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Erbil Training Event, 2nd May 2019

Food Safety and the Uptake of Nanomaterials Across the Gut

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Aims

- Outline how food safety is managed from “farm to fork”.
- Applications of nanotechnology in agriculture & fisheries, food production and drink.
- Examples of research to underpin nanosafety in the food chain.
- Research on the uptake mechanism across the gut.
- Responsible innovation of nanotechnology in the agri-food sector.

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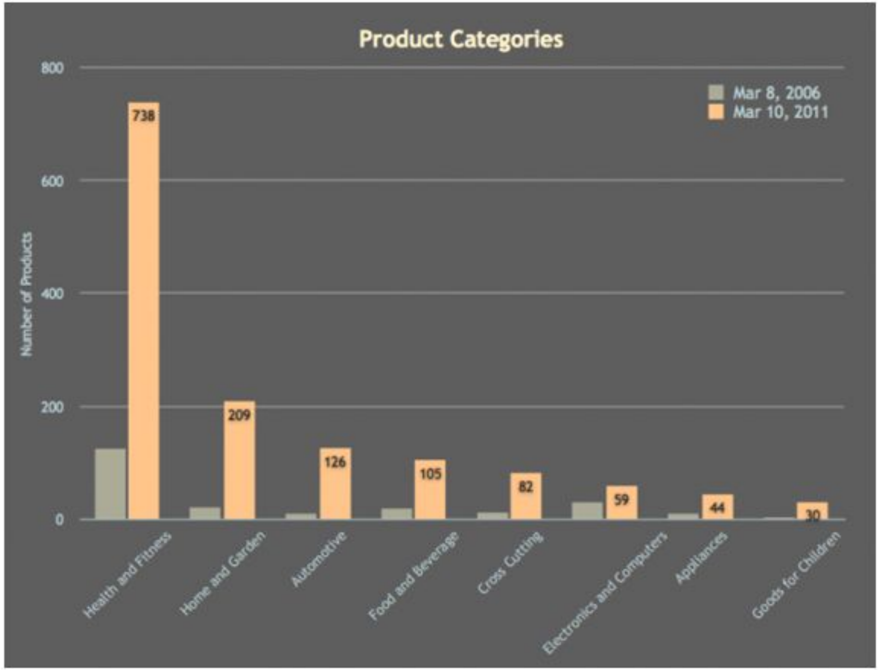
- Responsible innovation of nanotechnology in the agri-food sector.

Nano Inventory Product Types

http://www.nanotechproject.org/inventories/consumer/analysis_draft/



1317 different products on the market in 2011, 105 in food and drink

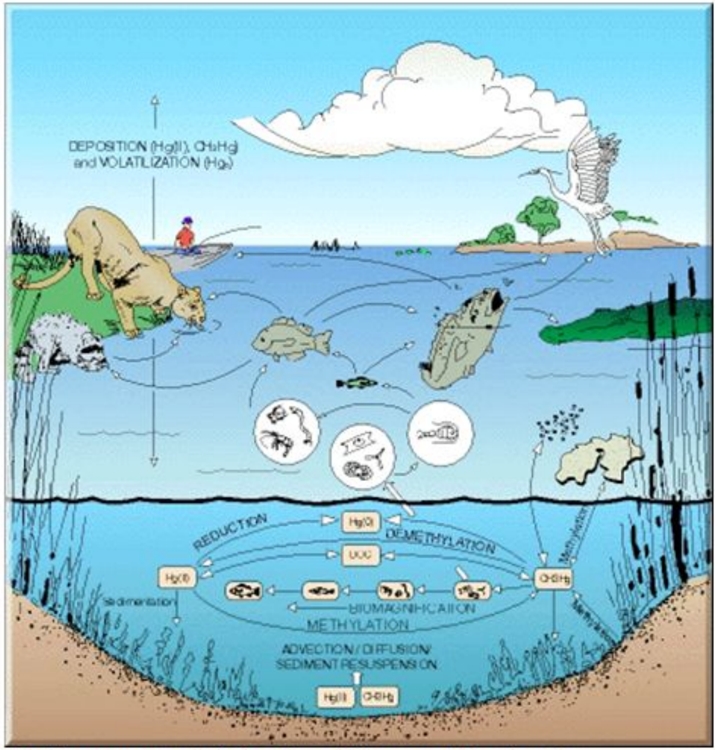
Safety Testing of Nanomaterials

Safe for the Environment

Safe for Human Health

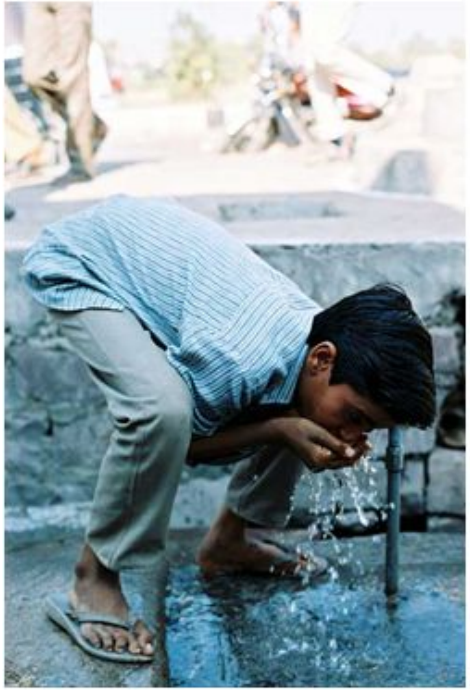
The Gut and Pollutant Exposure via Food Chains

- Chemicals get into the environment.
- Into the food chain.
- Risk to humans



http://sofia.usgs.gov/publications/fs/166-96/fig1.html

Ingestion: Food, Water, Soil.



Approaches to Food Safety

- Health surveillance (food poisoning)
- Quality standards and shelf life of foods
- Ensuring the human food chain
  - Animal feed quality
  - Animal health
  - Soil guidelines, contaminant levels in crops.
  - Fish and shellfish, farm animals.
- Bioaccumulation & Biomagnification

Agriculture & Live Stock

- Veterinary Laboratory Agency
- Food Standards Agency

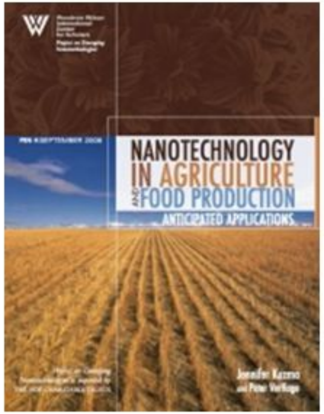
- Fisheries and Aquaculture-DEFRA

FOOD STANDARDS AGENCY

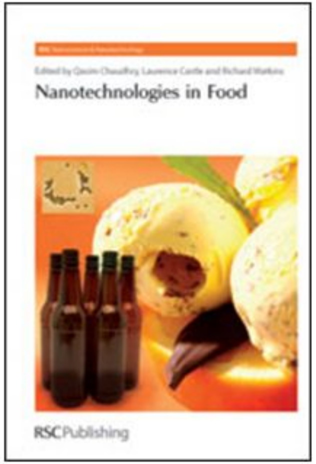
Applications of Nanotechnology in Food

- Health foods
- Mineral and vitamin supplements.
- Agents used in food manufacture
- Encapsulation technology
- Agriculture and food production
- Food packaging and labelling
- Drinking water technology

Reports on Nanotechnology in Food



http://www.nanotechproject.org/publications/archive/nanotechnology_in_agriculture_food/



http://www.rsc.org/shop/books/2010/9780854041695.asp

http://www.nanotechproject.org/publications/archive/nano_food_packaging/

Applications of Nanomaterial in Dentistry

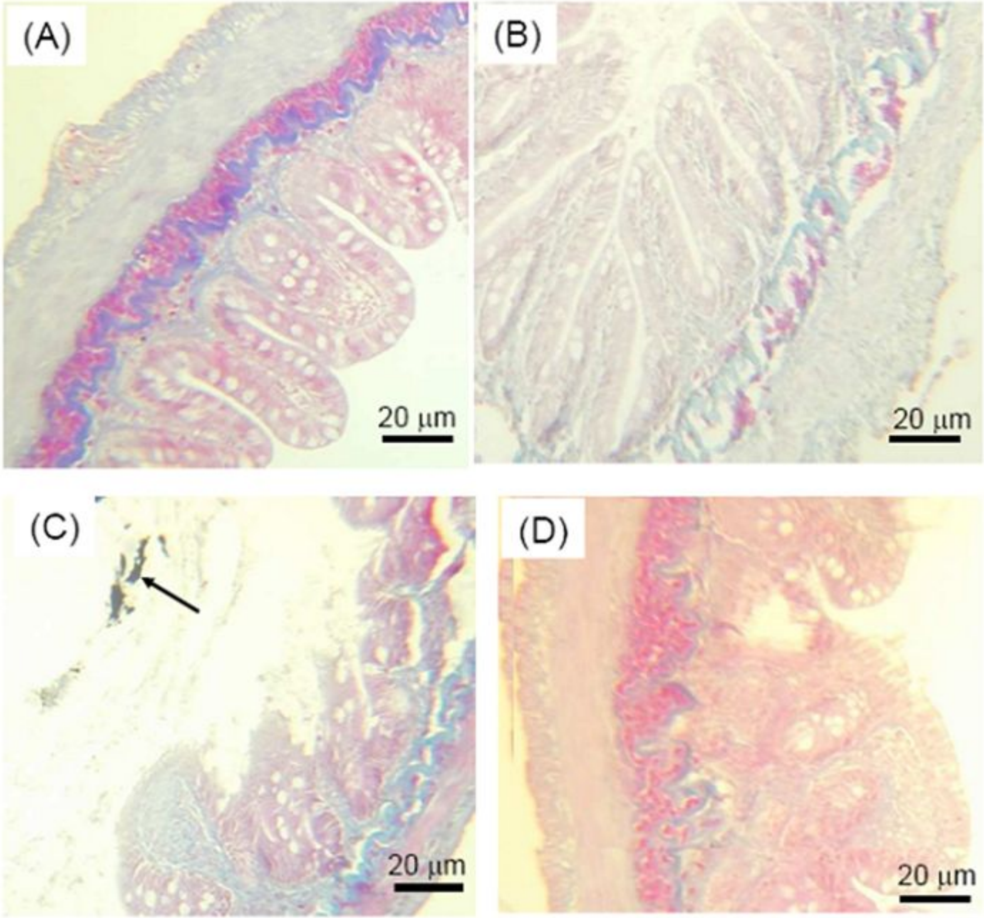
Nanocomposites

Dental implants

Antibacterial – Infection control

Personal health care products

Nutrition, Gut Function and Intestinal Pathology



Erosion of the Intestine in Trout Exposed to SWCNT

Smith et al. (2007) Aquatic Toxicology, 82, 94-109

Morphological Change in Trout Intestine: Waterborne Nano Copper

G.A. Al-Bairuty et al. / Aquatic Toxicology 126 (2013) 104–115

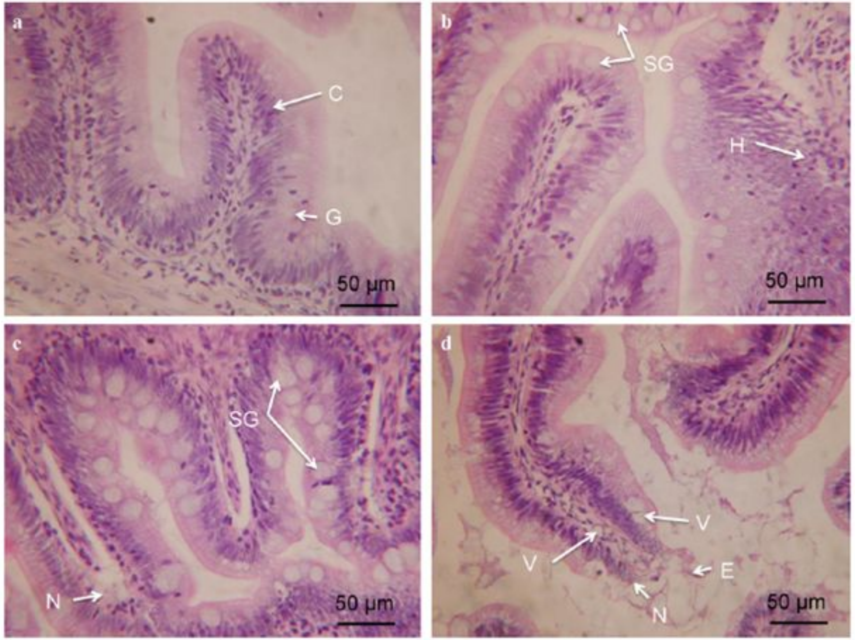
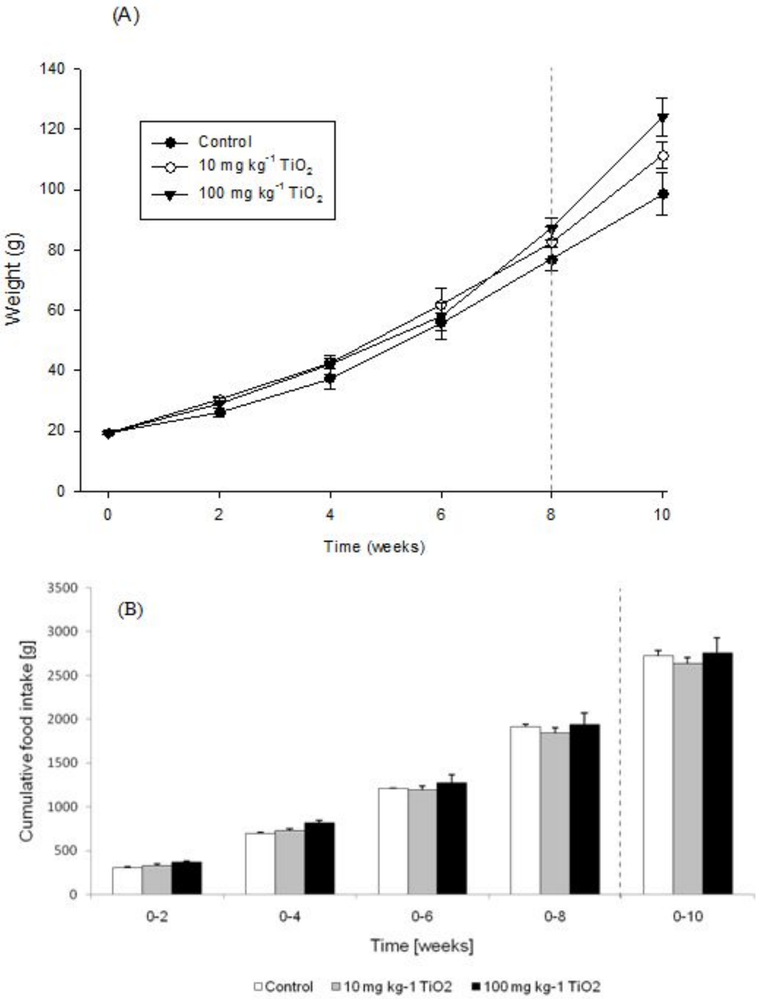


Fig. 2. Gut morphology in rainbow trout following waterborne exposure to (a) control, (b) 20 ug l^{-1} Cu, (c) 20 ug l^{-1} Cu as Cu-NPs, and (d) 100 ug l^{-1} Cu as Cu-NPs for 10 days. Columnar cells (C) and goblet cells (G). Vacuoles in the lamina propria (V), hyperplasia (H), necrosis in the mucosal layer (N), swelling of goblet cells (SG), and erosion of villi (E). Sections were 7 um, (H&E).

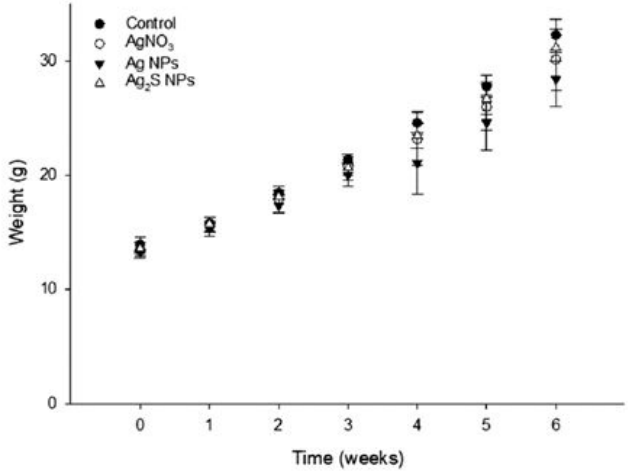


Dietary TiO_2 NPs: Growth & Food Intake in Trout

Ramsden et al. (2009) Ecotoxicology18:939-951.

No statistical differences between treatments (ANOVA, $P > 0.05$)

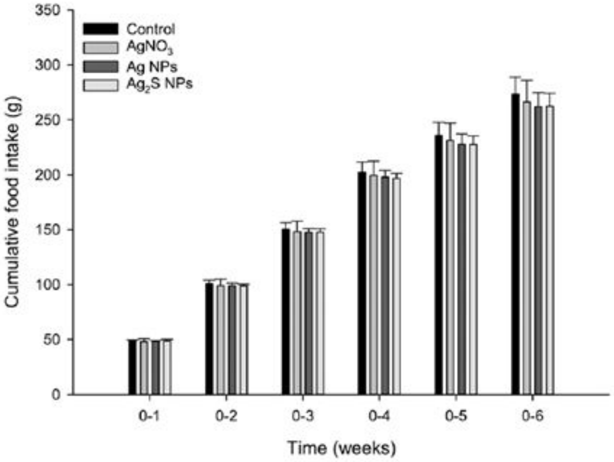
A



Dietary Ag NPs or Ag_2S NPs: Growth & Food Intake in Trout

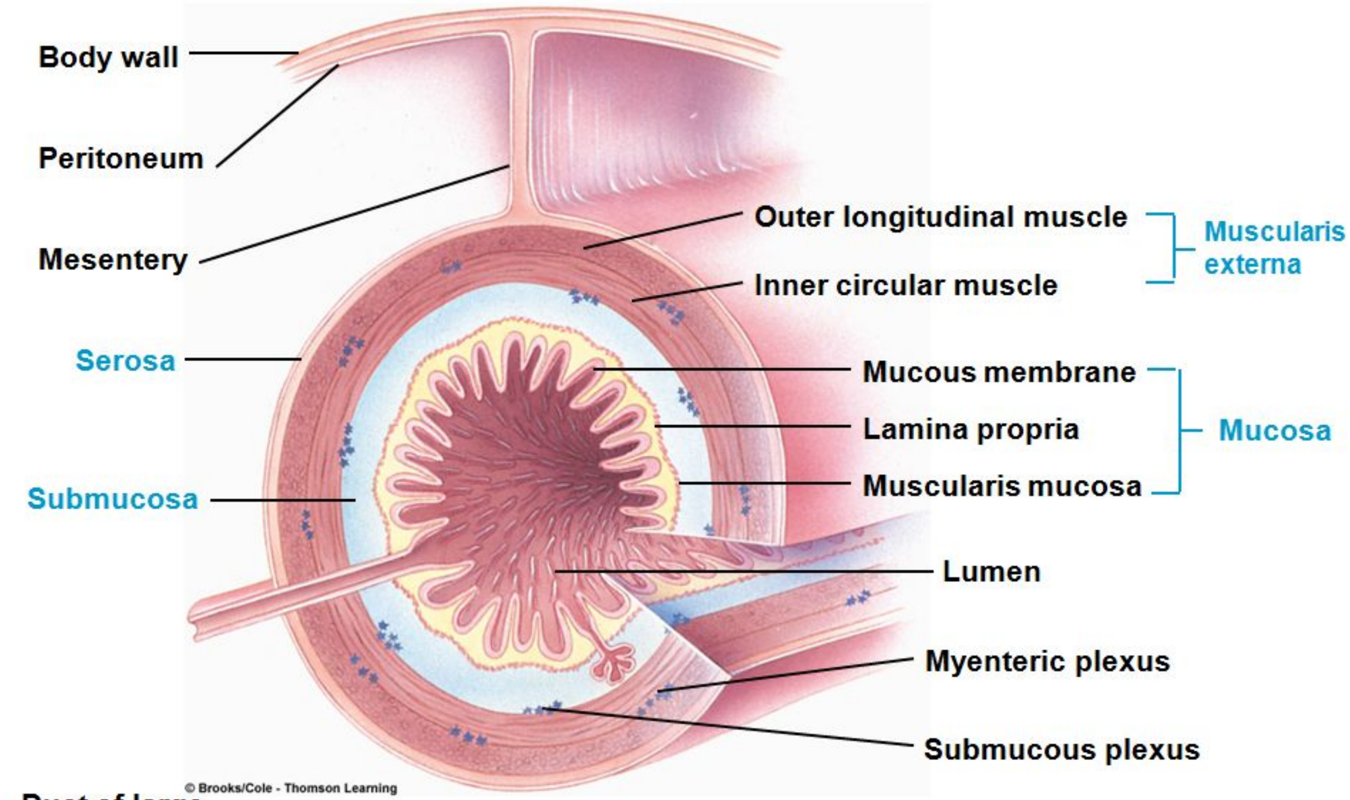
Clark et al. (2019) Environ. Sci. Nano.
DOI: 10.1039/c9en00261h

B



No statistical differences between treatments (ANOVA, $P > 0.05$)

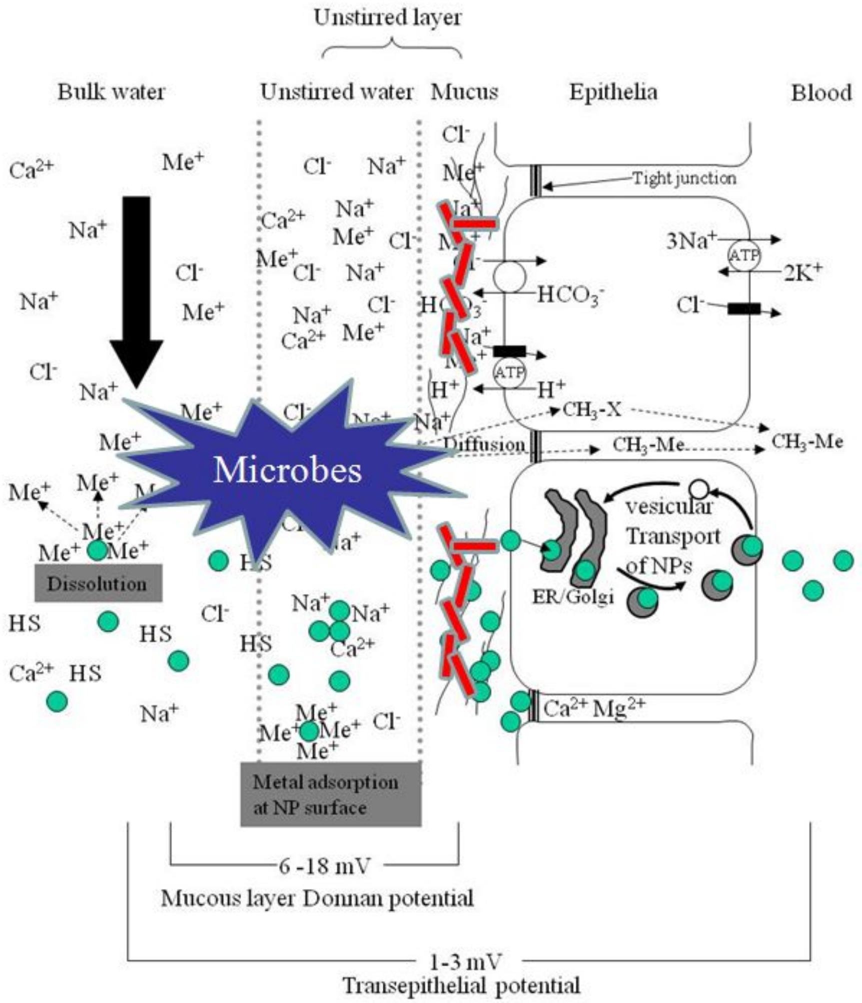
Absorption of Nanomaterials Across the Gut?



Duct of large accessory digestive gland (i.e., liver or pancreas) emptying into digestive tract lumen

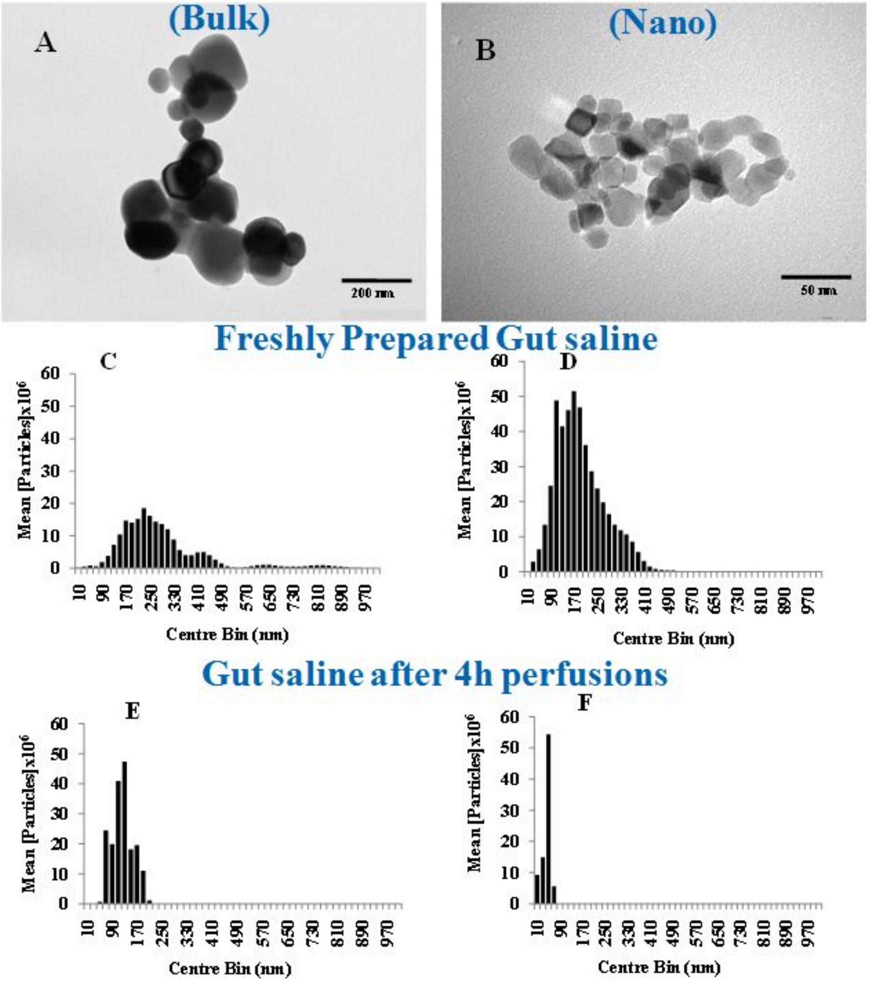
Sherwood, Human Physiology

Gut uptake by the patient?



Uptake Mechanisms

Shaw and Handy (2011) Environment International 37, 1083-1097.



Behaviour of TiO_2 in Gut Salines

Al-Jubory and Handy, (2013). Nanotoxicology, 7:8, 1282-1301

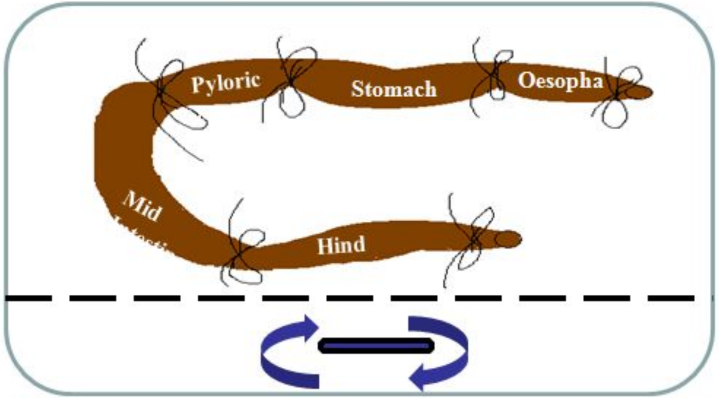
Electron micrographs show TiO_2 particles in a 1 g l^{-1} stock dispersion (water).

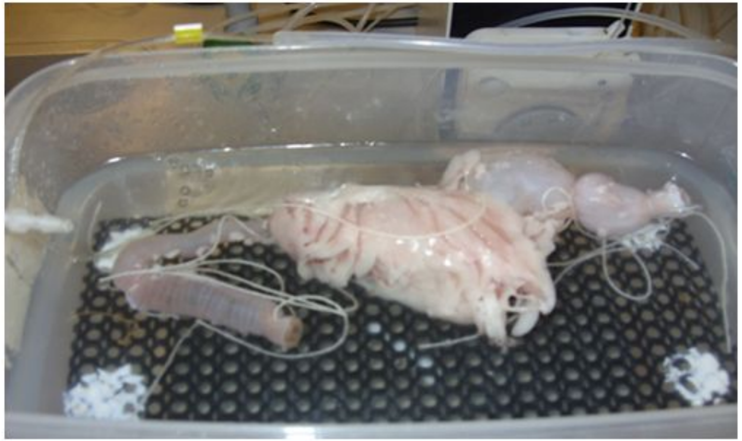
NTA: 1 mg l^{-1} stock dispersion in physiological saline, and the the same mucosal solution at the end of a 4 h perfusion, graphs are individual examples from triplicate measurements.

Whole Gut Sac Preparation

Modified from Hoyle & Handy (2005) Aquat. Tox. 72, 147-159.

- Dissect entire gut & fill lumen with test solution.
- Sutre off regions of the gut.
- Incubate for 4h.
- Measure metal concentrations in the mucosa removed from the underlying sub-mucosa/muscularis (stripped gut).





Al-Jubory and Handy, (2013). Nanotoxicology, 7:8, 1282-1301

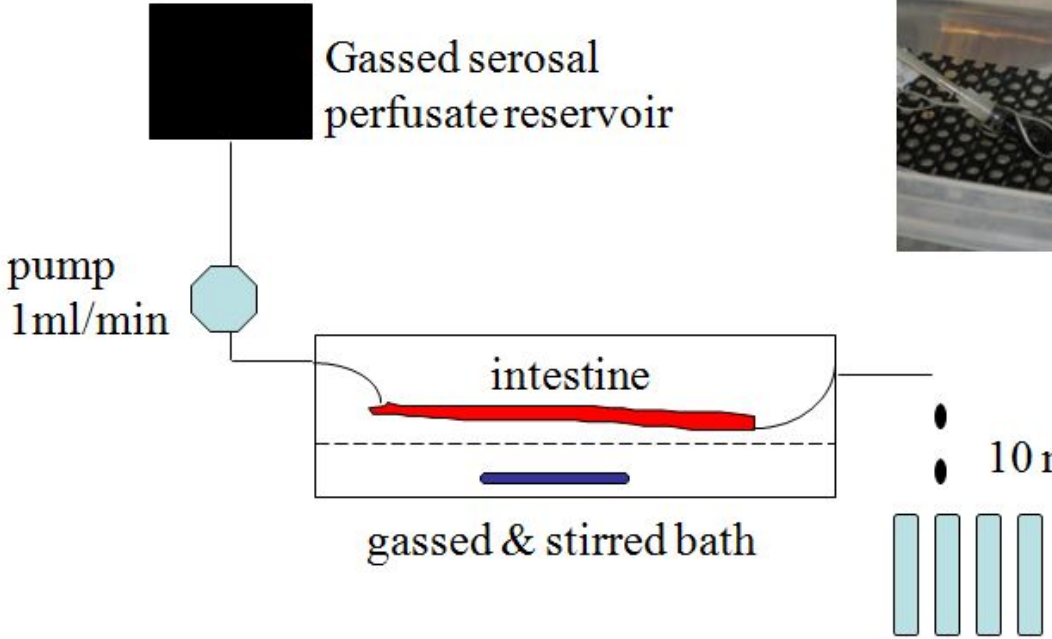
Titanium [Metal]: Gut Sac Tissue Following Exposure to 1 mg l^{-1} TiO_2 for 4 h.

[Ti] µmol g^{-1} dry mass					
Treatment		Oesophagus	Stomach	Mid intestine	Hind intestine
Control	Stripped	< 0.003[a]	< 0.003[a]	0.004 ± 0.001[a]	0.005 ± 0.002[a]
	Mucosa	0.018 ± 0.009[a]	0.010 ± 0.005[a]	0.012 ± 0.008[a]	0.019 ± 0.012[a]
	% in mucosa	90.0 ± 14.5[a]	83.3 ± 18.3[a]	75.0 ± 16.9[a]	79.2 ± 16.8[a]
BulkTiO_2	Stripped	0.018 ± 0.003[a,*]	0.030 ± 0.009[a]	0.135 ± 0.039[ab,*]	0.252 ± 0.131[b]
	Mucosa	0.128 ± 0.039[a,*]	0.065 ± 0.019[a,*]	0.159 ± 0.049[a,*]	0.148 ± 0.035[a,*]
	% in mucosa	87. 7 ± 6.6[a]	68.4 ± 5.9[a]	54.1 ± 3.1[b]	37.0 ± 10.8[b]
TiO_2 NP	Stripped	0.019 ± 0.005[a,*]	0.021 ± 0.004[ac,*]	0.056 ± 0.010[b,*]	0.040 ± 0.011[bc,*]
	Mucosa	0.174 ± 0.019[a,*]	0.060 ± 0.006[b,*]	0.136 ± 0.025[a,*]	0.165 ± 0.026[a,*]
	% in mucosa	90.2 ± 2.1[ac]	74.1 ± 4.3[bc]	70.8 ± 4.1[b]	80.1 ± 2.9[c]

Values are means ± S.E.M. (n = 6/treatment). * Statistically significant difference from the control within columns. Different letters (within rows) indicate a statistically significant difference between regions of the gut (Kruskal-Wallis or ANOVA, $p < 0.05$). There was no material-type effect within each region.

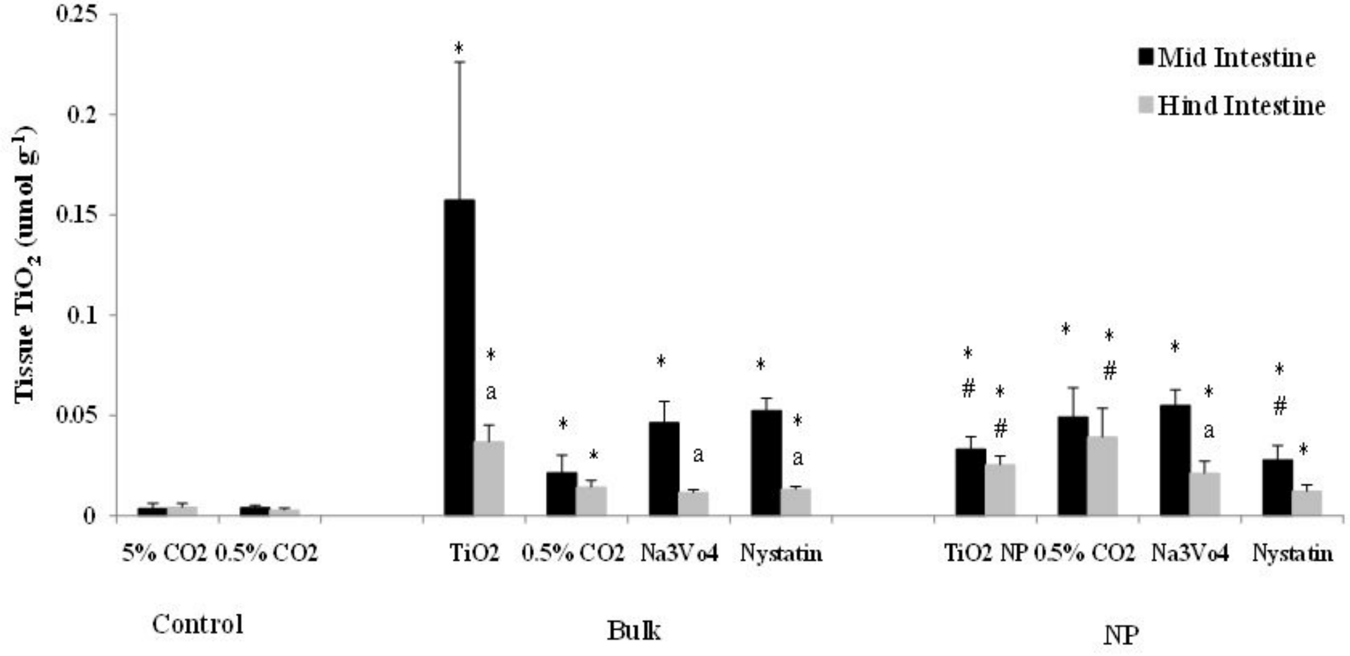
Isolated Perfused Intestine

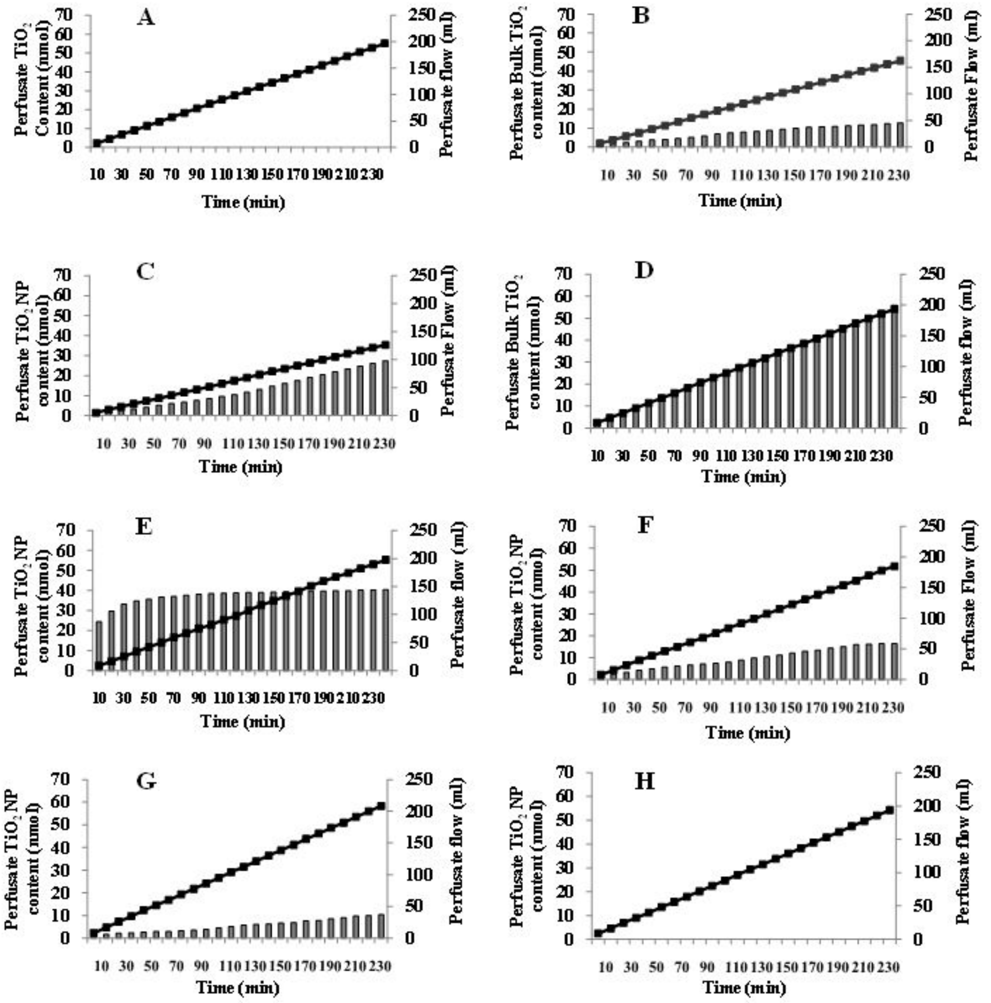
Handy et al. (2000) J. Exp. Biol. 203, 2365-2377



Ti Accumulation in the Perfused Trout Intestine

Al-Jubory and Handy, (2013). Nanotoxicology, 7:8, 1282-1301





Titanium Appearance in the Serosal Perfusate.

Al-Jubory and Handy, (2013). Nanotoxicology, 7:8, 1282-1301

(A) Control;
(B) Bulk TiO_2;
(C) TiO_2 NP;
(D) Bulk TiO_2 0.5% CO_2;
(E) TiO_2 NP 0.5 % CO_2
(F) Cyanide
(G) Vanadate
(H) Nystatin

Flux Rates of Water and Ti Across Perfused Trout Intestine

Al-Jubory and Handy, (2013). Nanotoxicology, 7:8, 1282-1301

Mucosal [TiO_2] (1mg l^{-1}) and Drugs Concentration	Net Ti flux, $J_{net,Ti}$ (nmol g^{-1} h^{-1})		Net water flux, $J_{net,H2O}$ (ml g^{-1} h^{-1})	
	Initial rate	Overall rate	Initial rate	Overall rate
Control	0.23 ± 0.16	0.26 ± 0.04	-6.22 ± 0.85	-7.50 ± 1.18
Bulk TiO_2	0.98 ± 0.47	0.85 ± 0.32	-26.28 ± 2.52*	-28.54 ± 3.19*
TiO_2 NP	1.55 ± 0.33*#	2.38 ± 0.68*#	-17.56 ± 4.00*#	-18.01 ± 4.16*#
0.5% CO_2 Control	0.51 ± 0.14	0.06 ± 0.03	-3.42 ± 1.71	-3.97 ± 0.97
0.5% CO_2 Bulk TiO_2	3.42 ± 0.74*‡	3.18 ± 0.42*‡	-7.05 ± 2.29‡	-8.95 ± 1.78*‡
0.5% CO_2 TiO_2 NP	21.16 ± 18.90*#‡	3.27 ± 1.60*a	-17.13 ± 2.37*#	-22.30 ± 6.40*#
Bulk TiO_2 Na_3Vo_4	<0.004†	<0.004†	-13.57 ± 1.83†	-38.41 ± 0.07†a
Bulk TiO_2 nystatin	<0.004†	<0.004†	-9.97 ± 2.05†	-31.66 ± 0.08a
TiO_2 NP KCN	2.56 ± 0.65†	0.85 ± 0.17†	-13.40 ± 3.66†	-44.51 ± 2.76†a
TiO_2 NP Na_3Vo_4	2.29 ± 0.64†	0.83 ± 0.22†	-16.26 ± 2.66†	-49.17 ± 3.88†a
TiO_2 NP nystatin	<0.004†	<0.004†	-13.96 ± 2.29†	-36.42 ± 2.03†a

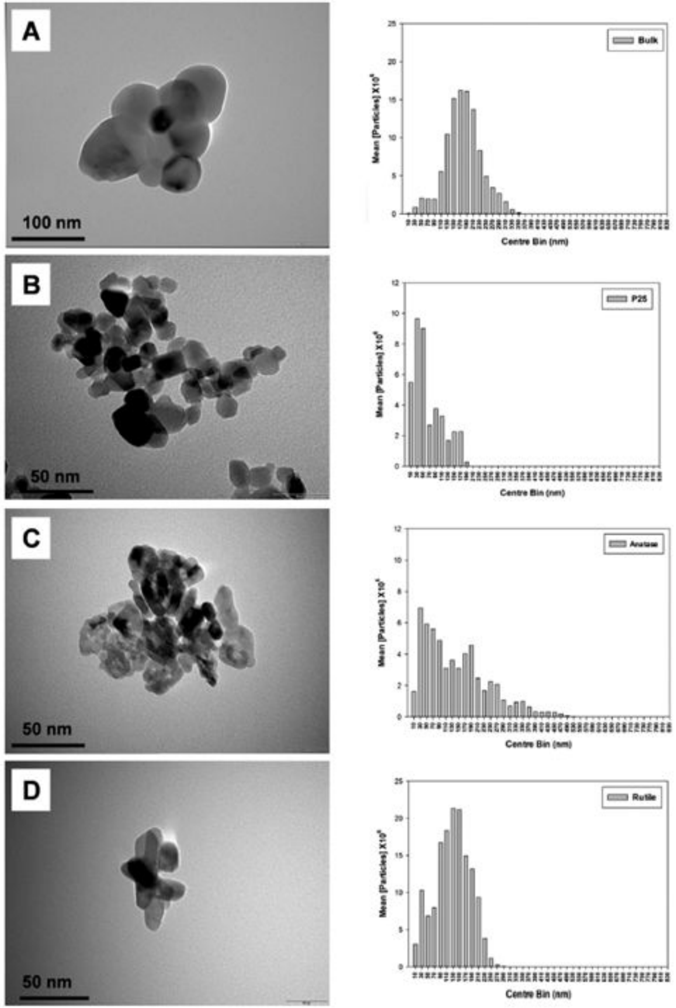
Values are means ± S.E.M. (n = 6-7). Negative values indicate a net loss from the serosal solution.* Statistically significant difference from the relevant control value within columns, # Statistically significant difference from bulk TiO_2 value within columns, ‡ Statistically significant difference from the standard gas mix group values within columns (Kruskal-Wallis or ANOVA, $p < 0.05$) and † Drugs effects statistically significant difference from the standard gas mix TiO_2 NP or bulk values within columns. Letter (a) within rows indicates a statistically significant difference between initial and overall rates (t-test, $p < 0.05$).

Titanium Uptake by Cultured Gut Epithelial Cells

Caco-2 cells

Do cells take up intact particles?

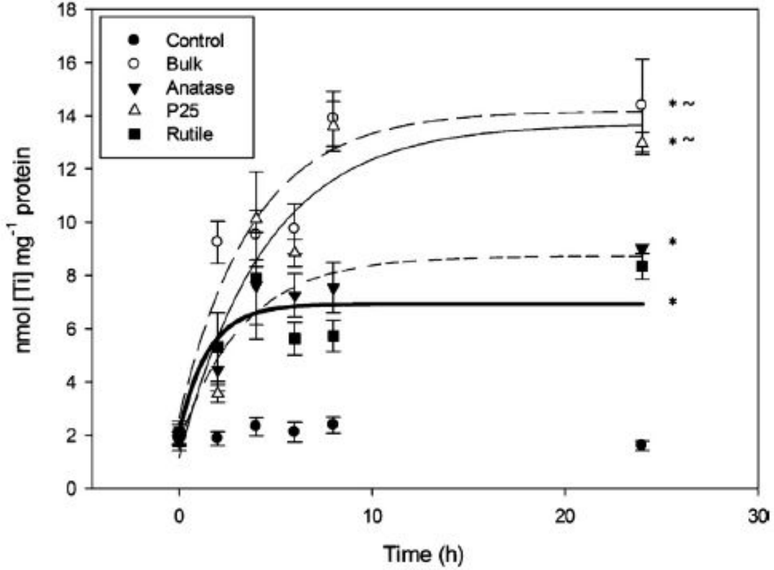
Can they select for shape or size?



Different Crystal Forms of TiO_2

Gitrowski et al. (2014) Toxicology Letters 226. 264–276.

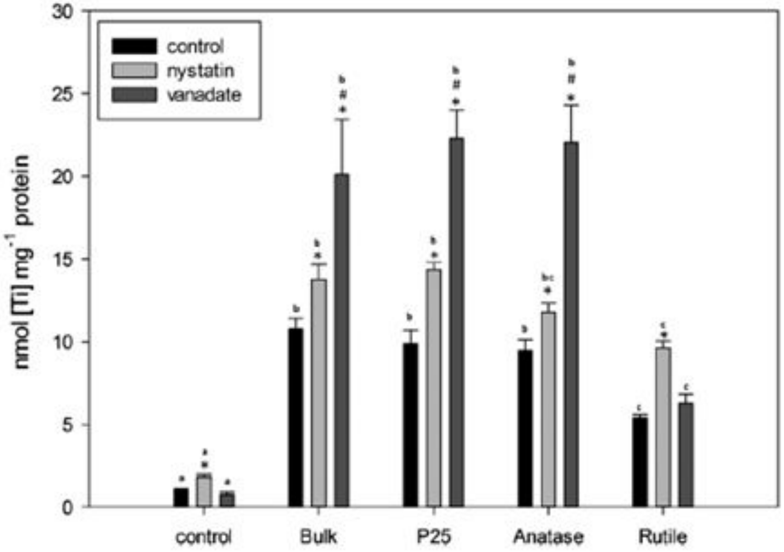
The P25 form of TiO_2 is Preferred Over Pure Anatase or Rutile by Caco-2 cells.

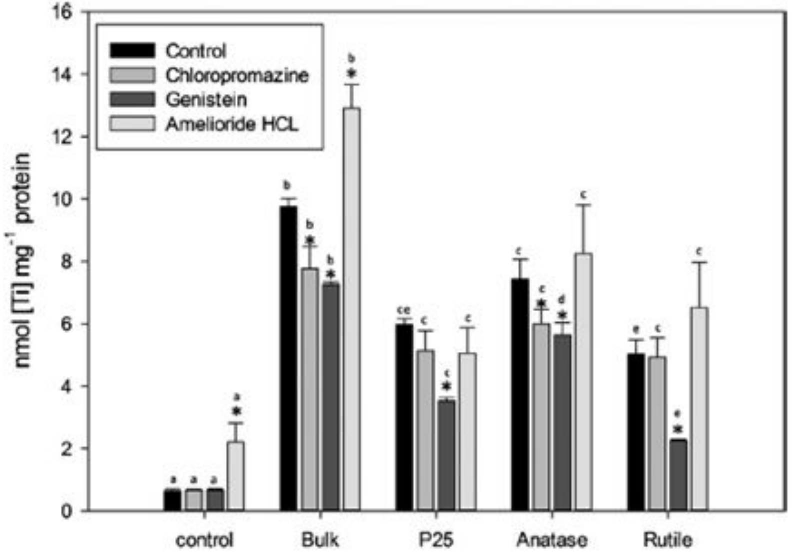


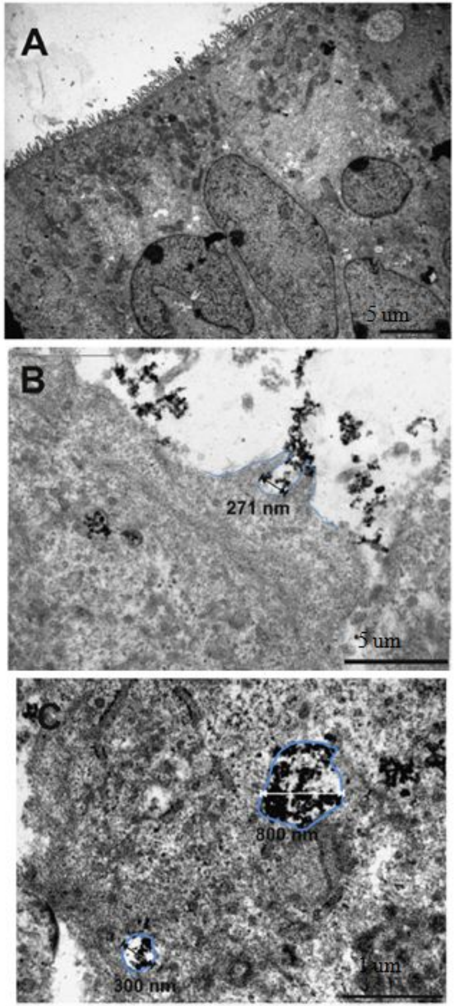
Gitrowski et al. (2014) Toxicology Letters 226. 264–276.

Effect of Inhibitors on Ti Accumulation by Caco-2 cells

Gitrowski et al. (2014) Toxicology Letters 226. 264–276.







Intact Particles Are Taken up by Caco-2 Cells.

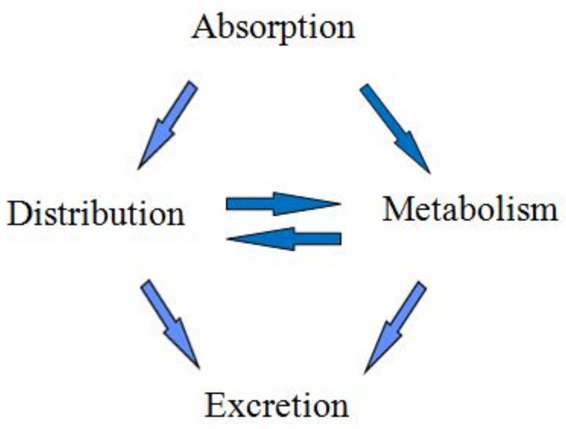
Gitrowski et al. (2014) Toxicology Letters 226. 264–276.

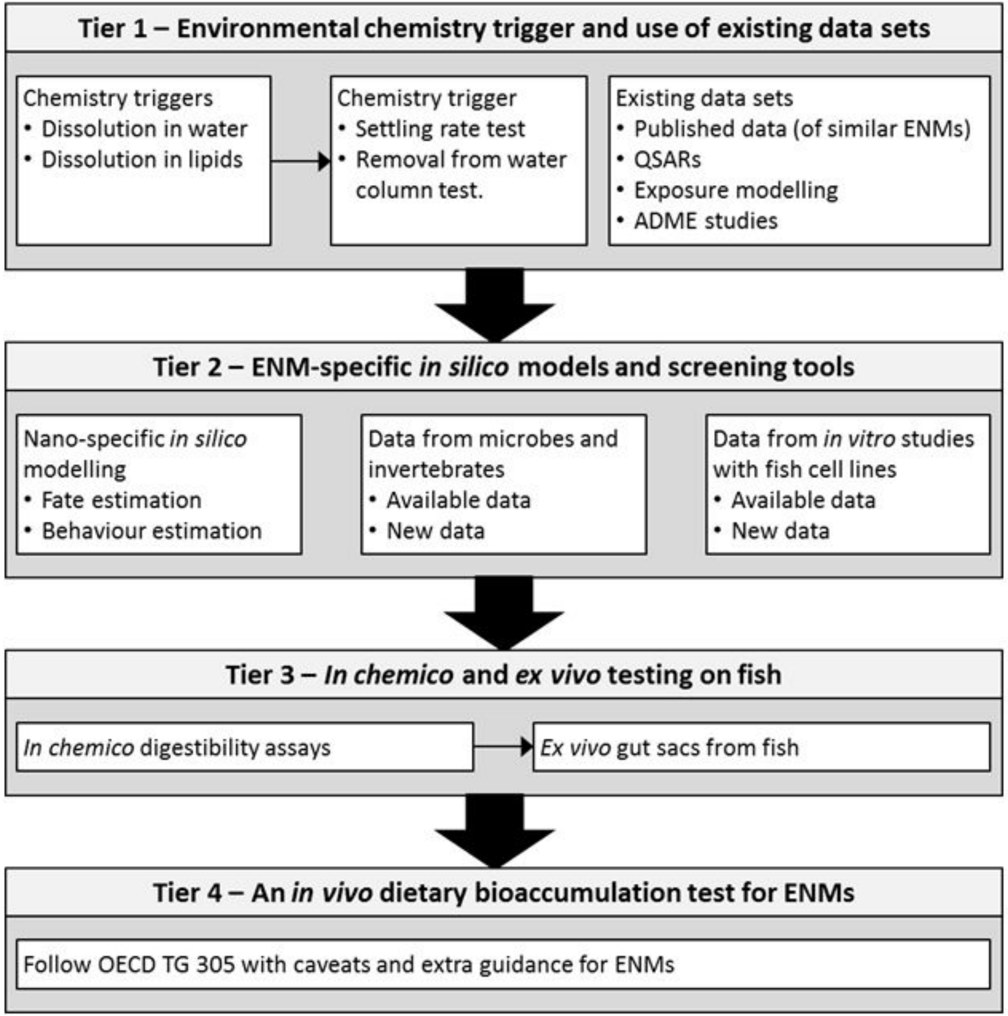
Calculation of Allowable Daily Intake for Humans

- Human requirements for nutrition
  - "Estimated safe and adequate daily dietary intakes" (ESDADDI)
- Allowable Daily Intake (ADI)
  - incorporates a safety factor which is x100 below the "No effect" level.
  - ADI = No effect level/100

Bioaccumulation Potential of ENMs

- Risk assessment triggers:
- Persistence in the Environment
- Bioaccumulation Potential
- Toxicity



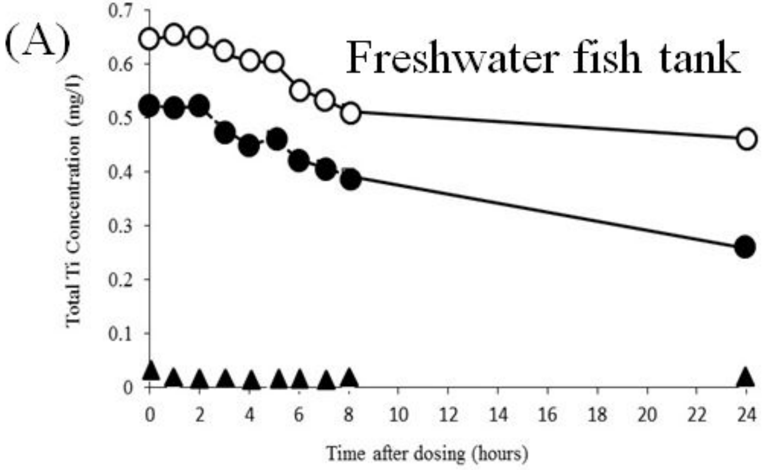


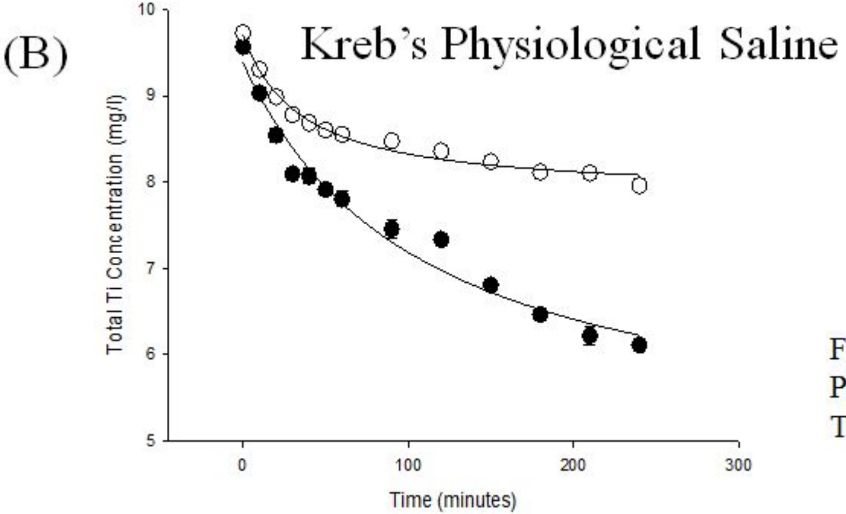
OECD

Dietary Bioaccumulation Potential Testing Strategy with Fish

Handy et al. (2018) Environ. Sci.: Nano, 5, 2030–2046.

Tier 1 New Chemical Triggers: Settling Rate Assay (~TG318)

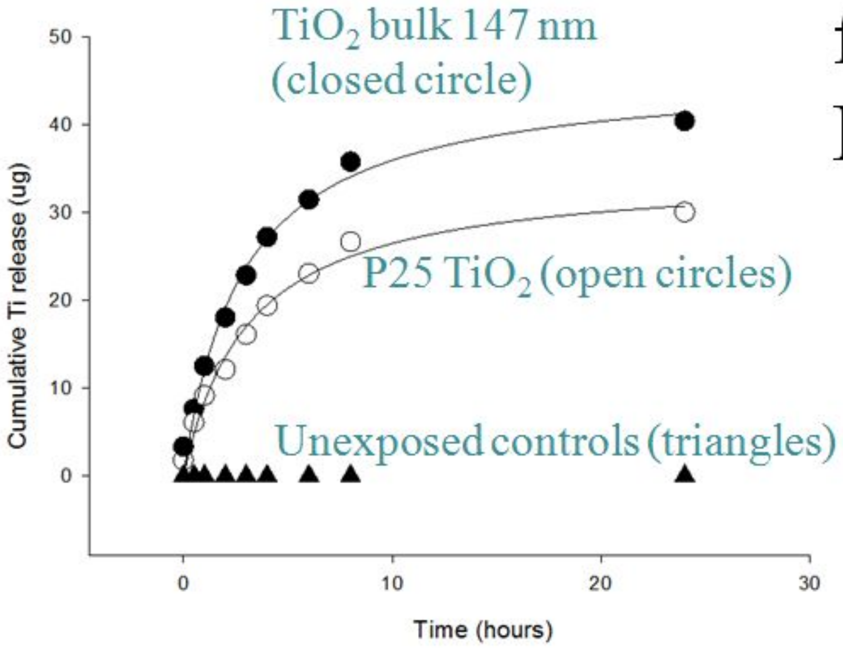




Handy et al. (2018) Environ. Sci.: Nano, 5, 2030–2046.

Figure: unexposed controls (triangles).
P25 TiO_2 (open circles),
TiO_2 bulk 147 nm (closed circle),

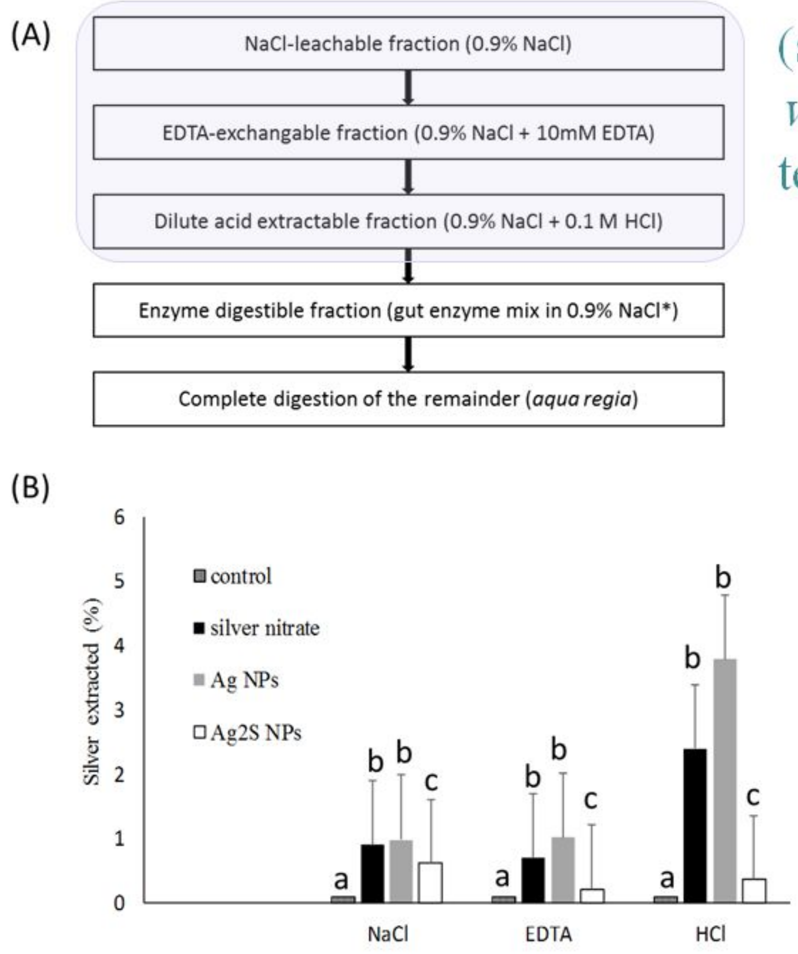
Fish Gut Saline



Tier 1 New Chemical Trigger for TG305: Max Dissolution rate. (New GD)

Handy et al. (2018) Environ. Sci.: Nano, 5, 2030–2046.

Figure: Measurement of dissolution by dialysis of 1 mg/L dispersions.



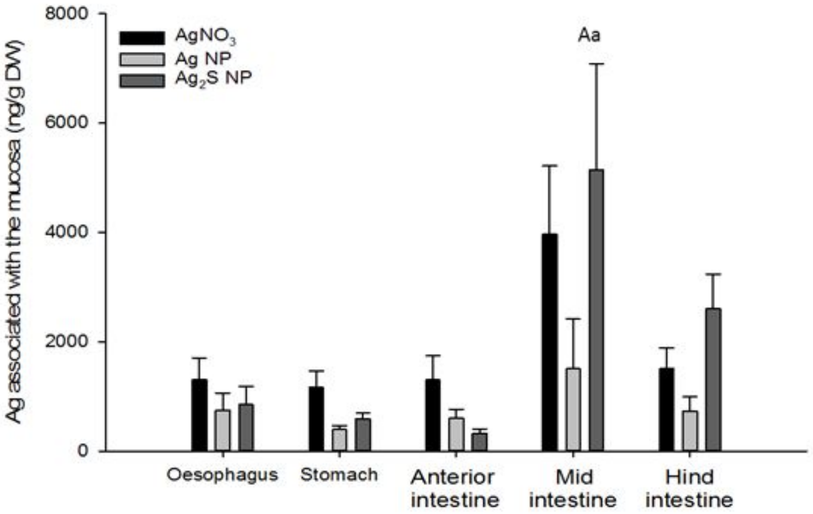
(similar to EFSA *in vitro* digestibility testing)

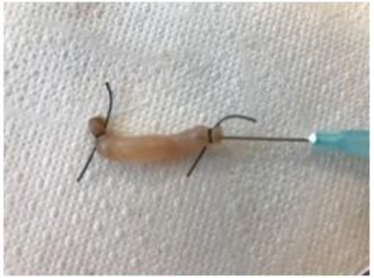
Tier 3A. New Trigger for TG305: *In Chemico* Digestibility Assay.

Handy et al. (2018) Environ. Sci.: Nano, 5, 2030–2046.

Nanofase Silver Accumulation in the Gut Mucosa of Individual Gut Sacs – Limited Uptake

Clark, Handy et al. (2019) ES Nano, 6, 646-660





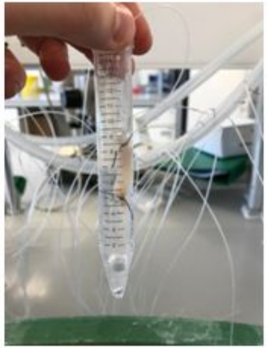
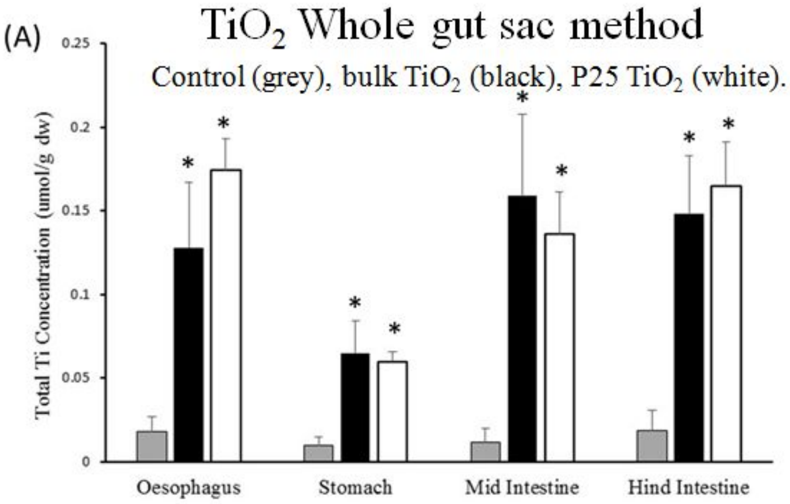


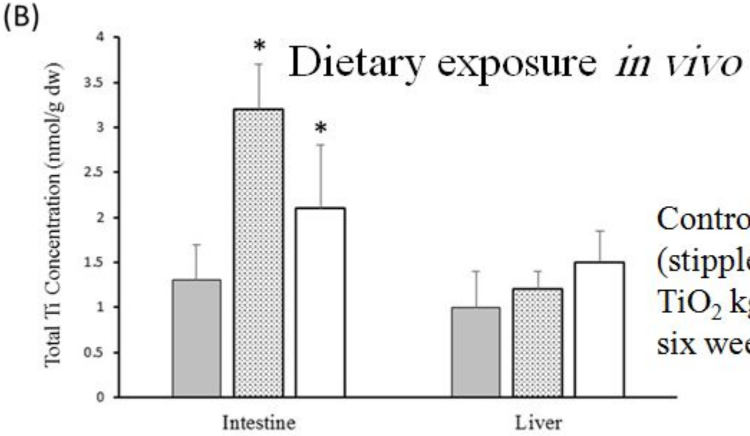
Figure 1. The mass of total Ag associated with the mucosa (ng/g dw) for each tissue compartment from a 1 mg/L exposure for 4h. (a) denotes significant difference from the Ag NP treatment for the same tissue. (A) denotes significant difference from the anterior intestine of the same treatment.



Handy et al. (2018)
Environ. Sci.: Nano,
5, 2030–2046.

In vitro (Tier 3B)

versus



In Vivo (Tier 4).

Control (grey), or 10 (stippled), or 100 mg P25 TiO_2 kg^{-1} food (white) for six weeks

Conclusions

- Nanomaterials are being used in the food sector for packaging, as food ingredients, and as preservatives.
- Framework for the safety testing of chemicals in food, includes nanomaterials in foods.
- Effort should also protect the food chain: soil quality, crops, farm animals and animal feed.
- In vivo studies show that animals will eat nanomaterials.
- Studies on the gut epithelium are starting to report uptake rates, and identify the form of the material absorbed.
- Long term bioaccumulation is a concern – the testing strategy is being developed at the OECD.
- Safe and allowable levels in food are yet to be determined for most materials.

Safe, Responsible Innovation…

Questions?

Thank You!