Willkommen
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NanoScreen: Assessing the impact of SAS particles on advanced *in vitro* intestine models

Particles & Health 2021, October 20 – 21, 2021, Paris France

Dr. Peter Wick, Head Particles – Biology Interactions, Empa

Disclaimer

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- The study was performed within the frame of CCMX Materials Challenge Nanoscreen and was co-financed by Evonik

Nanomaterials as food additives and in packaging materials

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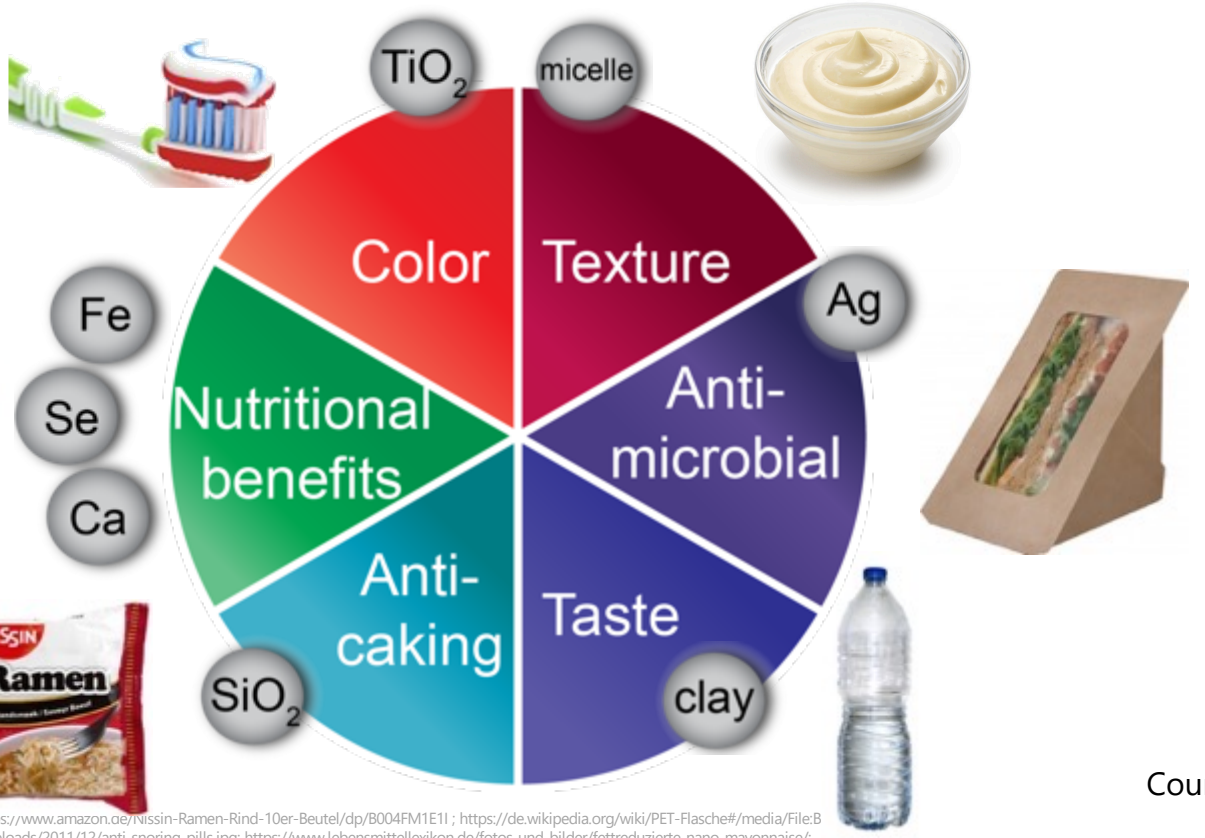

Courtesy of C. Hempt

https://de.wikipedia.org/wiki/Kaugummi ; https://www.amazon.de/Nissin-Ramen-Rind-10er-Beutel/dp/B004FM1E1I ; https://de.wikipedia.org/wiki/PET-Flasche#/media/File:B
http://mysnoringsolutions.info/wp-content/uploads/2011/12/anti-snoring-pills.jpg; https://www.lebensmittellexikon.de/fotos-und-bilder/fettreduzierte-nano-mayonnaise/;
http://www.redorbit.com/news/space/1113218472/toothpaste-fluorine-may-come-from-space-082314/

Debate of nano-enabled food additives re-launched

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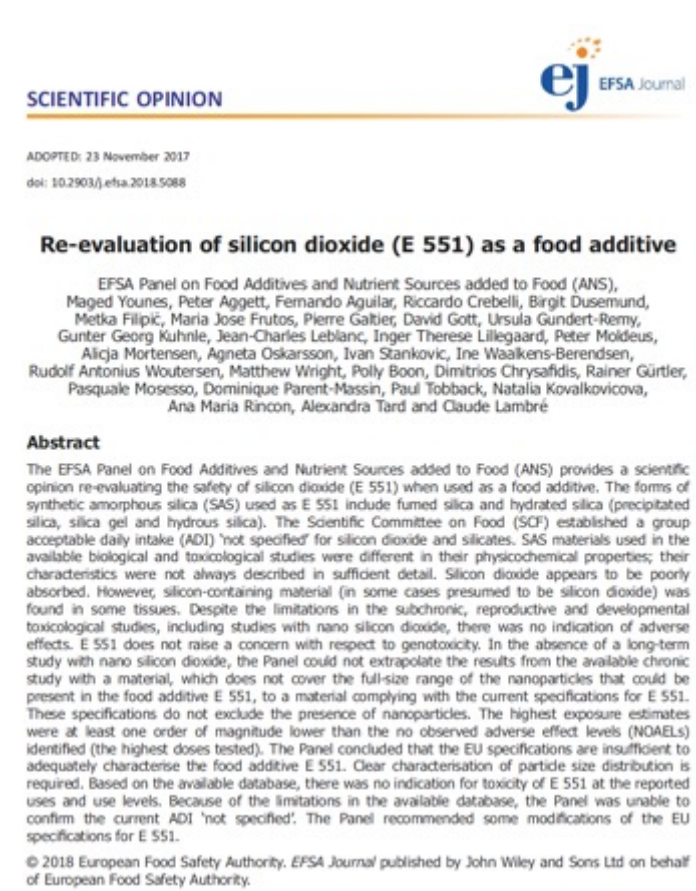

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 November 2017

doi: 10.2903/j.efsa.2018.5088

Re-evaluation of silicon dioxide (E 551) as a food additive

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Maged Younes, Peter Aggett, Fernando Aguilar, Riccardo Crebelli, Birgit Dusemund, Metka Filipič, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Gunter Georg Kuhnle, Jean-Charles Leblanc, Inger Therese Lillegaard, Peter Moldeus, Alicja Mortensen, Agneta Oskarsson, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright, Polly Boon, Dimitrios Chrysafidis, Rainer Gürtler, Pasquale Mosesso, Dominique Parent-Massin, Paul Tobback, Natalia Kovalkovicova, Ana Maria Rincon, Alexandra Tard and Claude Lambré

Abstract

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of silicon dioxide (E 551) when used as a food additive. The forms of synthetic amorphous silica (SAS) used as E 551 include fumed silica and hydrated silica (precipitated silica, silica gel and hydrous silica). The Scientific Committee on Food (SCF) established a group acceptable daily intake (ADI) 'not specified' for silicon dioxide and silicates. SAS materials used in the available biological and toxicological studies were different in their physicochemical properties; their characteristics were not always described in sufficient detail. Silicon dioxide appears to be poorly absorbed. However, silicon-containing material (in some cases presumed to be silicon dioxide) was found in some tissues. Despite the limitations in the subchronic, reproductive and developmental toxicological studies, including studies with nano silicon dioxide, there was no indication of adverse effects. E 551 does not raise a concern with respect to genotoxicity. In the absence of a long-term study with nano silicon dioxide, the Panel could not extrapolate the results from the available chronic study with a material, which does not cover the full-size range of the nanoparticles that could be present in the food additive E 551, to a material complying with the current specifications for E 551. These specifications do not exclude the presence of nanoparticles. The highest exposure estimates were at least one order of magnitude lower than the no observed adverse effect levels (NOAELs) identified (the highest doses tested). The Panel concluded that the EU specifications are insufficient to adequately characterise the food additive E 551. Clear characterisation of particle size distribution is required. Based on the available database, there was no indication for toxicity of E 551 at the reported uses and use levels. Because of the limitations in the available database, the Panel was unable to confirm the current ADI 'not specified'. The Panel recommended some modifications of the EU specifications for E 551.

Aim:
find a structure – activity relationship between differently produced SAS particles and their bioresponses using an adv human GI *in vitro* model

Set of synthetic amorphous silica particles (SAS)

Material	Code[I]	Production process	Primary structure size/ nm[II]	Aggregate size (ECD)/nm[II]	Specific surface area/(m^2/g)[III]	Material density/ (g/cm^3)[IV]	Agglomerate effective density/(g/cm^3)[V]	Agglomerate size/ nm[VI]	Point of zero charge/pH[VII]	Zeta potential/ mV[VIII]
SIPERNAT® 350		precipitated	30.3 ± 6.8	276.3	55	2.15	1.33	623 ± 15	1.7	n.a.
SIPERNAT® 22	P-5	precipitated	10 ± 2.6	82.2	180	2.12	1.65	325 ± 37	1.7	−37.1
SIPERNAT® 22 S		precipitated	10 ± 2.6	82.2	185	2.14	1.20	743 ± 38	2.0	−37.1
SIPERNAT® 2200		precipitated	9 ± 1.4	44.9	185	2.12	1.25	324 ± 13	1.5	n.a.
SIPERNAT® 160	P-1	precipitated	12.2 ± 2.7	58.3	180	2.16	1.18	555 ± 32	1.7	−41.2
SIPERNAT® 50	P-2	precipitated	3.1 ± 0.7	59.8	460	2.12	1.15	645 ± 79	1.7	−13.7
SIPERNAT® 50 S		precipitated	3.1 ± 0.7	59.8	460	2.12	1.16	747 ± 86	1.7	−13.7
AEROSIL® OX50	F-1	fumed	41.4 ± 18.3	233.7	45	2.32	1.25	299 ± 6	2.3	−34.0
AEROSIL® 200 Pharma		fumed	7.8 ± 1.8	161.1	210	2.38	1.07	294 ± 5	2.3	−11.8
AEROSIL® 380 F	F-3	fumed	8 ± 2.7	101.9	390	2.29	1.09	318 ± 15	2.4	−10.6

Dispersion protocol:

Stock dispersions of SAS (10mg/ml) were prepared in double distilled water (ddH2O) using an ULTRA-TURRAX T25 at 14’600 rpm for 1 min, which results in a particle size distribution as expected in food matrix

Dilutions of the stock dispersions were applied in protein containing cell culture medium to cell cultures

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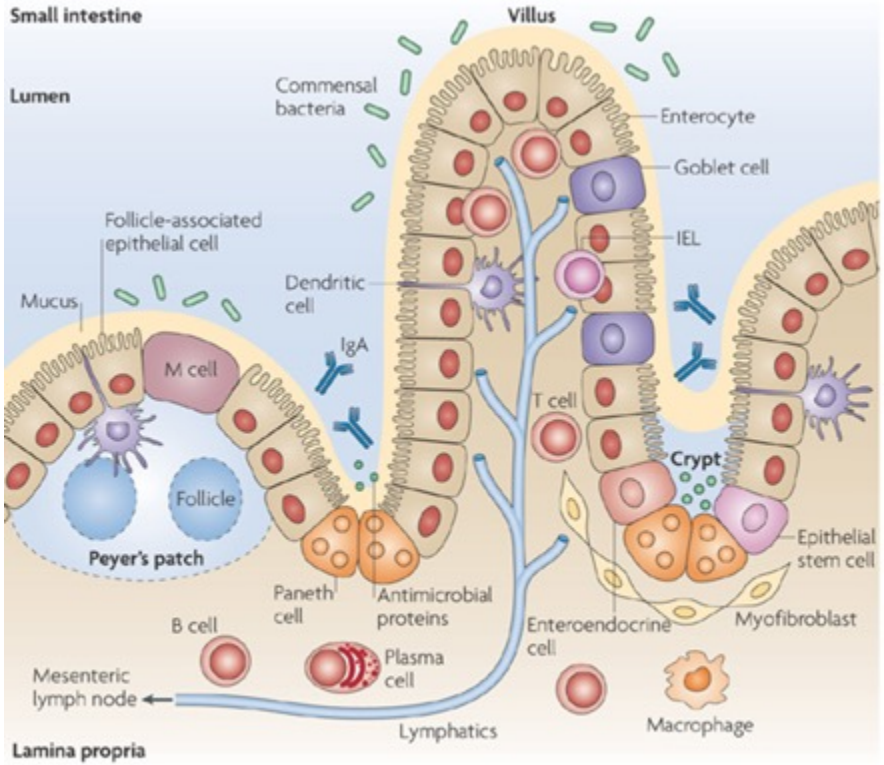
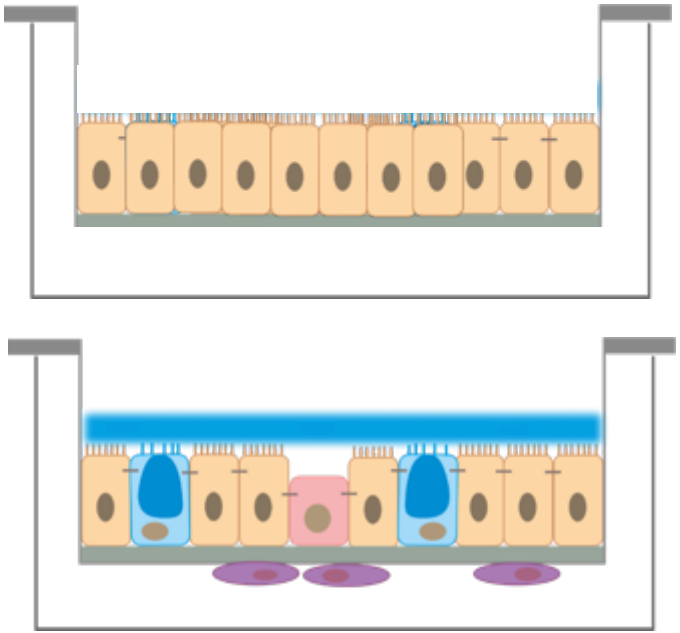
In vitro co-culture model applied



Modified after Abreu M.T. et al., Nature Reviews Immunology, 2010



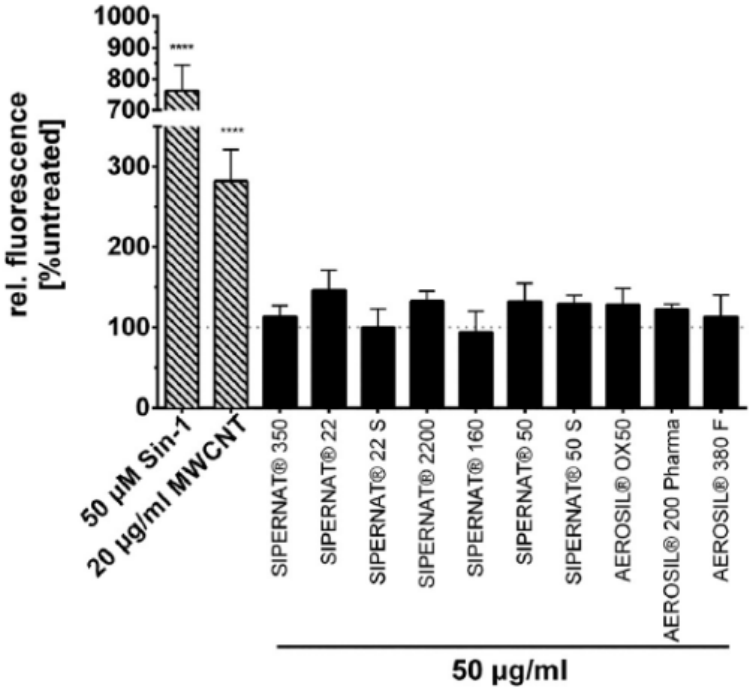
Enterocytes

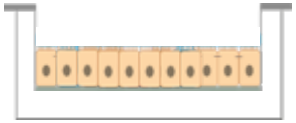
B-lymphocytes

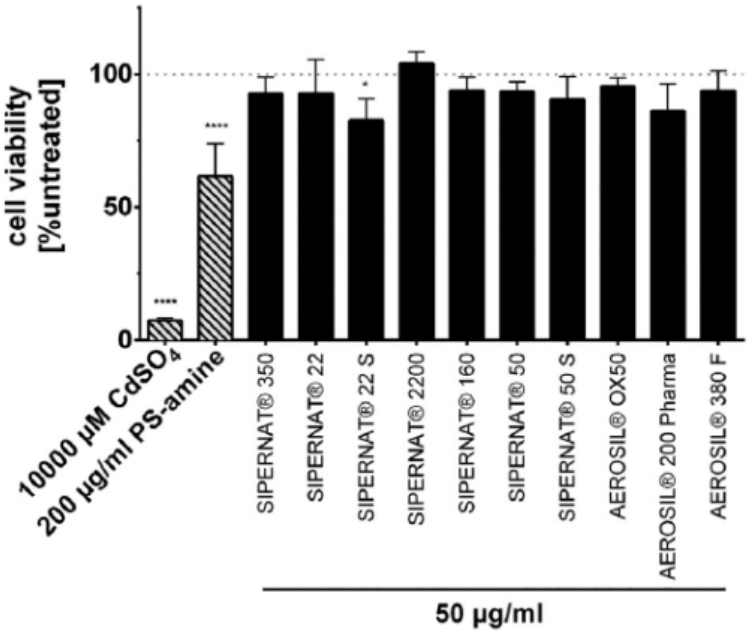
Goblet cells

M-cells

The 10 different SAS particles do not induce any acute cytotoxicity effects…







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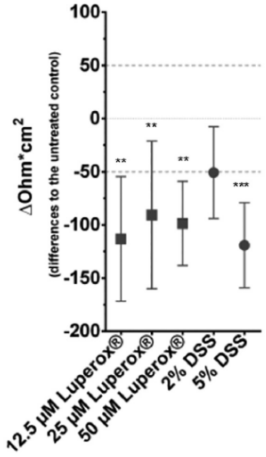
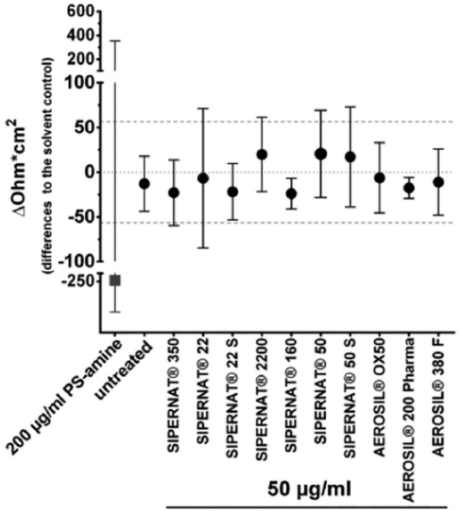
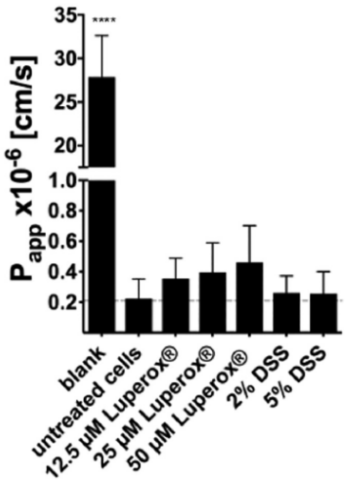
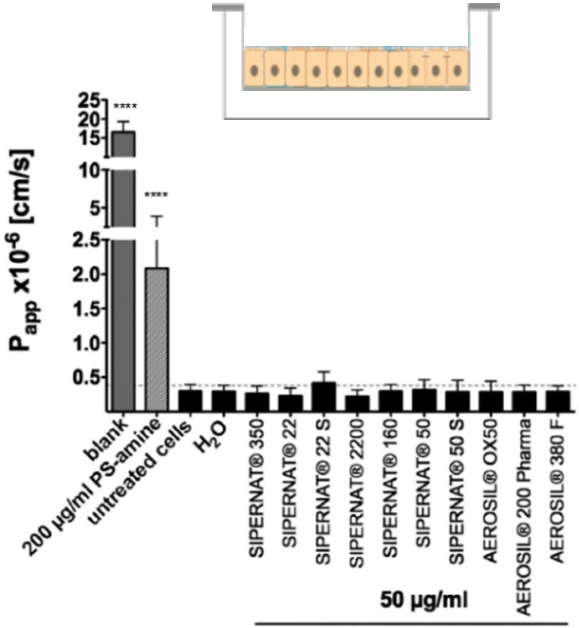
… nor an acute effect on the barrier function









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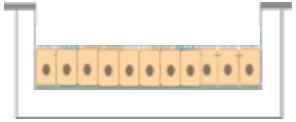
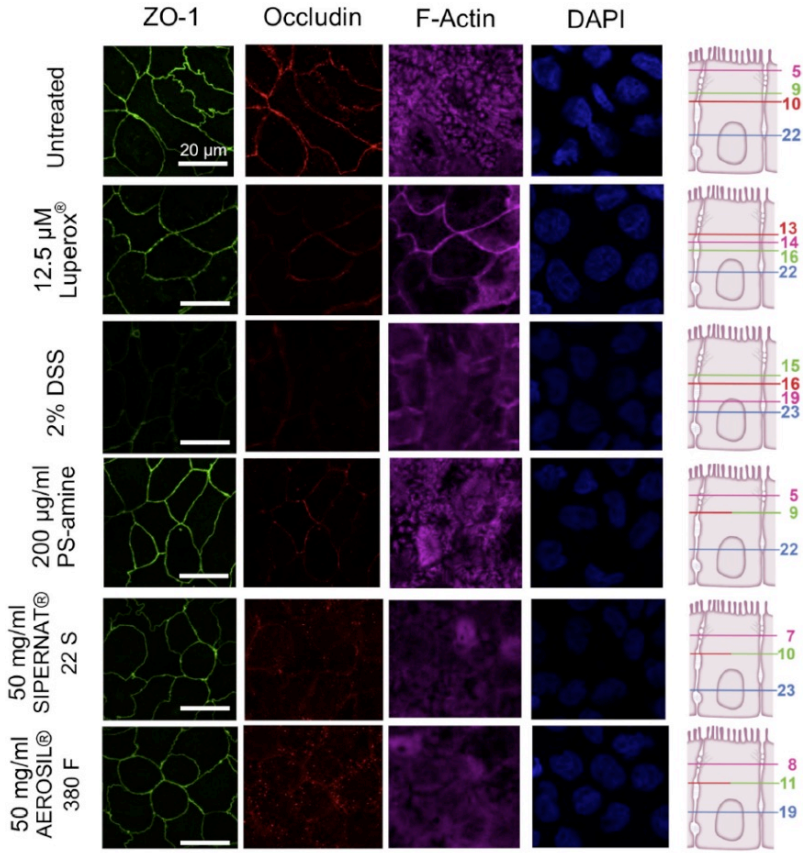
No visible changes of the enterocyte morphology and layer integrity





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Analysis of sub-toxic events and influence on barrier functions

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Material	Production process	Primary structure size/ nm[I]	Aggregate size (ECD)/ nm[II]	Specific surface area/ (m^2/g)[III]	Material density/ (g/cm^3)[IV]	Agglomerate effective density/ (g/cm^3)[V]	Agglomerate size/ nm[VI]	Point of zero charge/ pH[VII]	Zeta potential/ mV[VIII]
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AEROSIL® 380 F	fumed	8 ± 2.7	101.9	390	2.29	1.09	318 ± 15	2.4	-10.6

Quadrupel GI *in vitro* model

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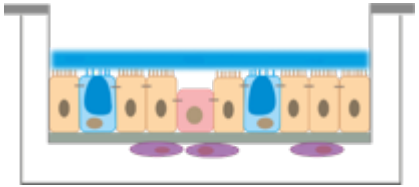
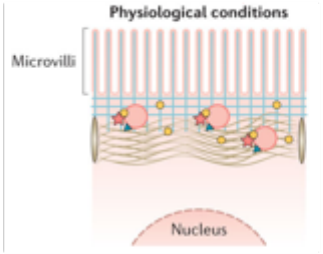


Microvilli function

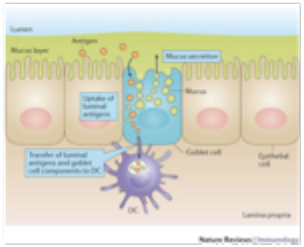
Alkaline phosphatase activity



Delacour D. et al., 2016
doi:10.1038/nrgastro.2016.5

Mucus coverage

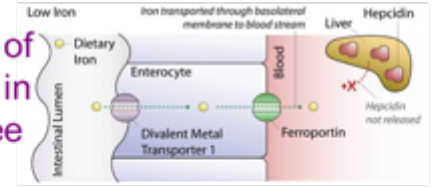
Alcian blue staining



Johansson M. & Hansson G., 2016
doi:10.1038/nri.2016.88

Iron uptake

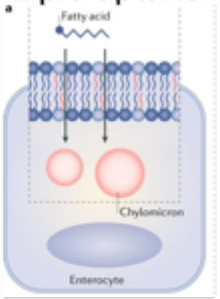
Uptake of Ferritin in iron free liquid



Naigamwalla D.Z., Webb J.A., and Giger U, 2012., PMC3280776

Lipid uptake

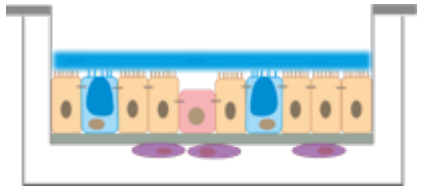
Uptake of fluores-cent model Lipid C_{12}



Wang B. and Tontonoz P., 2018
doi.:10.1038s41574-018-0037-x

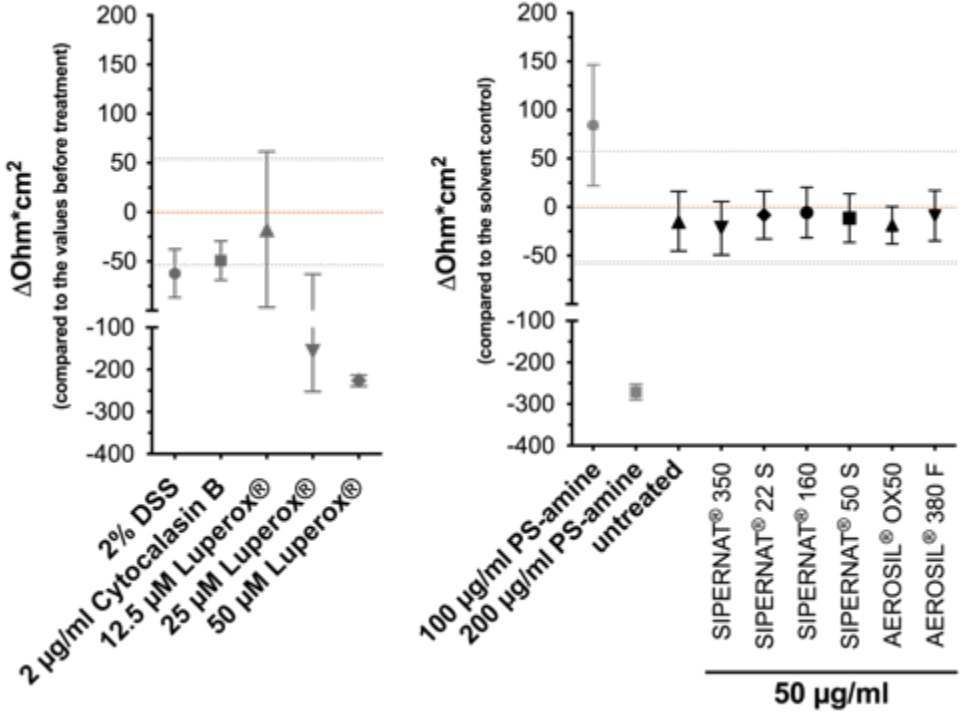
pNPP: para-Nitrophenylphosphate; ALPL: alkaline phosphatase; SI: sucrase-isomaltase;MUC1: Mucus protein 1; MUC5AC: mucus protein 5AC; DTM1: solute carrier family 11 member 2; TfRC: transferrin receptor; HFE: homeostatic iron regulator; FPN: solute carrier family 40 member 1; GPR40: free fatty acid receptor 1; GPR120: free fatty acid receptor 4

No effect on barrier integritiy



After 1 h

After 48 h



SAS N=6; PS-amine N=2-4

DSS: Dextran sulfate sodium
Luperox®: tert-Butyl hydroperoxide solution
TEER: Transepithelial electrical resistance

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No acute effects on micro villi function

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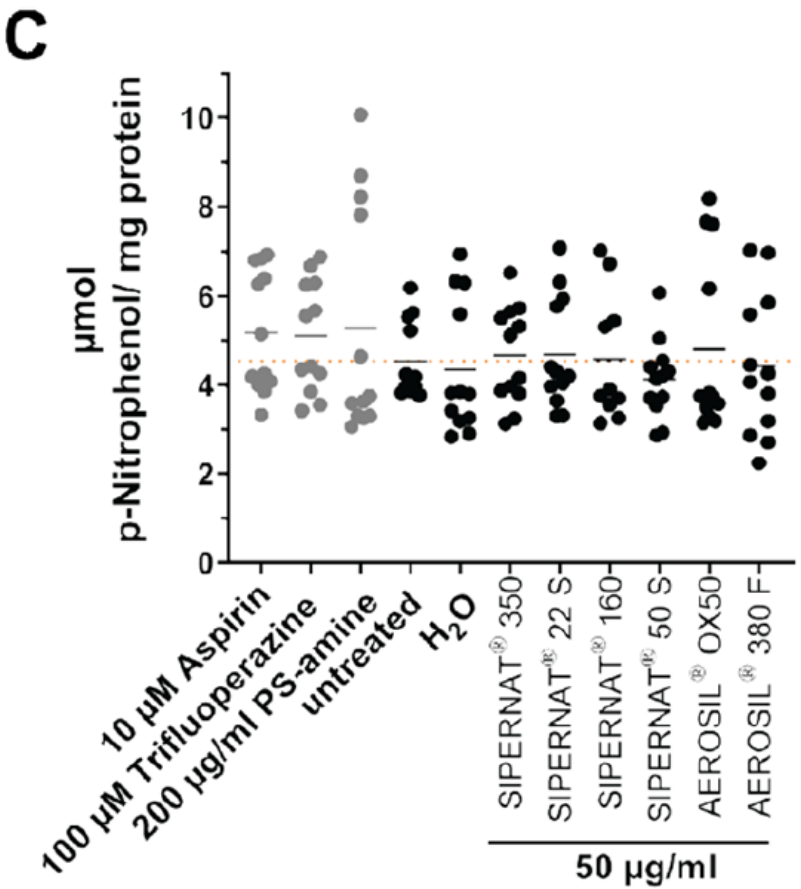

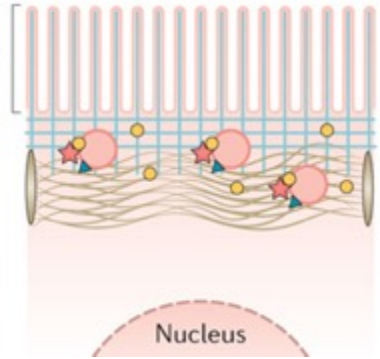

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No SAS influence on lipid uptake

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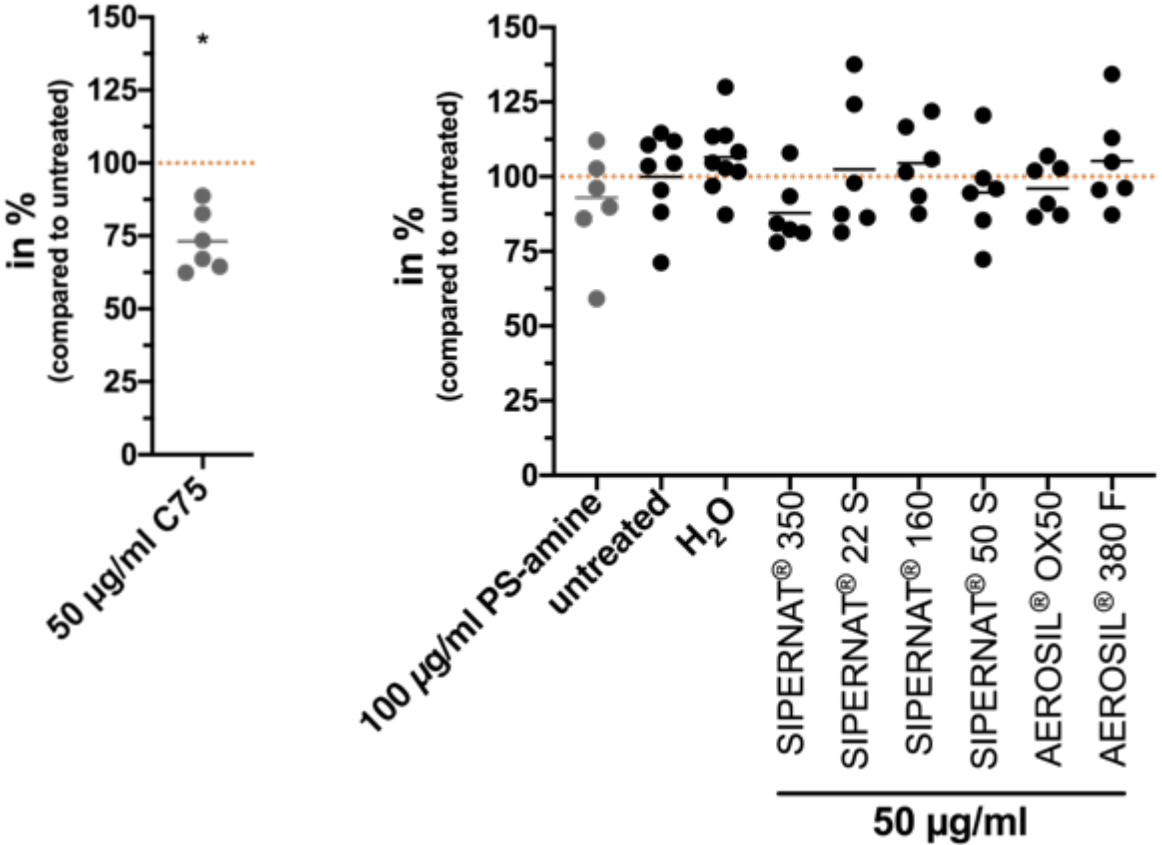

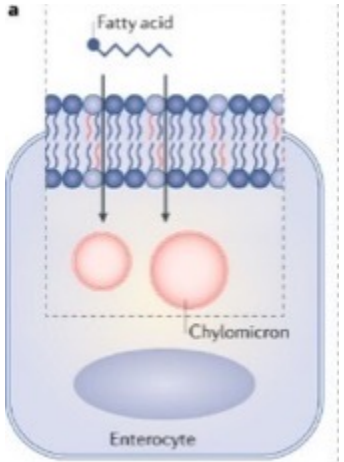

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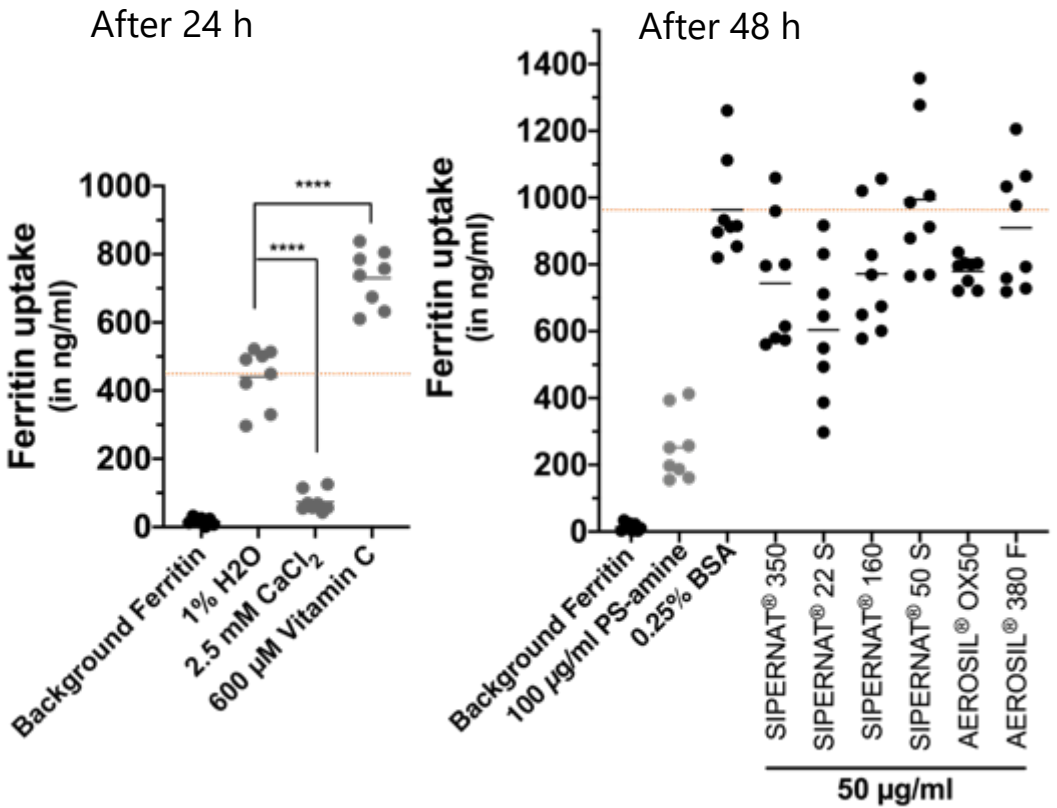
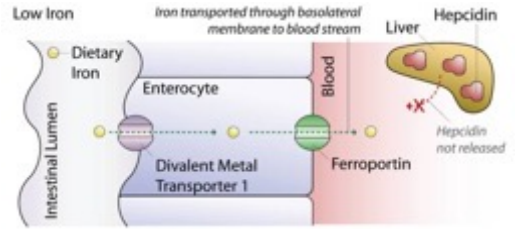
SAS influence on iron uptake





No release of (pro-)inflammatory factors

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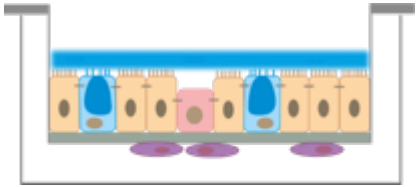

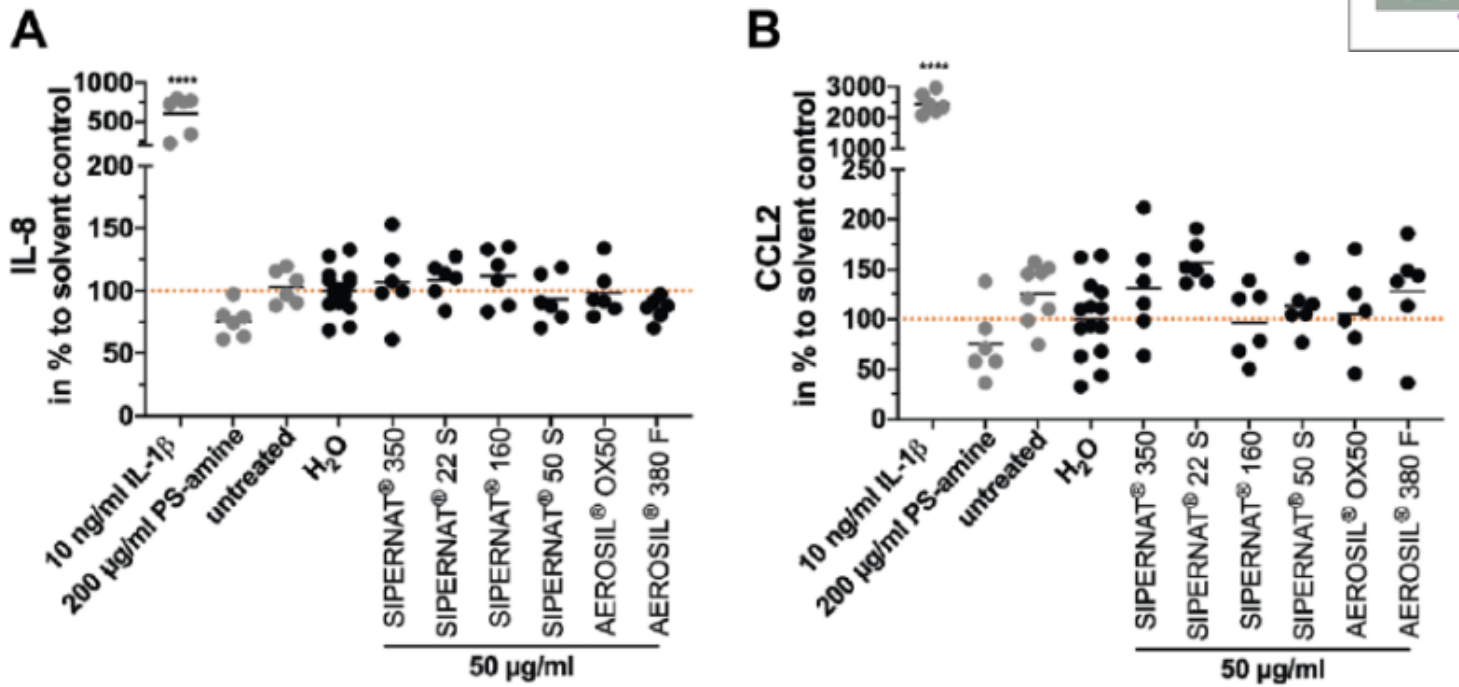

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Summary

Endpoint		Material: SIPERNAT® 350	SIPERNAT® 22 S	SIPERNAT® 160	SIPERNAT® 50 S	AEROSIL® OX50	AEROSIL® 380 F	PS-amine
Viability								
TEER								↓
inflammation (IL-8)	cytokine							
	mRNA							
mucus	function							
	mRNA			↓				↓
microvilli	function (ALP)							
	mRNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
lipid	function							
	mRNA	↑						
iron	function	↓	↓					↓
	mRNA							↓

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Conclusion

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- Despite considerable differences in production process, specific surface area or silanol content, SAS neither induced severe acute cytotoxicity nor had an effect on barrier integrity and functionality.
- The mucus acts somehow as a protective layer with additional barrier properties, seen by the reduced cytotoxicity of our pos. controls
- The role of the mucus layer on nanomaterial interactions with the intestinal barrier should be investigated in more detail

Thank you for your attention

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ETH zürich

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Mirjam Schneider

INM

Annette Kraegeloh
Carmen Schmitz
Jana Fleddermann
Isabella Tavernaro

HZI HELMHOLTZ Centre for Infection Research

Claus-Michael Lehr

EPFL

Heinrich Hofmann

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Fonds National Suisse
Schweizerischer Nationalfonds
Fondo Nazionale Svizzero
Swiss National Science Foundation

CCMX
Competence Centre for Materials Science and Technology

Bundesamt für Umwelt, Wald und Landschaft BUWAL

Schweizerische Herzstiftung

Chancen und Risiken von Nanomaterialien
Nationales Forschungsprogramm NFP 64

KTI/CTI

Bundesamt für Gesundheit

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