeren, Rob J. Vandebriel, Wim H. de Jong & Agnes G. Oomen



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Oral intake of added titanium dioxide and its nanofraction from food products, food supplements and toothpaste by the Dutch population

Cathy Rompelberg, Minne B. Heringa, Gerda van Donkersgoed, José Drijvers, Agnes Roos, Susanne Westenbrink, Ruud Peters, Greet van Bemmel, Walter Brand & Agnes G. Oomen

Heringa et al. Particle and Fibre Toxicology (2018) 15:15
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Particle and Fibre Toxicology

RESEARCH Open Access

Detection of titanium particles in human liver and spleen and possible health implications

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Overview of main findings: intake

- Aim: realistic estimation of oral intake of added titanium dioxide (TiO_2) and its nanofraction ($nTiO_2$) from food products, food supplements and toothpaste by the Dutch population (Rompelberg et al., 2016)
- To calculate the $nTiO_2$ fraction (<100 nm) ingested, we used a fraction of 0.31% (by mass, corresponding to 15% by number) of nano-sized particles calculated from the data of Peters et al. (2014)

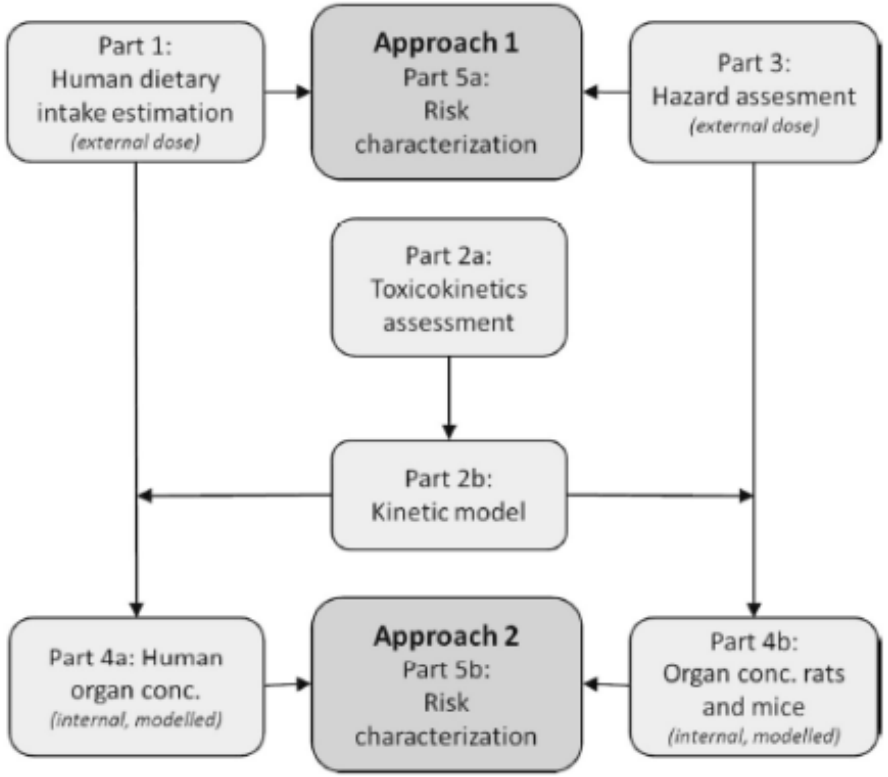
	TiO_2			TiO_2 nanoparticles (TiO_2 NPs)		
Age groups	**Percentiles of intake*** *(mg/kg bw per day)*		**Mean*** *(mg/kg bw per day)*	**Percentiles of intake*** *(µg/kg bw per day)*		**Mean*** *(µg/kg bw per day)*
	P50	**P95**		**P50**	**P95**	
2-6 y old	0.59 (0.57- 0.60)	1.29 (1.19 – 1.40)	0.67 (0.66 - 0.70)	1.90 (1.80 – 1.94)	4.16 (3.84 – 4.52)	2.16 (2.13 – 2.26)
7-69 y old	0.08 (0.07 –0.08)	0.50 (0.47 – 0.54)	0.17 (0.16 – 0.18)	0.26 (0.23 – 0.26)	1.61 (1.52 – 1.74)	0.55 (0.52 – 0.58)
70+	0.03 (0.02 –0.03)	0.23 (0.20 – 0.28)	0.06 (0.05 – 0.07)	0.10 (0.06 – 0.10)	0.74 (0.65 – 0.90)	0.19 (0.16 – 0.23)
lifelong daily intake**	0.11	0.52	0.19	0.36	1.67	0.62

Rompelberg et al. Nanotoxicology. 2016 10(10):1404-1414.
Peters et al. J Agric Food Chem. 2014, 62(27):6285-93.

Overview of main findings: risk assessment

- Risk assessment based on internal organ concentrations using a kinetic model in order to account for accumulation over time.

- Margins between estimated/measured tissue concentrations in humans and the (no) effect concentrations in tissues obtained from toxicity studies in rodents (MoE_i).



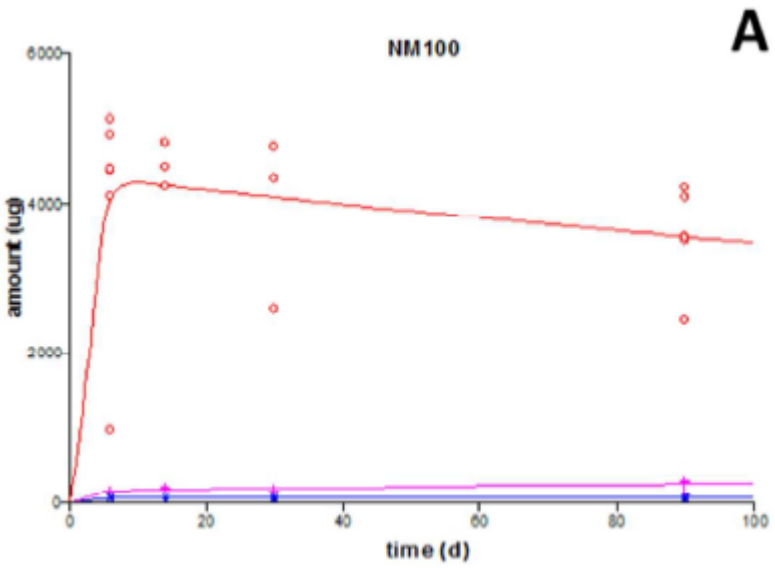
E171 and $nTiO_2$ in risk assessment approach

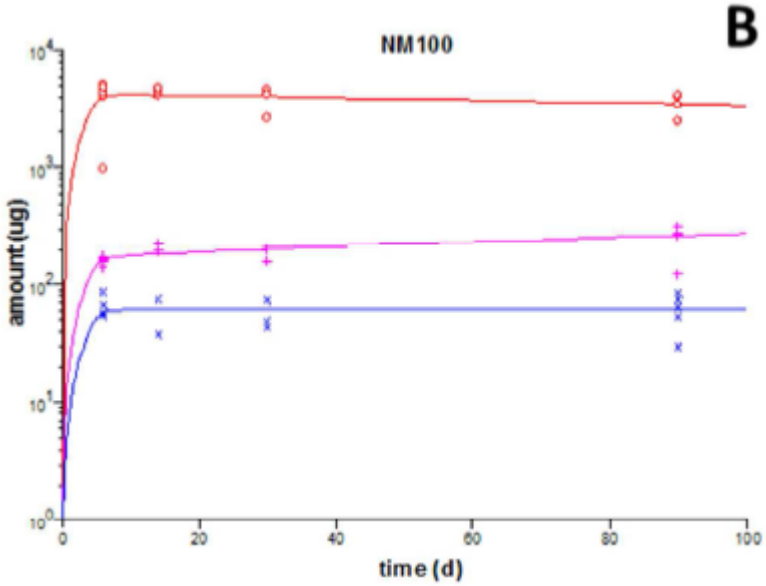
- E171 used in food (and other products) for its white colour and brightness
  - Particles 200-300 nm optimal light diffraction
- A small fraction of the pigment is present as nanoparticles (NPs)
  - Various studies: 10-37% of number of particles <100 nm

- **In the present approach it is assumed that any toxicity is caused by the nanoparticles (<100 nm) present in E171.**
  - Artificial boundary
    - In human tissue it was found that many of the TiO_2 particles were below or around 100 nm
- **Toxicity studies with E171 as well as with $nTiO_2$ are used**
  - Exposure/dose is, if needed, converted to nanoparticles

Kinetic data and model *

Red = liver
pink = spleen
blue = rest

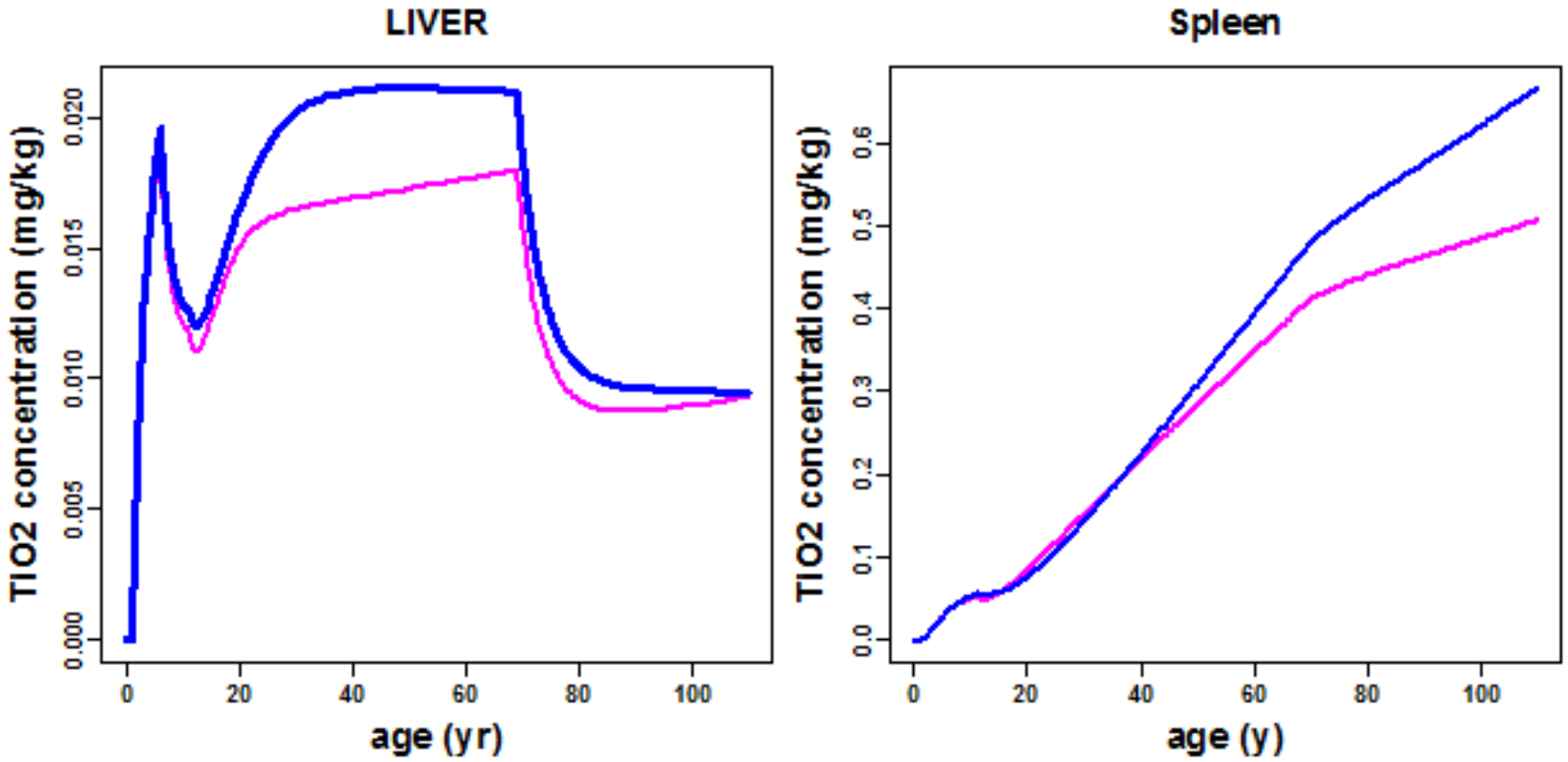




- Fast removal from blood
- Distribution mainly to liver, spleen and lung
- In liver limited elimination
- In spleen, no elimination, even small increase
- Slow total elimination
- Oral study: very low absorption: 0.02%

* Data from Geraets et al. Part Fibre Toxicol 11: 30 (2014), NanoGenotox project, 4 forms of TiO2

Estimated $nTiO_2$ concentrations in human organs



$nTiO_2$ concentration in different human organs plotted against age

Key toxicity study – oral, rat

Study details	TiO_2 details	NOAEL or LOAEL	Effects at LOAEL	Source
• 30 days • Sprague Dawley rats • 7 animals/sex/dose • 0; 10; 50; 200 mg/kg bw/day • Intragastric in water • Young rats (3 weeks old) and adult rats (8 weeks old)	• anatase • 75 nm average diameter • 63.95 m^2/g (BET)	Liver (young + adult): NOAEL: 10 mg/kg bw/day Spleen (young + adult): NOAEL: 200 mg/kg bw/day	Liver in young rats: edema and biochemical markers Liver in adult rats: biochemical markers (no effect in spleen, testes, lung, brain) Biochemical markers kidney tox adults Biochemical markers heart injury young	Wang et al., 2013

Key toxicity studies

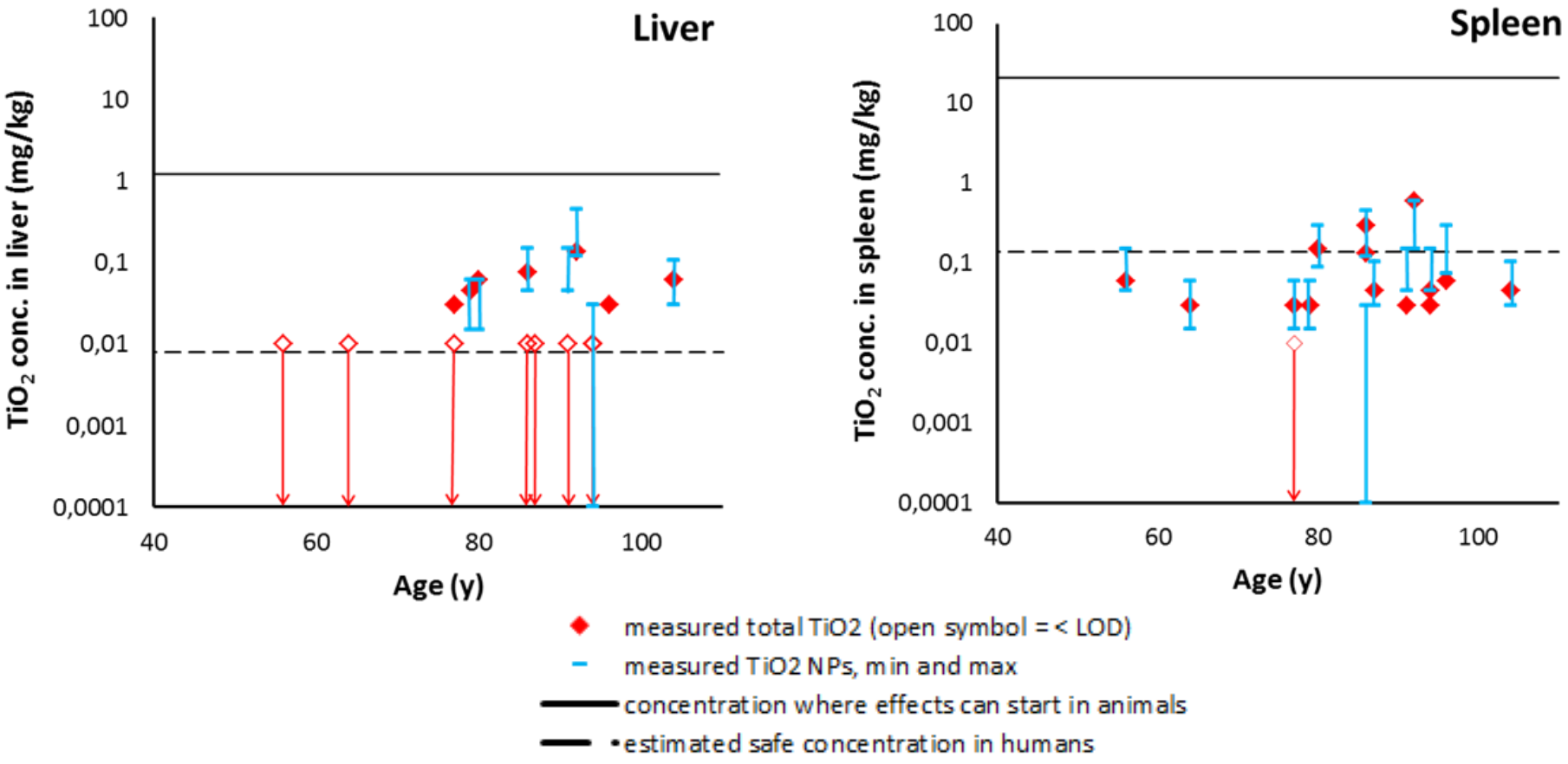
Study details	TiO_2 details[1]	Study-specific NOAEL or LOAEL (*self- derived levels in italics*)	Critical effects at LOAEL (not exhaustive)	Source
• 103 weeks • F344 rats, females and males • 50 animals/sex/dose • 0; 1250; 2500 mg/kg bw/day • Via diet	• Anatase, uncoated, Unitane® 0-220 • (No further information; interpretation of the authors: probably standard food-grade white pigment)	NOAEL: 2500 mg/kg bw/d *LOAEL: 1250 mg/kg bw/d*	No significant carcinogenic effects or macroscopic lesions In annexes (without historic control range): - Congestion and hemorrhage in lung of male and female rats - Fibrosis in heart of male rats - Hyperplasia of bile ducts in male rats ↑, but no clear dose-response in female rat - Atrophy of the seminal vesicles and possibly testes of male rats - Galactocele in mammary gland of female rats	NCI, 1979
• 30 days • Sprague Dawley rats, males only • 7 animals/dose • 0; 10; 50; 200 mg/kg bw/day • Intragastric in water • Young rats (3 weeks old) and adult rats (8 weeks old)	• Anatase • 75 ± 15 nm average diameter • 63.95 m^2/g (BET) • Hydroxyl groups on surface	*Liver young: NOAEL: 10 mg/kg bw/day* *Liver adult: NOAEL: 50 mg/kg bw/d* *Spleen (young + adult): NOAEL: 200 mg/kg bw/day*	Young rats: Liver edema AST ↓ and ALT/AST ↑ (ALT unchanged) → liver damage HBDH[2] ↓ → heart injury, but not dose-related Glucose and LDL-C[2] ↑, but not dose-related Adult rats: TBIL ↑ → liver damage (multiple possible causes)	Wang et al., 2013
• 42 days Kunming mice, males only • 15 animals/dose • 0; 10; 50; 250 mg/kg bw/day • Intragastric in PBS with 0.5 % Tween 80 • 4 weeks old at start of study	• Anatase • 25 nm (indicated by supplier , no further details)	*NOAEL: 10 mg/kg bw/d*	Sperm abnormalities ↑ Testosterone levels in serum ↓ Vacuoles in seminiferous tubules	Jia et al., 2014
• 5 days • Sprague Dawley rats, f + m • 7 animals/sex/dose • 0; 1; 2 mg/kg bw/day • Intragastric in water	• Anatase • Two different morphologies in tested material: o Spherical NPs with primary size 20-60 nm o Irregular NPs with primary size 40-60 nm • Mostly present as large agglomerates (mean diameter 1.6 µm) • BET surface area 45-55 m^2/g	*LOAEL: 1 mg/kg bw/day*	Histopathological effects in thyroid, adrenal and ovary Testosterone in serum ♂↑ Testosterone in serum ♀ ↓ Triiodothyronine (T_3) in serum ♂ ↓ → thyroid system disrupted	Tassinari et al., 2014
• 14 days • Swiss albino mice, males only • 5 animal/dose • 0; 10; 50; 100 mg/kg bw/d • Oral in Milli-Q water	• Anatase • 2-50 nm primary size • Hydrodynamic diameter varied per dose: 281, 294 and 301 nm, respectively	*LOAEL: 10 mg/kg bw/d*	ALT and ALP ↑→ liver damage Olive tail moment (comet assay) ↑ → genotoxicity	Shukla et al., 2014

TiO_2 particle concentration in post-mortem human liver and spleen*

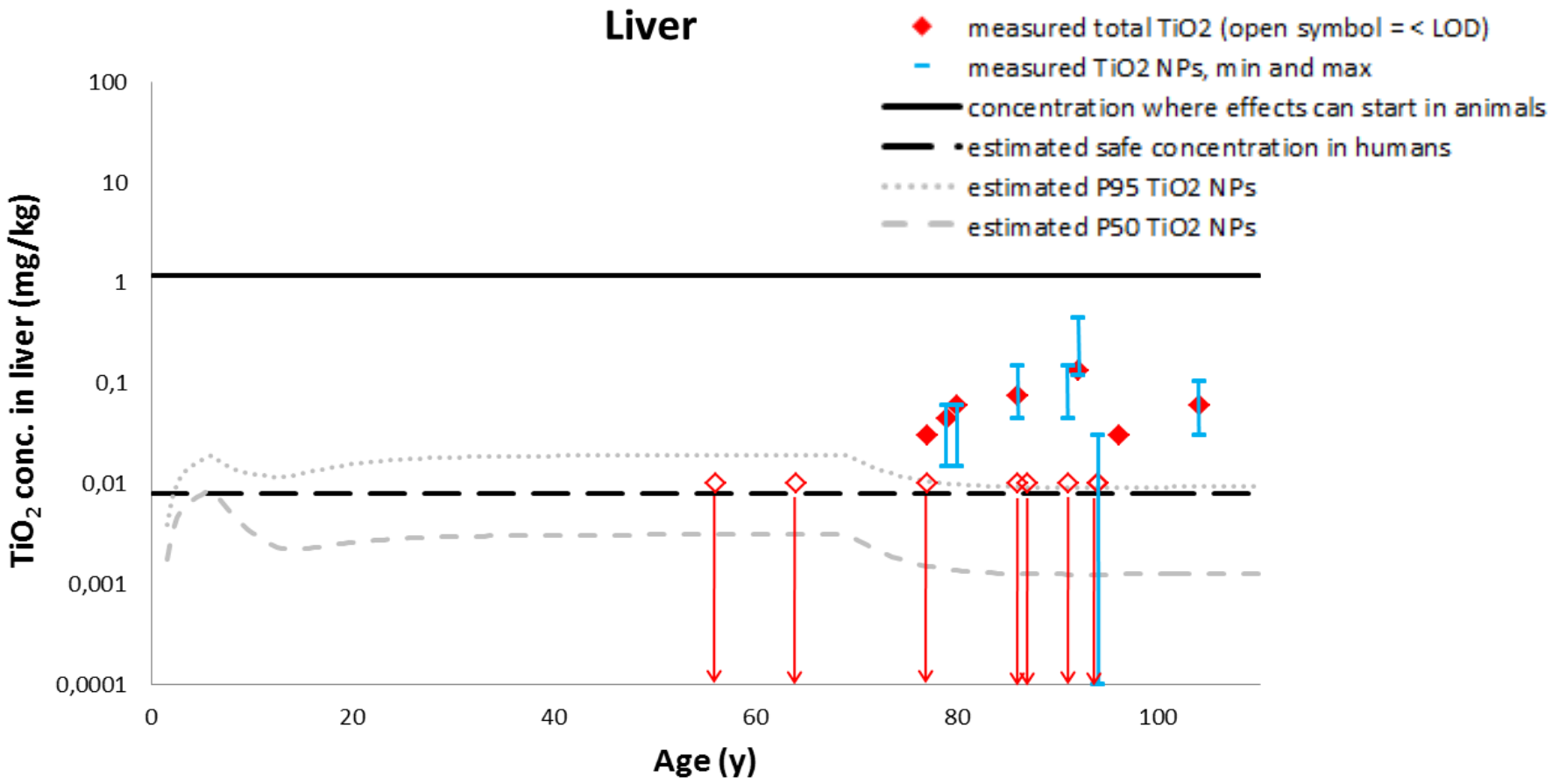
- 15 post-mortem liver and spleen
  - Recorded age, gender, ethnicity
- Analysis
  - weighed and minced
  - acid digestion of a minced sample, total Ti measured with ICP-HRMS
  - Depolymerisation and protein digestion, measurement of TiO_2 particles with sp-ICP-HRMS
  - SEM-EDX analysis of minced piece
- Method for Ti and TiO_2 analysis validated (by RIKILT)

 * Heringa et al. Part Fibre Toxicol. 2018 15(1):15

Results: levels and risk assessment

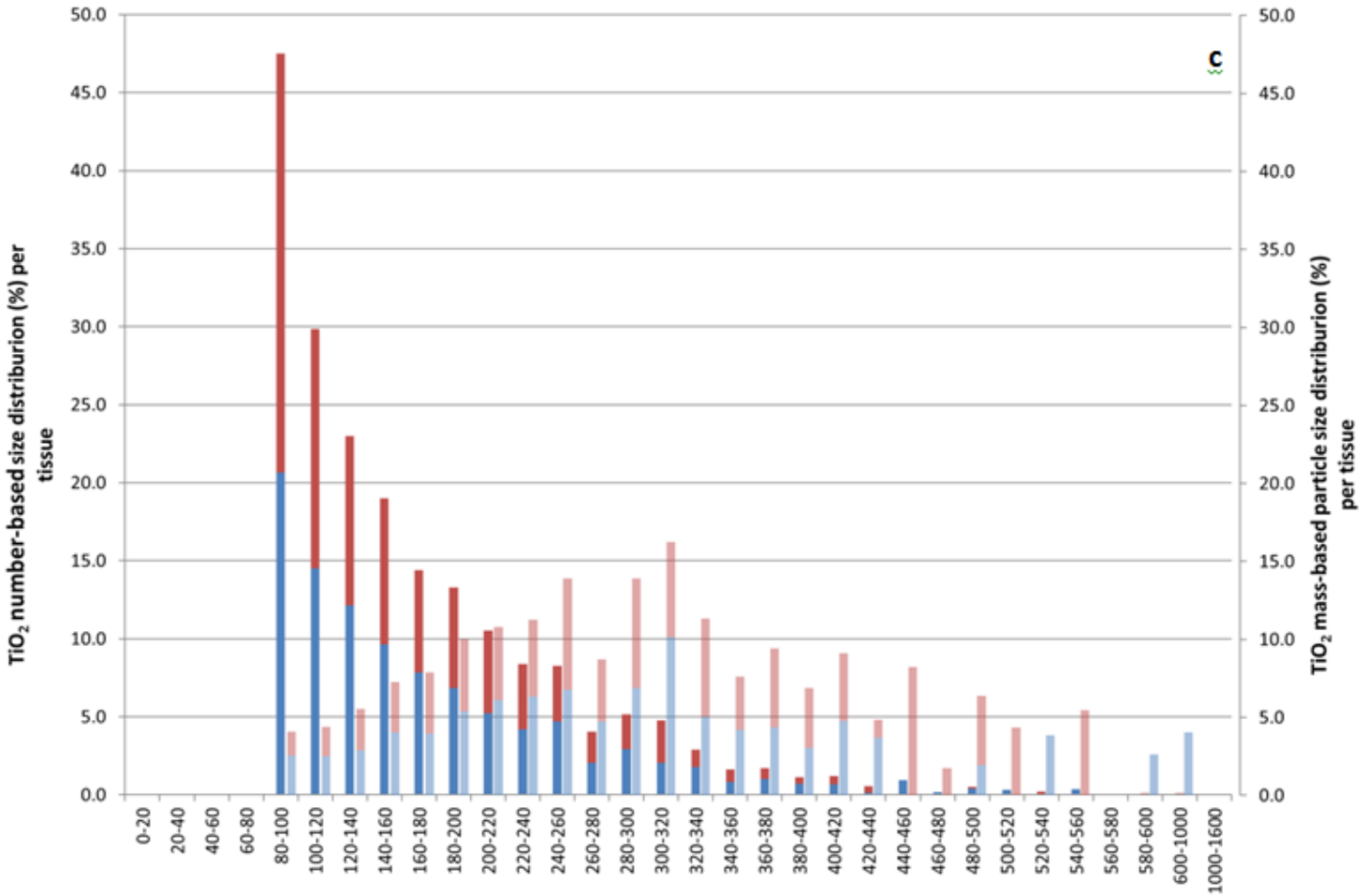


Comparison to model predictions

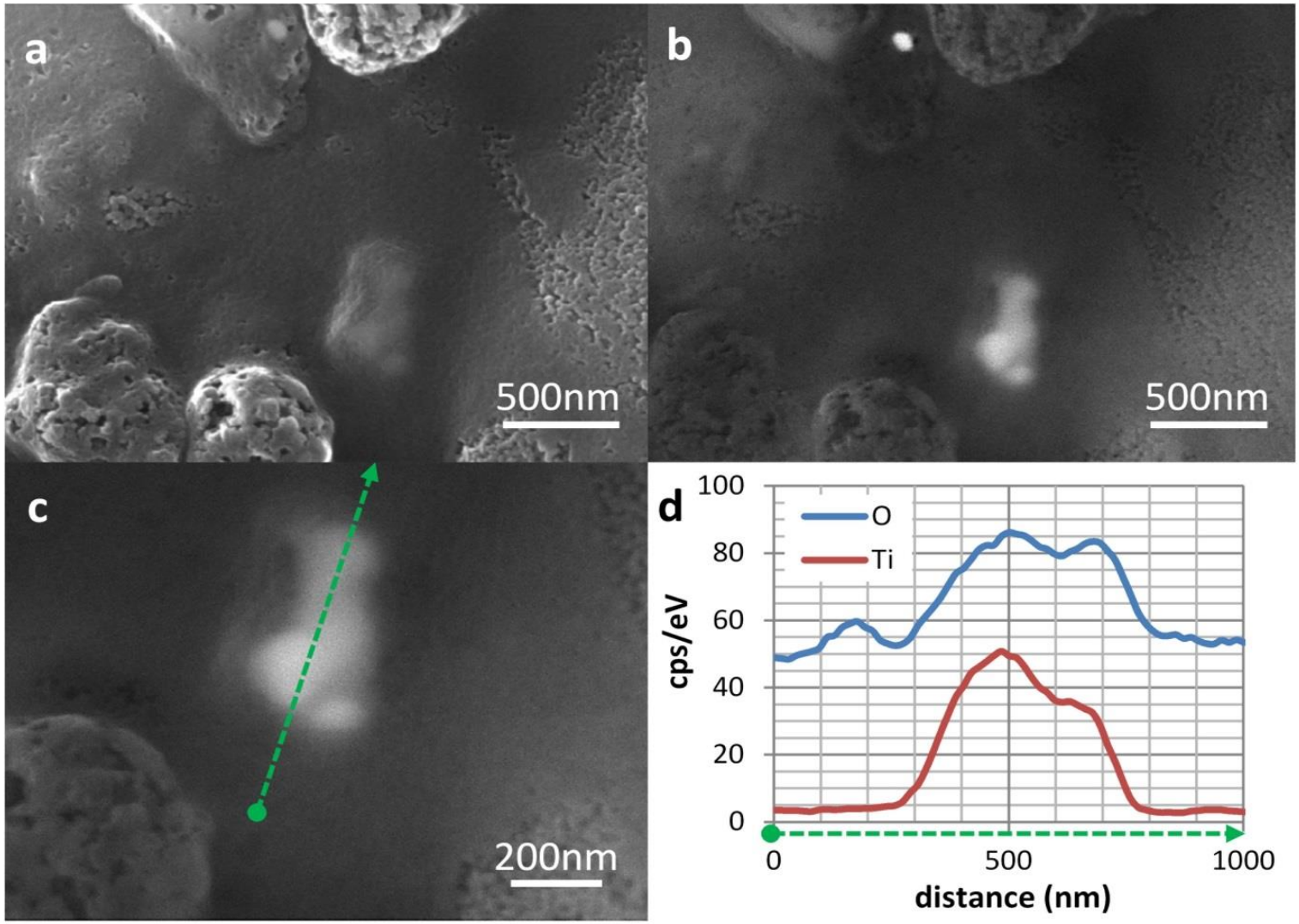


Particle size distribution
number-based: left axis and dark colours
mass-based: right axis and light colours

Liver: red bars
Spleen: blue bars



≥24% of nanosize (<100 nm), but may be underestimation as LOD_{size} is 85 nm


a
500nm
b
500nm
c
200nm
d
100
80
60
40
20
0
cps/eV
O
Ti
0
500
1000
distance (nm)

Discussion TiO_2 in human liver and spleen

- Still may miss particles <85 nm
- Size range particles in organs (i.e. 86-421 and 88-445 nm) falls within that of particles in food products (30-600 nm diameter)
  - Particles can be single nanoparticles as well as aggregates/agglomerates
  - ≥24% of particles nanosize (< 100 nm)
- It can be assumed that these levels come from oral exposure
- Organ levels not far off from predicted levels
  - Measured levels slightly higher than predicted for liver

Conclusions

TiO_2 (nano)particles are present in human liver and spleen

The liver Ti/TiO_2 concentrations in humans are below the liver concentrations related to adverse effects in toxicity studies. However, the MoE_i is limited.

If adverse (liver) effects occur due to exposure to TiO_2 remains unclear.

Conclusion/Recommendations

- Risk assessment should be based on internal concentrations
- Further chronic oral toxicity studies with TiO_2 as used in food are needed
  - Include assessment on tissue concentrations
  - Include markers of liver damage and liver pathology
  - Apply benchmark dose approach to get a good dose-response curve which includes both lower dose groups that are more representative for human exposure as well as higher dose groups
    - Small particles may agglomerate at higher doses leading to a decrease in the fraction absorbed.
  - Consider that TiO_2 in E171 can be present in different 'forms' and that the risk assessment of E171 should cover all
    - Anatase, rutile, mixture, coated with silica or alumina, different size distribution

Acknowledgement

- RIVM: Minne Heringa, Cathy Rompelberg, Jan van Eijkeren, Walter Brand, Petra van Kesteren, Liesbeth Geraets, Rob Vandebriel, Wim de Jong, Gerda van Donkersgoed, José Drijvers, Agnes Roos, Susanne Westenbrink, Greet van Bemmel, Agnes Oomen
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