Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

Netherlands Food and Consumer Product Safety Authority (NVWA)
-
Office for Risk Assessment and Research (BuRO)

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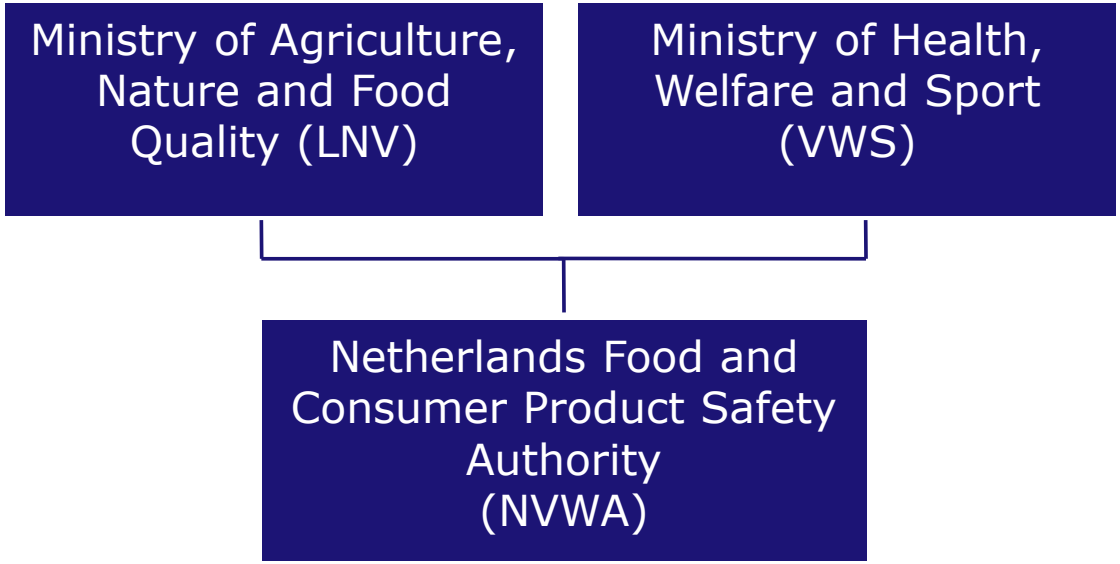
Welcome



- NVWA is part of the central government, within LNV
- assignments by and budget from two ministries (LNV, VWS)
- laboratories (food safety; product safety; plant health)

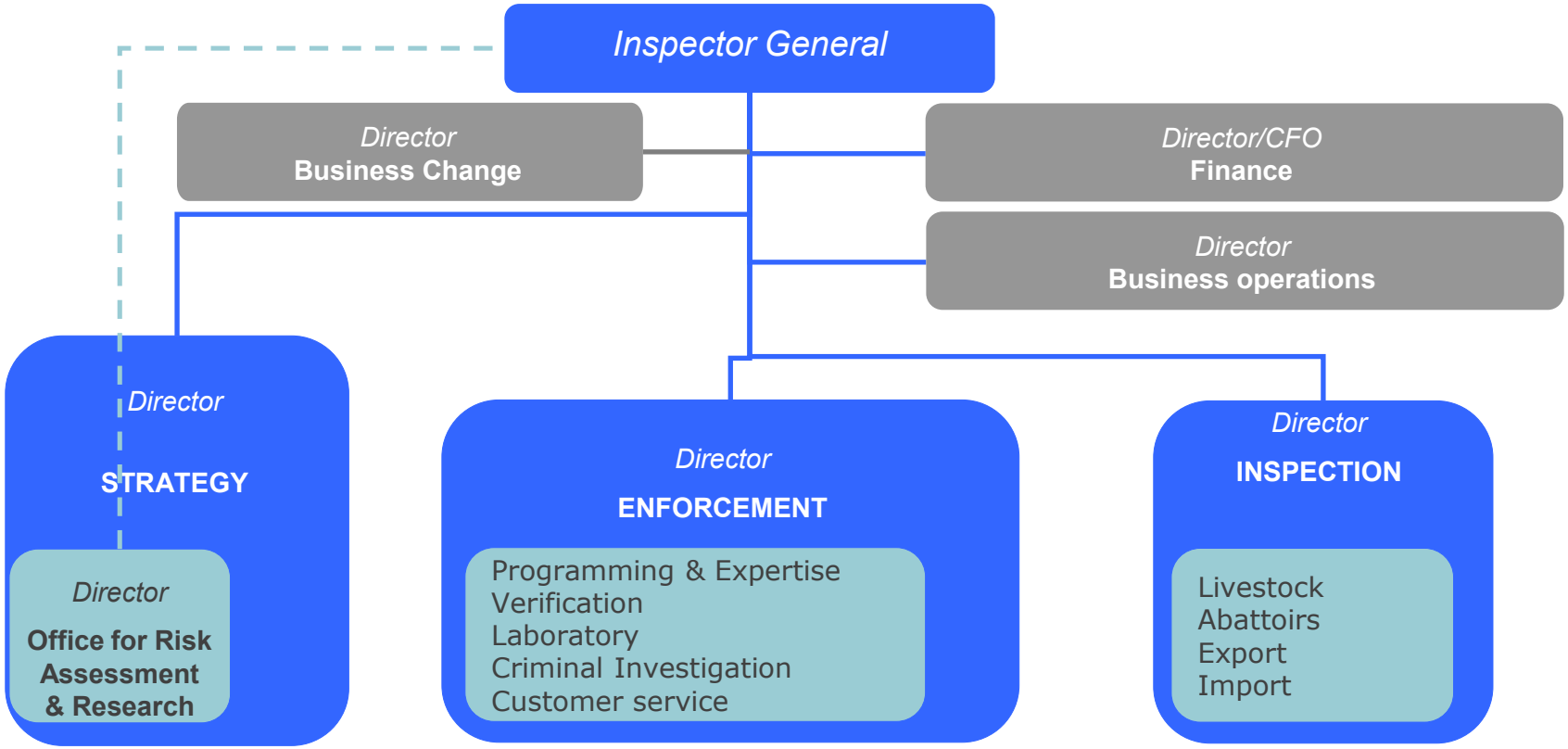
Mission and role

The NVWA safeguards food safety, consumer product safety, animal health, animal welfare, plant health and maintains the legislation in the field of nature

Main tasks:

- risk assessment and communication
- verification: inspections, audits
- enforcement

NVWA


Inspector General
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NVWA – key facts and figures in 2016

- Budget: € 333 million
- 2471 total number staff
- number of controls: 160,455
- samples: ± 510,000
- warnings, fines, etc.: ± 22,000

More facts and figures available on our website:

https://www.nvwa.nl/onderwerpen/toezicht-nvwa-eten-en-drinken/dossier/meerjarenprogramma-nationale-controles-mancp/jaarverslagen-mancp

Approach of inspection and enforcement

The supply chain comprises many links, all vulnerable to introduction of risks.

Therefore we need to:

- assess those risks throughout the chain
- prioritise those risks to act most efficient
- work together to be most effective
- discover criminal hazardous activities

Knowledge-based and risk-oriented!

NVWA and EFSA

EFSA mission (EC/178/2000):
- Scientific advice concerning food safety;
- Communication on risks.

NVWA responsibilities:
- Identical, but includes law enforcement;
- Liaison office.

Office for Risk Assessment & Research

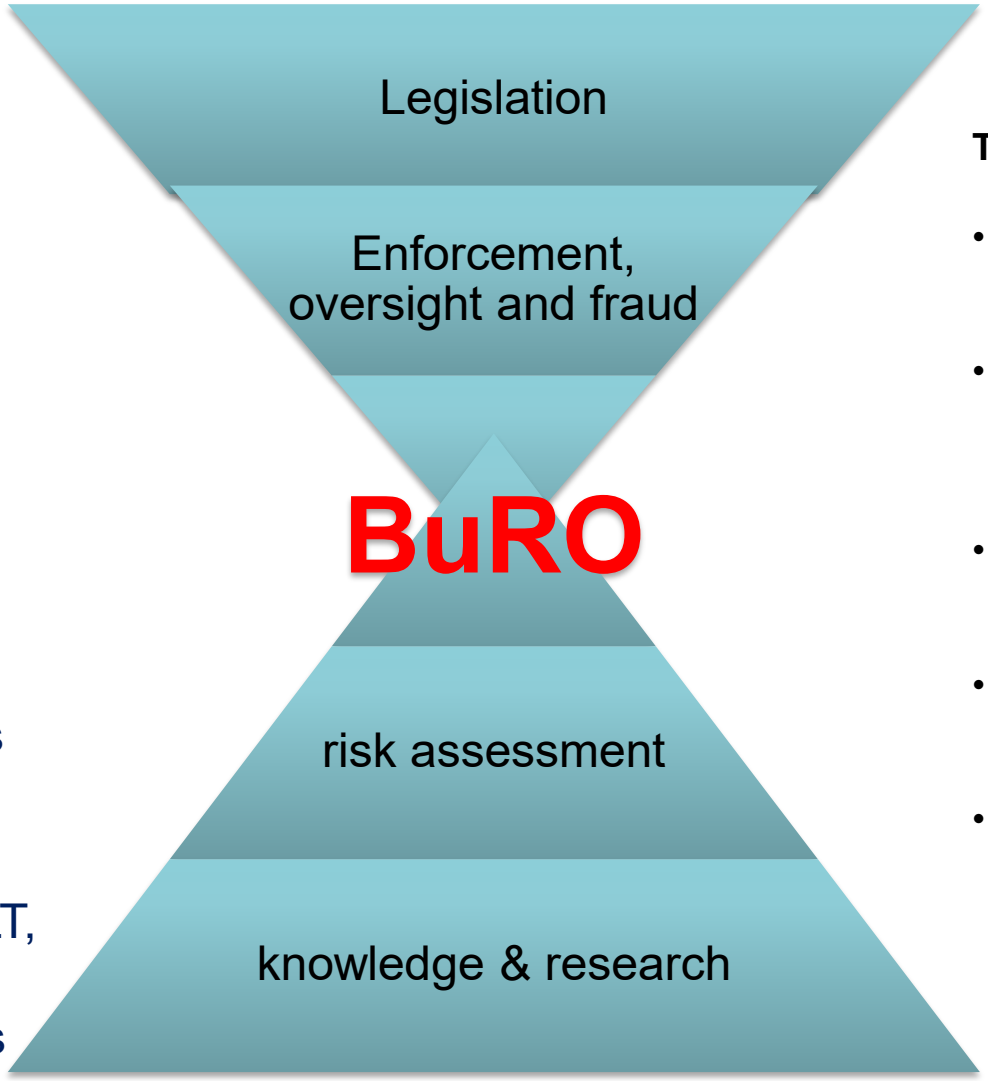
BuRO

Ministries

NVWA

Public values

RIVM, RIKILT, WUR, universities



Tasks:

- To direct pro-active risk assessment (early warning)
- To act as knowledge broker and manager towards research institutions
- To design and supervise research
- To acknowledge research proposals
- To co-ordinate and promote cohesion and process efficacy

BuRO and NVWA

Distinct tasks of risk assessment (BuRO) and risk management (NVWA) requires functional separation:

- To ensure that scientific assessment is independent and not influenced by other interests
- To demarcate that the acceptability of a risk is a matter for decision-makers, not risk assessors
- To ascertain that different issues are assessed by scientists with relevant competence

WOR

- Law on the Independent Risk Assessment VWA

(*Wet Onafhankelijke Risicobeoordeling NVWA [WOR]*), April 26 2006, nr 247, is focussed on public health

  - Food Safety
  - Product Safety
  - Intention as of 2014: include Animal Welfare in WOR

- BuRO-employee ≠ enforcer, inspector!

- Risk Assessment
  - Hazard Identification
  - Hazard Characterization (dose/response)
  - Exposure Assessment
  - Risk Characterization

→ Advice, with action perspective & risk communication

BuRO and EFSA

EFSA

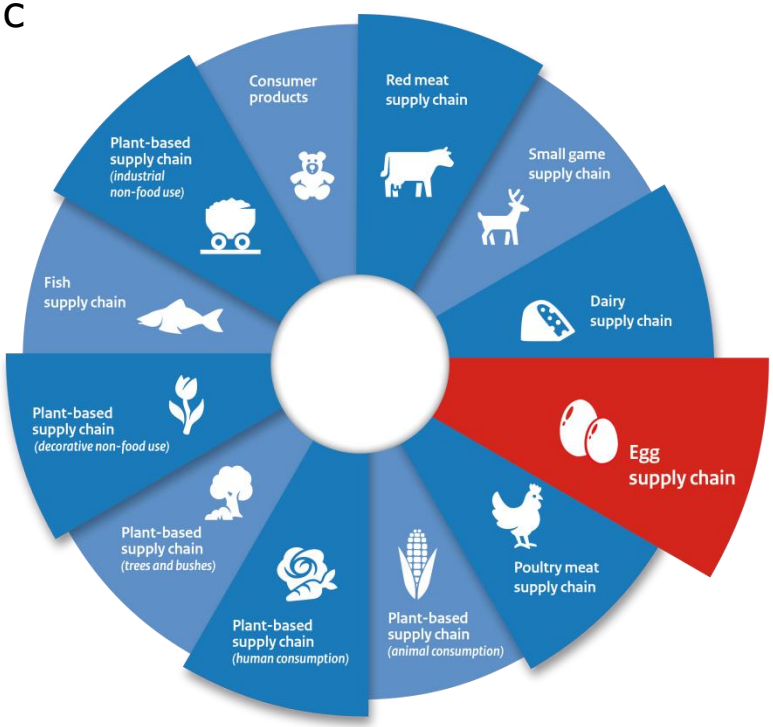
- EFSA should be a depository for the best expertise available
- Harmonisation of risk assessment methodology, generic assessment, EU food consumption/occurrence data bases, communication and ICT structure, EREN network, midterm planning ……

BuRO

- Advisory Forum member
- EFSA Focal Point
- Panels
- Networks

Output BuRO

- (Short) Advices
  - Varying from emails (minutes), written contributions (hours to days), to public advices (weeks to years)
  - Generally, publicly available
- Contributions to 'the State of' (Product Safety, Food Safety)
- Risk Communication
- Risk Assessment of Supply (or Production) Chains

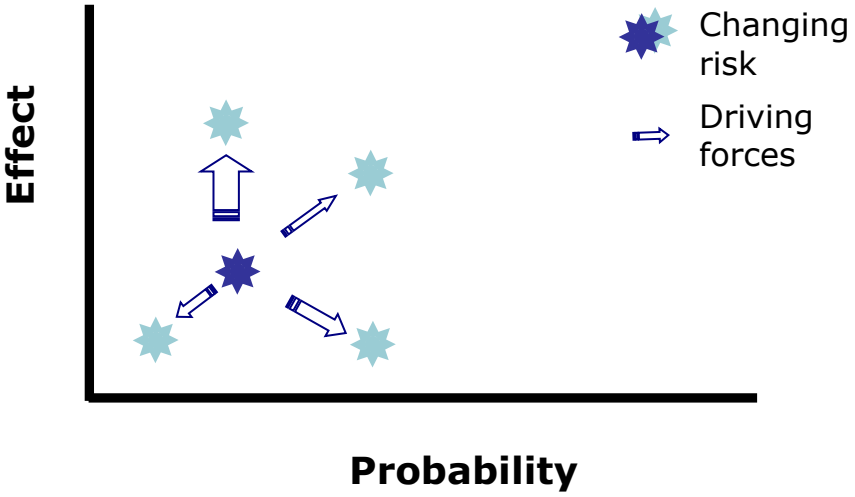


New and emerging risks

Early warnings - networks

- National (e.g., on zoönoses, chemical risks)
- EFSA (Advisory Forum, Focal Point, EREN)

→ Too many signals, weighing needed using risk matrix!



Active enforcement	Incidents and crisis control
Regular enforcement	Risk communication

Effect →

→ Probability

Axes can comprise various parameters!

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

Risk assessment of TiO_2 nanoparticles in food, incl. toxicokinetic considerations

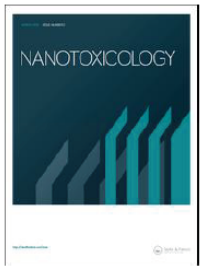
Minne Heringa et al.

NVWA workshop TiO2 | 4-5 July 2018

Three papers:

- Intake
- Risk assessment
  - Based on modelled organ levels
  - Based on measured organ levels in humans

Then current work

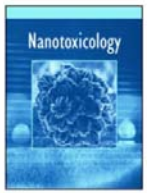


Nanotoxicology

ISSN: 1743-5390 (Print) 1743-5404 (Online) Journal homepage: http://www.tandfonline.com/loi/inan20

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



Nanotoxicology

ISSN: 1743-5390 (Print) 1743-5404 (Online) Journal homepage: http://www.tandfonline.com/loi/inan20

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
https://doi.org/10.1186/s12989-018-0251-7

Particle and Fibre Toxicology

RESEARCH Open Access

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

M. B. Heringa[1*], R. J. B. Peters[2], R. L. A. W. Bleys[3], M. K. van der Lee[2], P. C. Tromp[4], P. C. E. van Kesteren[1], J. C. H. van Eijkeren[1], A. K. Undas[2], A. G. Oomen[1] and H. Bouwmeester[2,5]

Intake: method

- 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)
- TiO_2 levels from literature and analyses RIKILT
- Food product intake from Dutch National Food Consumption Survey, 3 age groups

- To calculate the $nTiO_2$ fraction (<100 nm) ingested, 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)

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

Intake: main findings amount

	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

- P95: 95% of population has exposure at or below this level
- Natural sources not included, but have very low levels

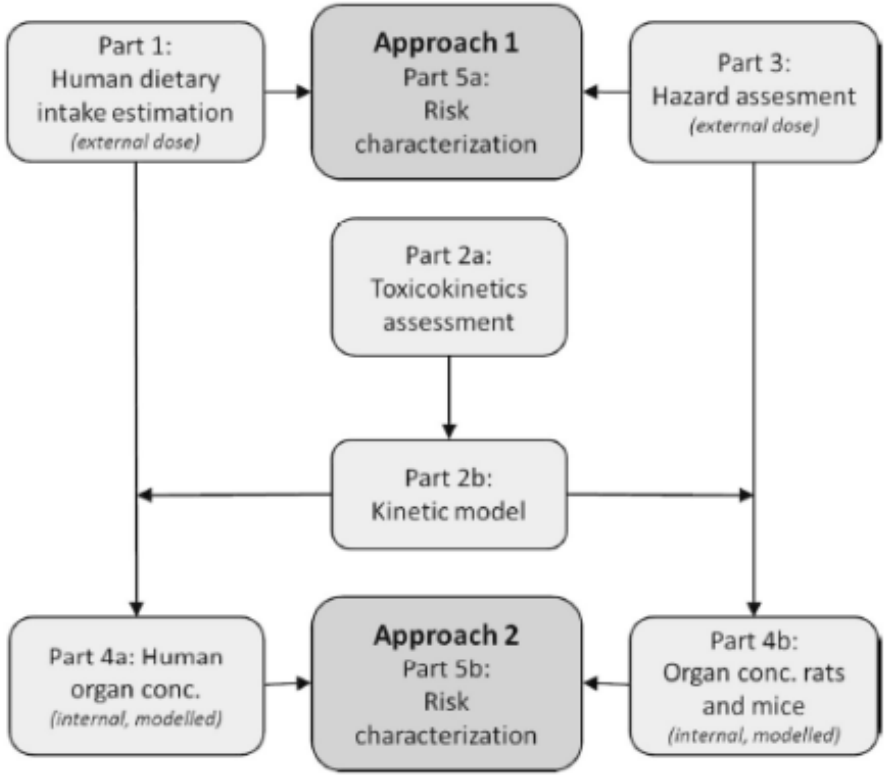
Intake: main findings sources

Top ten	DNFCS-Children (2-6 y old)	DNFCS-2007-2010 (7-69 y old)	DNFCS-older adults (70+)
1	Toothpaste (57%)	Chewing gum (14%)	Coffee creamer (13%)
2	Hard candy with sugar (4%)	Coffee creamer (11%)	Thickened milk for coffee, powdered (8%)
3	Sugar-coated chocolate confectionery (3%)	Mayonnaise normal (7%)	Chewing gum (4%)
4	Chewing gum with sugar (2%)	Sauce, garlic, mayonnaise based (5%)	Cappuccino with cafein (3%)
5	Sugar-coated chocolates (2%)	Cappuccino (4%)	Cappuccino instant ready to drink (3%)
6	Biscuit with flavoured milk-filling (2%)	Thickened milk for coffee, powdered (3%)	Cappuccino (3%)
7	Biscuit with milk-filling (2%)	Iced cake 'glacekoek' (3%)	Sauce, garlic, mayonnaise based (2%)
8	Marsh mellow big (2%)	Cappuccino instant ready to drink (3%)	Pastries with cream (2%)
9	Biscuits with coffee glaze (2%)	Cappuccino with cafein (3%)	Sauce based on mayonnaise (2%)
10	Chewing gum sugar free (2%)	Coffee liquid with sugar and milk (machine) (2%)	Mayonnaise (2%)

Rompelberg et al. Nanotoxicology. 2016 10(10):1404-1414.

Risk assessment: approach

- Approach 1: Margins between external exposure dose and external safe dose
- Approach 2: 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

- **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 $nTiO_2$ used**
  - Exposure/dose was, if needed, converted to nanoparticles

Key toxicity studies

Study details	TiO_2 details	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	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): - Fibrosis in heart of male rats - Hyperplasia of bile ducts in male rats ↑, but no clear dose-response in female rat - (and more)	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 w) and adult rats (8 w)	• 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*	<u>Young rats</u>: Liver edema AST ↓ and ALT/AST ↑ (ALT unchanged) → liver damage $HBDH^2$ ↓ → heart injury, but not dose-related Glucose and $LDL\text{-}C^2$ ↑, but not dose-related <u>Adult rats:</u> TBIL ↑ → liver damage (multiple possible causes)	Wang et al., 2013

Key studies (continued)

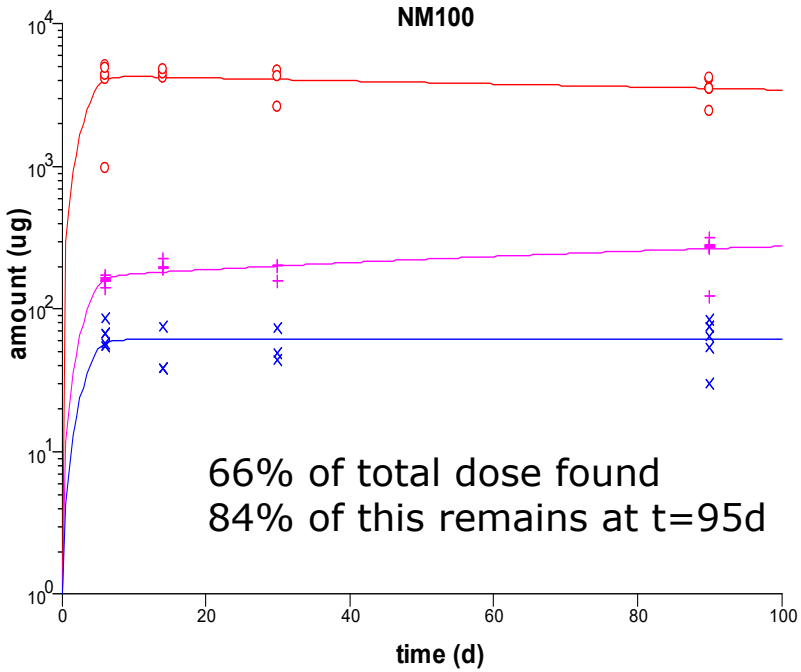
Study details	TiO_2 details	Study-specific NOAEL or LOAEL (*self- derived levels in italics*)	Critical effects at LOAEL (not exhaustive)	Source
• 42 days Kunming mice, males only • 15 animals/dose • 0; 10; 50; 250 mg/kg bw/day • Intragastric in PBS with 0.5 % Tween 80 • 4 weeks old at start of study	• 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:
	  ○ Spherical NPs with primary size 20-60 nm
	  ○ Irregular NPs with primary size 40-60 nm
		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

Toxicokinetics:

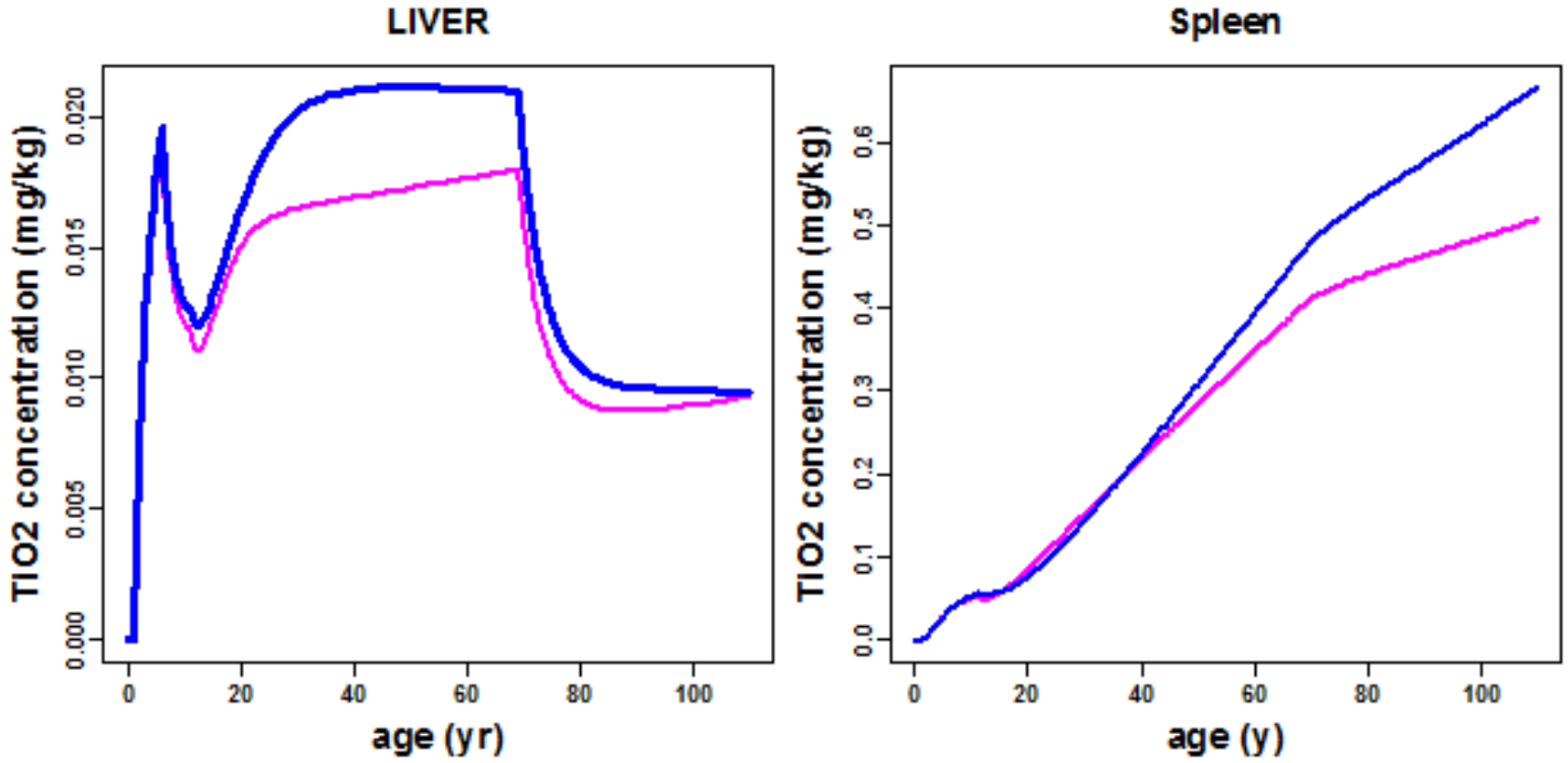
- Based on Geraets et al. (2014)
  - 4 TiO_2 materials, oral and IV, rat, 5 d exposure, 85 d post-exposure
  - 30% loss (dosing materials?)
  - Oral study: very low absorption: 0.02%
  - Highest levels in liver and spleen
  - Some elimination from liver, none from spleen → redistribution, accumulation
  - Hardly any total elimination

(logscale)



Liver, spleen, rest

Estimated $nTiO_2$ concentrations in human organs



$nTiO_2$ concentration in different human organs plotted against age

Risk assessment: results

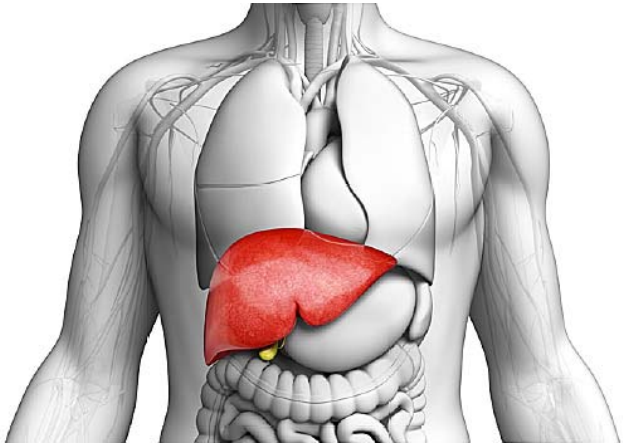
- Traditional approach: margins of safety (MoS) large enough, except for effects on repro-organs found by Tassinari et al.(2014)
- Approach incl. toxicokinetics: MoS too small, except for NCI study (1979)
  - Due to accumulation in organs in time

Conclusions:

- Risk cannot be excluded
- Important to include toxicokinetics as this changes risk assessment outcome for this accumulating substance

Emerging question

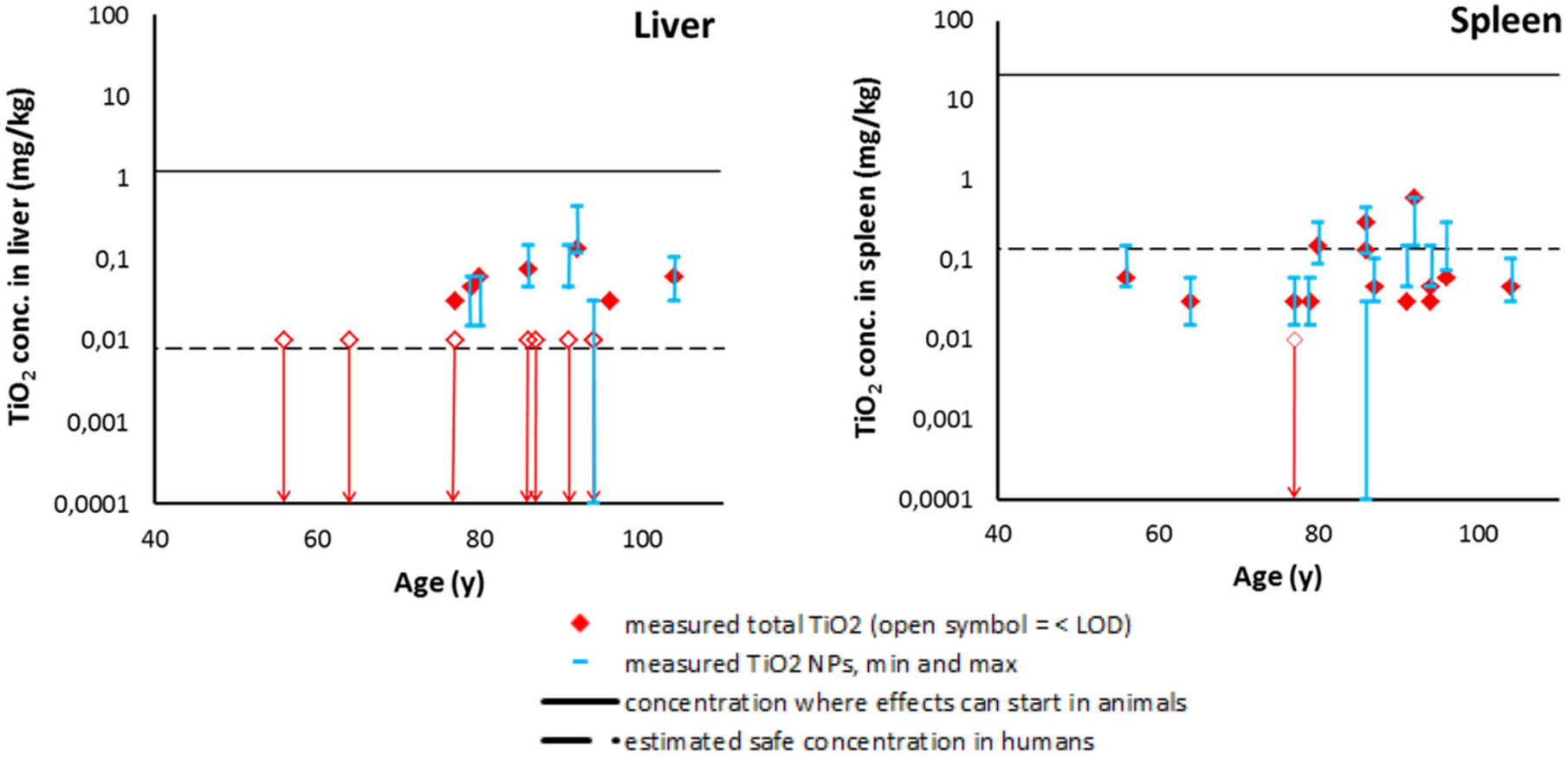
What are the real organ levels in humans?
(Did we estimate them correctly?)



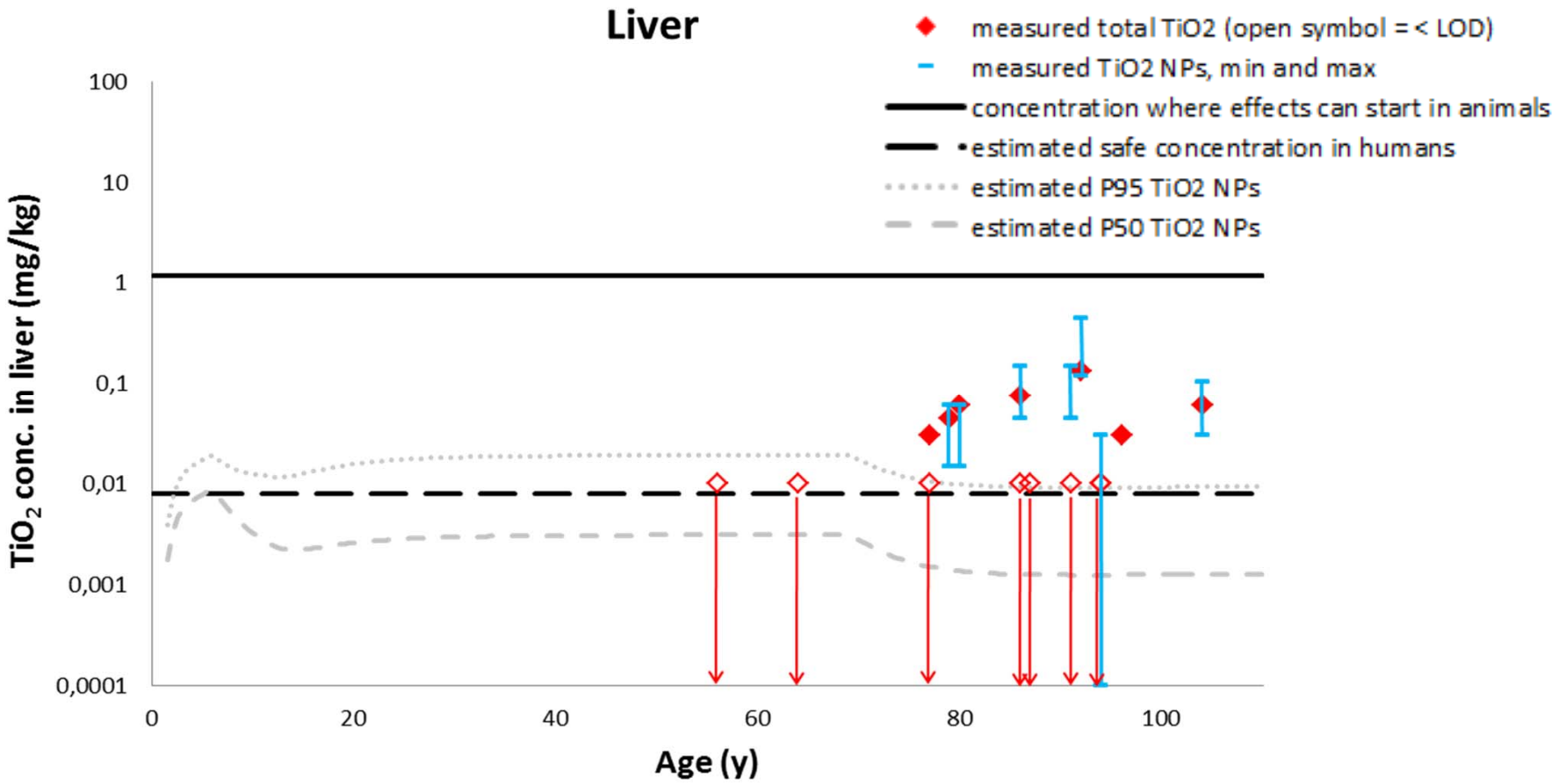
Measured TiO_2 particle concentration in post-mortem human liver and spleen

- 15 post-mortem liver and spleen
  - Recorded age, gender, ethnicity
- Analysis (by RIKILT)
  - 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)

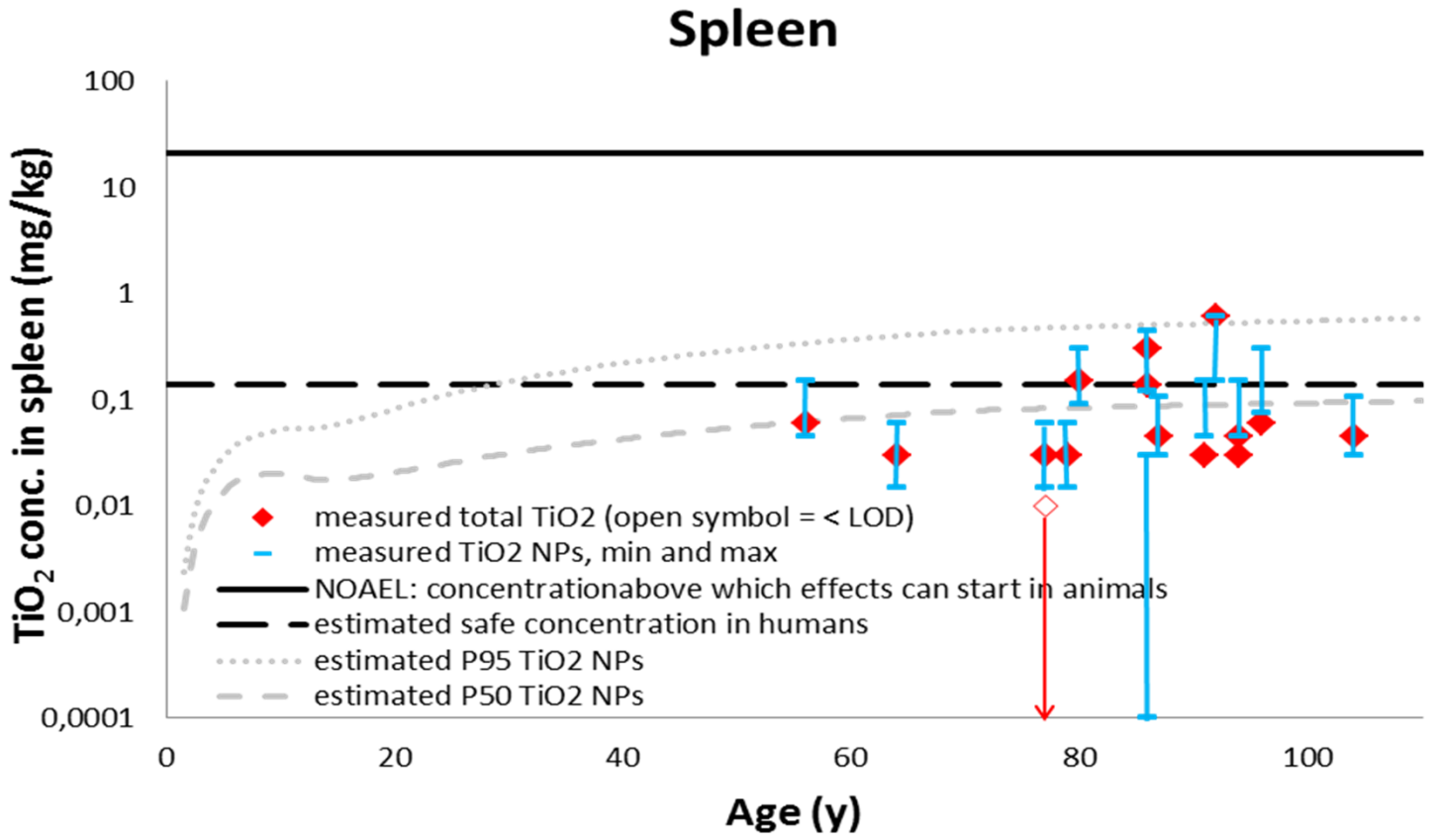
Risk assessment with measured levels: results



Comparison to model predictions: liver



Comparison to model predictions: spleen



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

Whole risk assessment:

- Extrapolation from short-term studies
- All NP sizes lumped together, and assumed any toxicity is caused only by NPs

Conclusions RIVM work

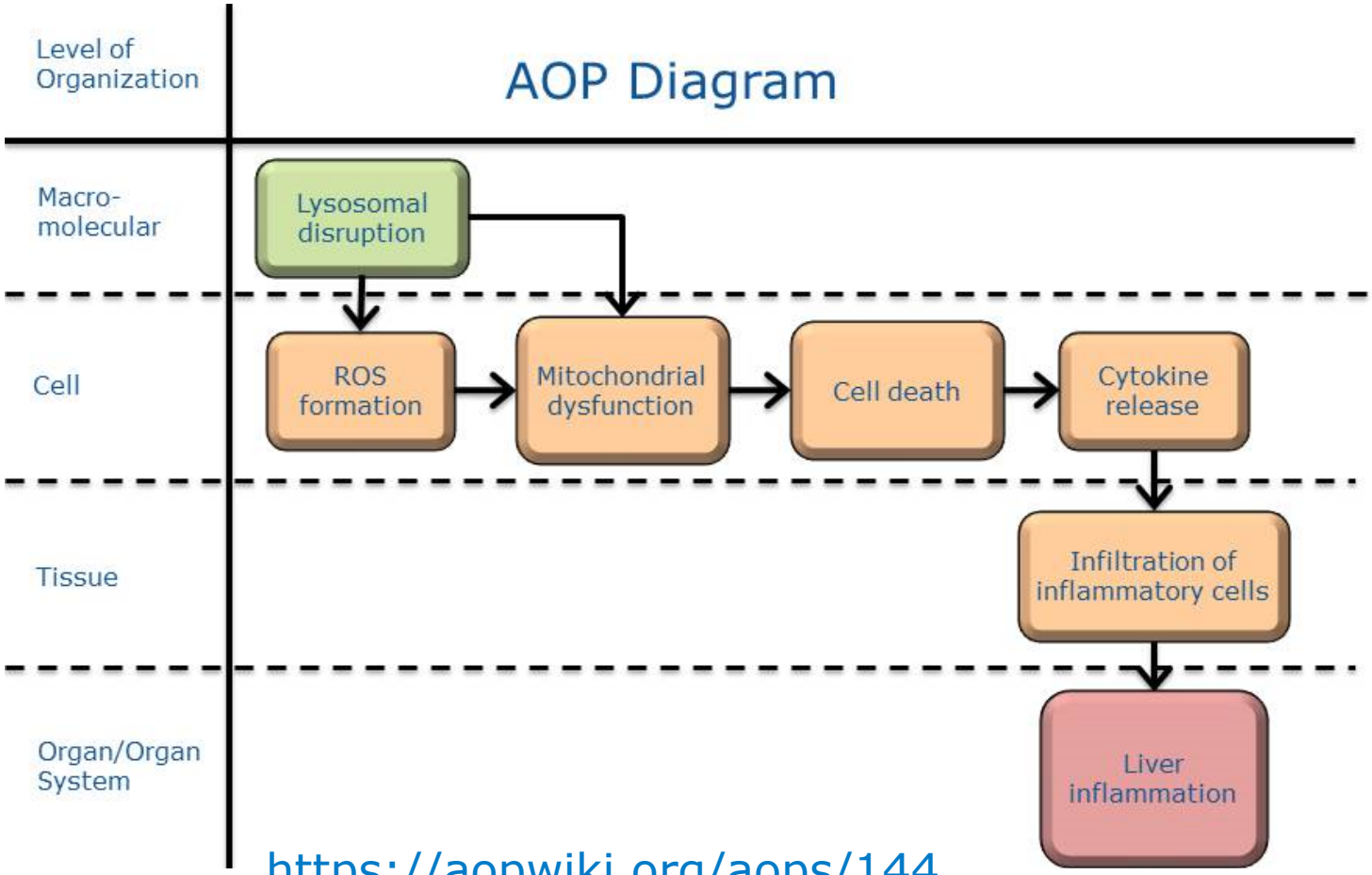
TiO_2 (nano)particles are present in human liver and spleen

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, thus **risk cannot be excluded**.

If adverse (liver) effects occur due to exposure to TiO_2 remains unclear. Hazard studies have limitations.

Risk assessment of NPs better based on internal concentrations

Closer look on liver effects: mechanisms



https://aopwiki.org/aops/144

Recommendation

- Further (sub)chronic oral toxicity study with TiO_2 as used in food
  - Include determination of 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.

- KRISS performed oral 90-d study (GLP, OECD TG 408)
  - No internal concentrations
  - OECD TG 408: measure >2 of 5 enzymes as marker of liver toxicity

Present

I. EOGRT test was requested by EFSA ANS Panel, based on 2016 evaluation, now awaiting results.

II. Intestine results:

- Urrutia-Ortega et al. (2016), Bettini et al. (2017) and Ruiz et al. (2017): TiO_2 exacerbates colitis, causes tumours in colitis-induced mice, causes increase in intestinal lesions in healthy mice.
- Proquin et al. (2018): gene expression changes in colon after TiO2 exposure

→ Can TiO_2 NP (and thus E171) cause colon cancer? (analogy with lung cancer?)

→ 3 underlying questions identified

Question 1: absorption in colon?

- Ruiz et al. (2017), mouse: No TiO_2 in colon, only in small intestine
  - Induced colititis worsened (in colon)
- Bettini et al. (2017), rat: TiO_2 in cells of PP and in colon mucosa
  - Initiation and promotion of preneoplastic lesions in colon
- Urrutia-Ortega et al. (2016), mouse: TiO_2 in colon cells
  - only colon investigated: preneoplastic lesions in distal colon and particles in colon cells
- Proquin et al. (2018), mouse: TiO_2 not analyzed
  - only distal colon investigated: gene expression changes and hyperplasia

→ Why inconsistency in detection TiO_2 in colon cells?

→ Is absorption in colon cells necessary?

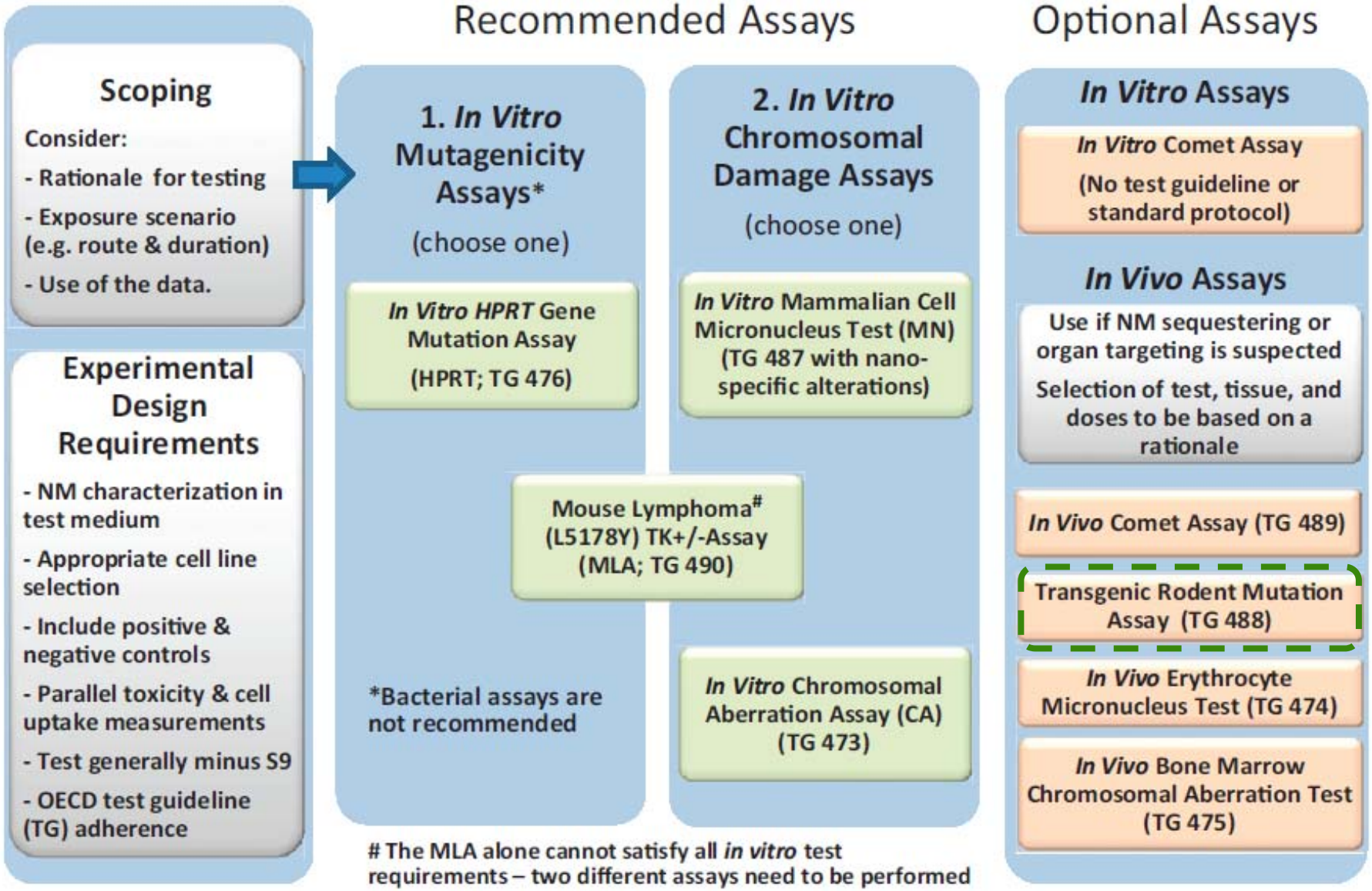
Question 2: genotoxic mechanism (initiation)?

- Analysis genotoxicity Möller et al. (2017): WoE *in vitro* points to DNA strand breaking potential, *in vivo* insufficient quality data
- Meta-analysis *in vitro* genotoxicity data by Charles et al (2018): unclear if genotoxic potential (88 tests in 36 publications included after quality check)
- GCCT 2018: in vitro comet assay not reliable, in vitro MN only with some additional requirements, for in vivo MN assay show exposure of bone marrow/erythrocytes

Shareen Doak: seems to be indirect mechanism:

Current in vitro tests not built to pick up indirect genotox → 3D?

NANOMATERIAL GENOTOXICITY TESTING *ROADMAP*



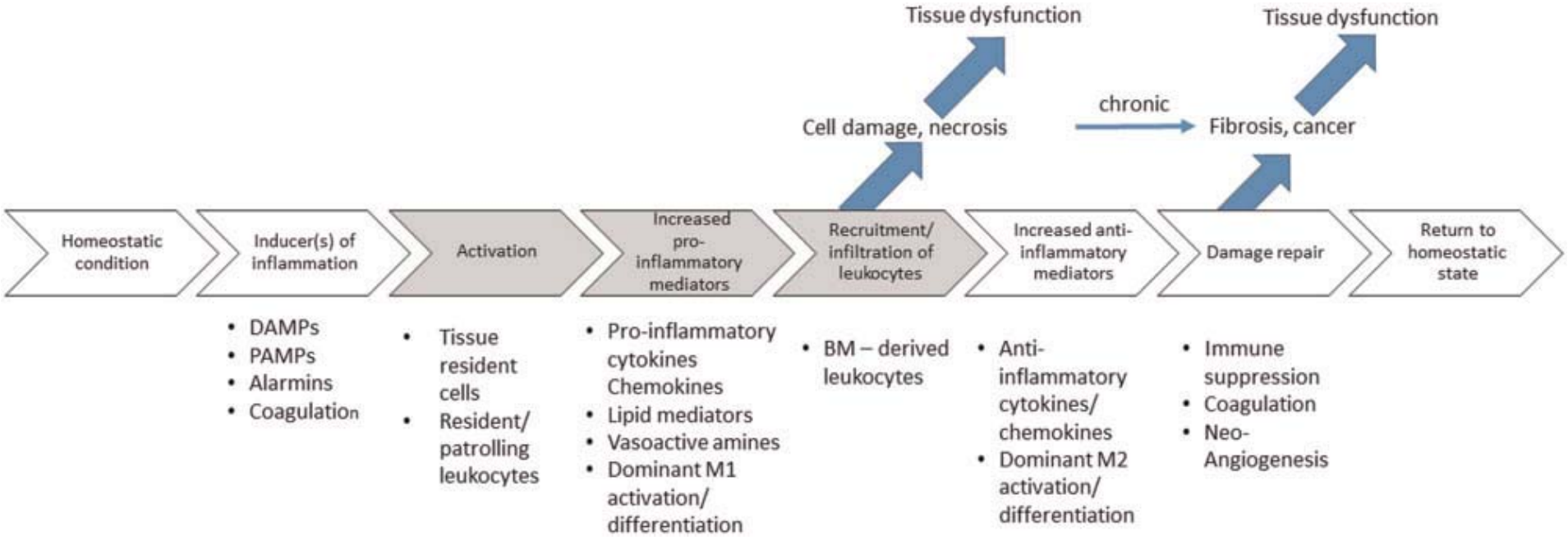
Q3: immunotoxic mechanism (progression)?

Two possible mechanisms hypothesized in literature:

1. Inflammation → cell death → tissue renewal through cell divisions → increased chance mutations are formed and passed on → tumour cells
2. Proquin et al. 2018: immune suppression → tumour cells not destroyed → more tumours

Impression: hardly information on immune suppression: data gap?

AOP inflammation



- Question for participants: how much evidence is there for this mechanism?

Conclusion on current data gaps

- Reprotox, specifically fertility part
- (sub)chronic oral study with internal conc., according to OECD TG, with E171/anatase
  - Include all measurable events of AOPs
- Appropriate genotoxicity study? For different types of TiO_2 NPs
  - First review exact state of data
  - In vivo desirable due to indirect effects
- Immune suppression study
- Review evidence for inflammation pathway? → possibly specific studies recommended

Acknowledgements

Co-authors:

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Min. VWS (Kees Planken) was also closely involved.

July 5th 2018, Amsterdam

Workshop on Possible adverse effects of food additive E171 (titanium dioxide)

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

Inflammatory responses induced by titanium dioxide in the gut

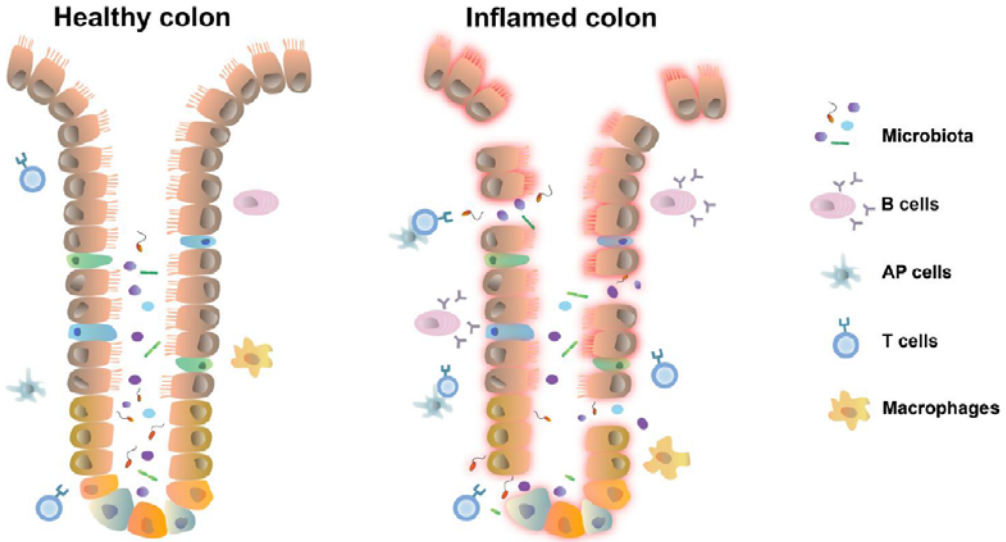
Gerhard Rogler, Department of Gastroenterology and Hepatology, UniversitätsSpital Zürich

UniversitätsSpital Zürich

Universität Zürich[UZH]

Inflammatory bowel disease (IBD)

- Chronic, relapsing autoinflammtion of the colon
- Comprises Colitis ulcerosa and Morbus Crohn
- Increasing prevalence
- Switzerland: 12.000 – 15.000 adult cases
- Severe morbidty / restrictions on quality of life
- Insufficient treatment options / medication



- Causative factor remains unkown
- Multifactorial disease – environmental influences – polygenic risk variants

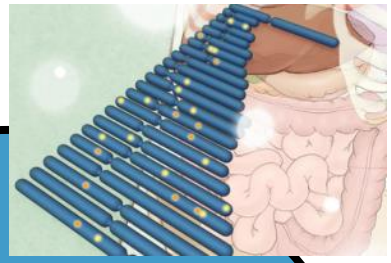
Understanding IBD pathogenesis is essential to improve conditions for patients and health care

Rogler et al. Swiss Medical weekly 2018, Molodecky et al. Gastroenterology 2012, Braegger et al. Gastroenterology 2011

UniversitätsSpital Zürich

Environmental factors and the incidence and course of IBD

Lifestyle/Environ-mental factors in industrialized countries 70%



„genetic susceptibility“ 30%

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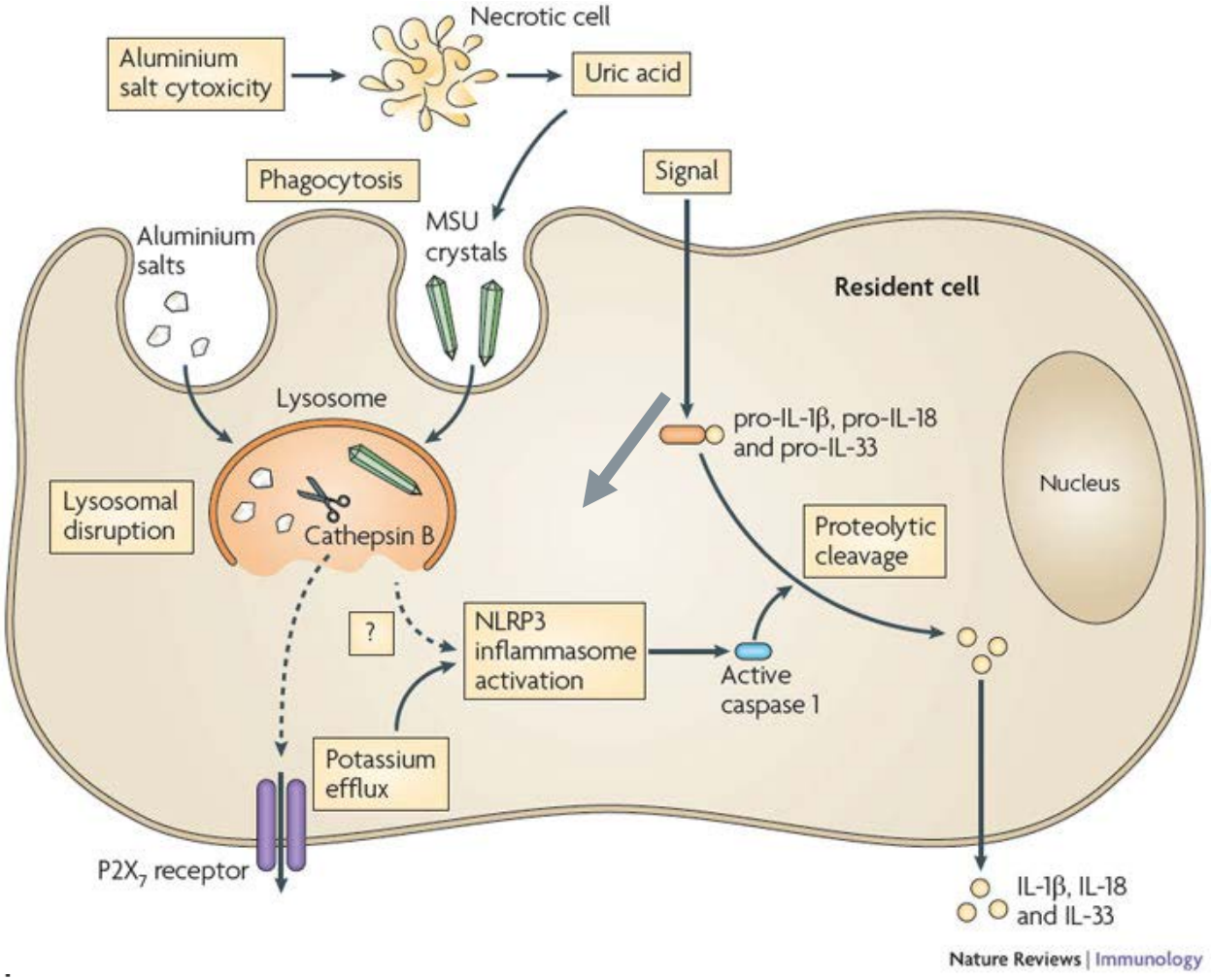
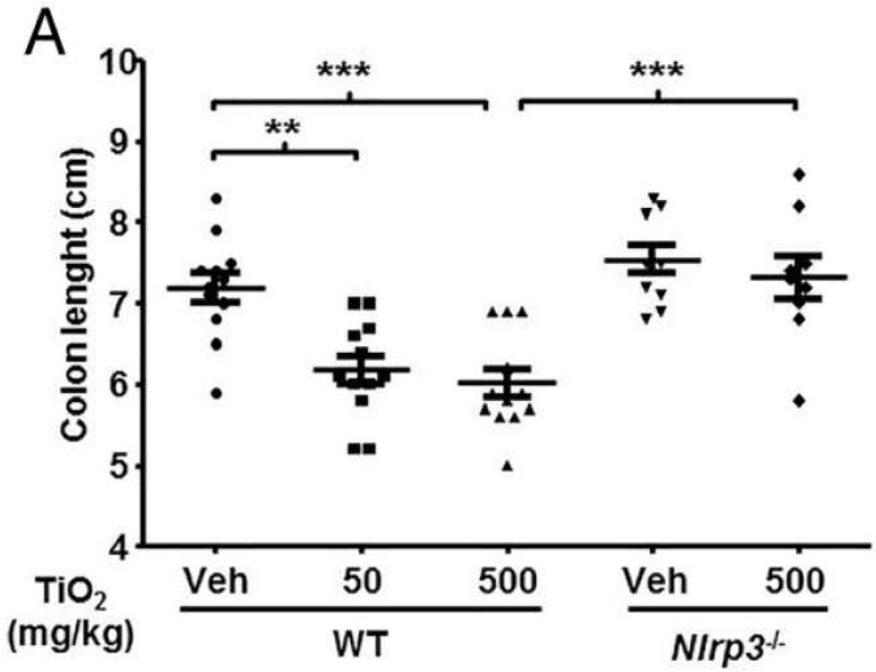
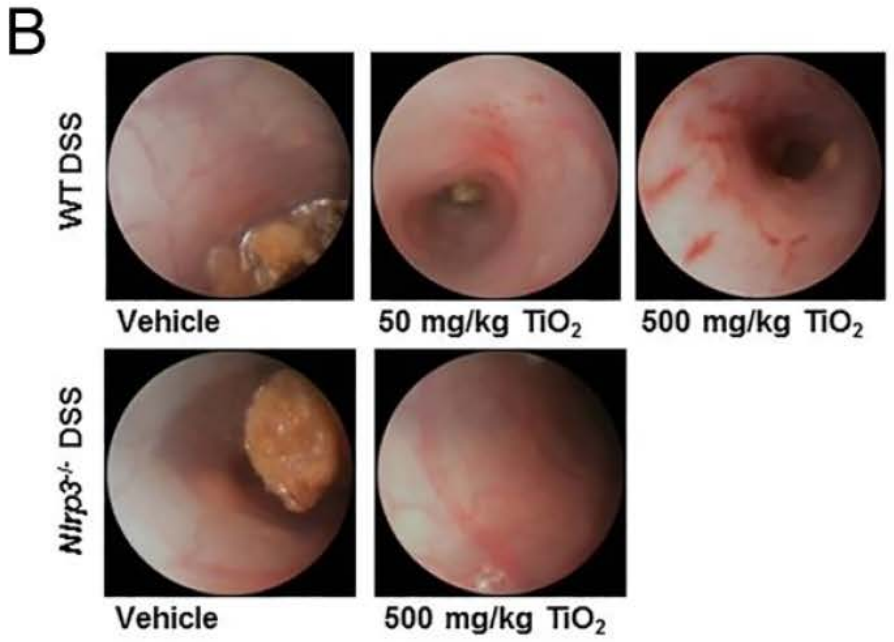
Potential mechanisms of diet: Inflammasome-activation via anorganic particles (not just microbiota)



Adair-Kirk L. et al.(2008)

Administration of TiO2 nanoparticles aggravates DSS colitis





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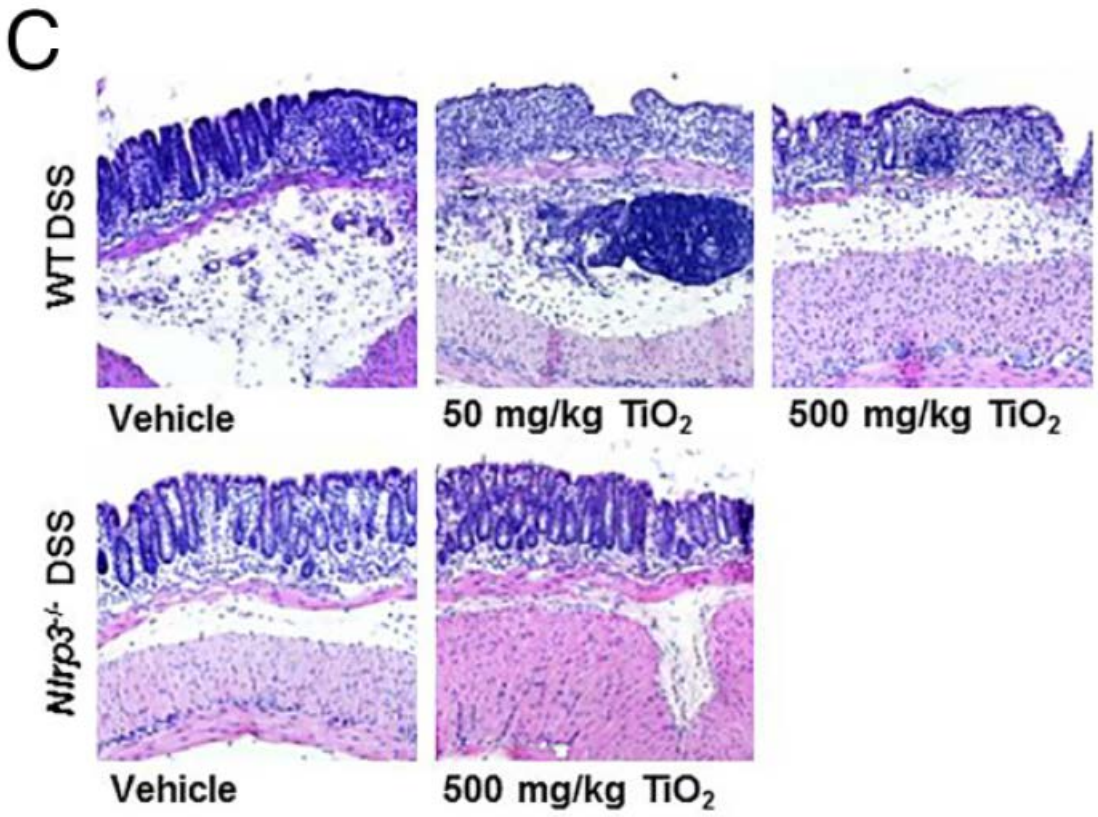
Ruiz PA, et al. Gut 2017;**66**:1216–1224

Administration of TiO2 nanoparticles aggravates DSS colitis



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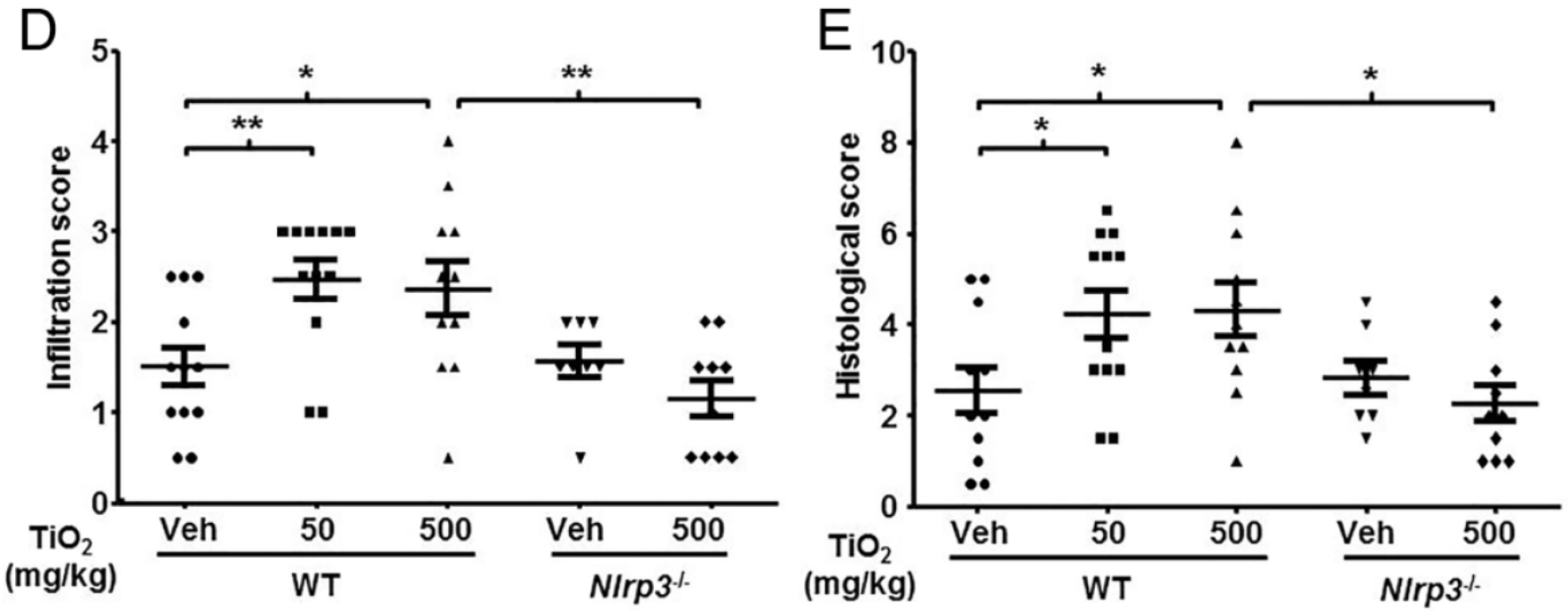
Ruiz PA, et al. Gut 2017;**66**:1216–1224

Administration of TiO2 nanoparticles aggravates DSS colitis



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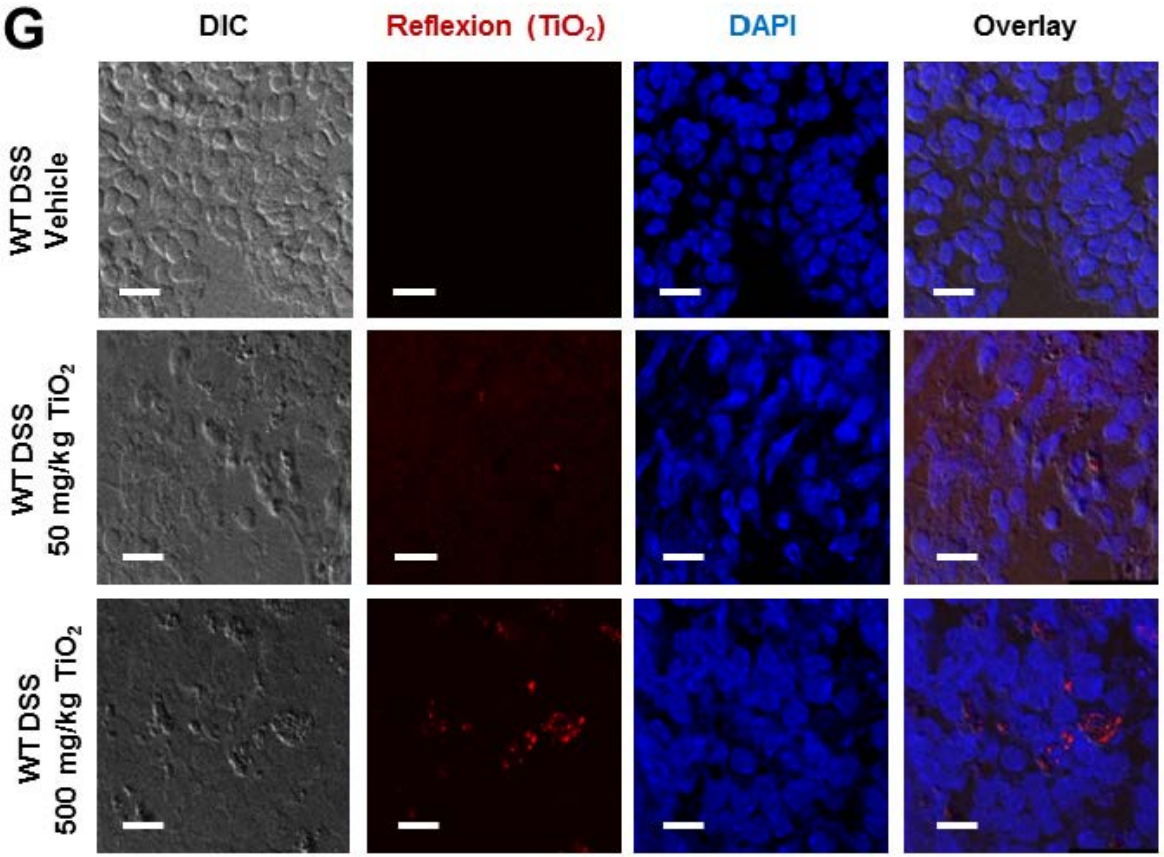
Ruiz PA, et al. Gut 2017;**66**:1216–1224

TiO2 nanoparticles accumulate between enterocytes



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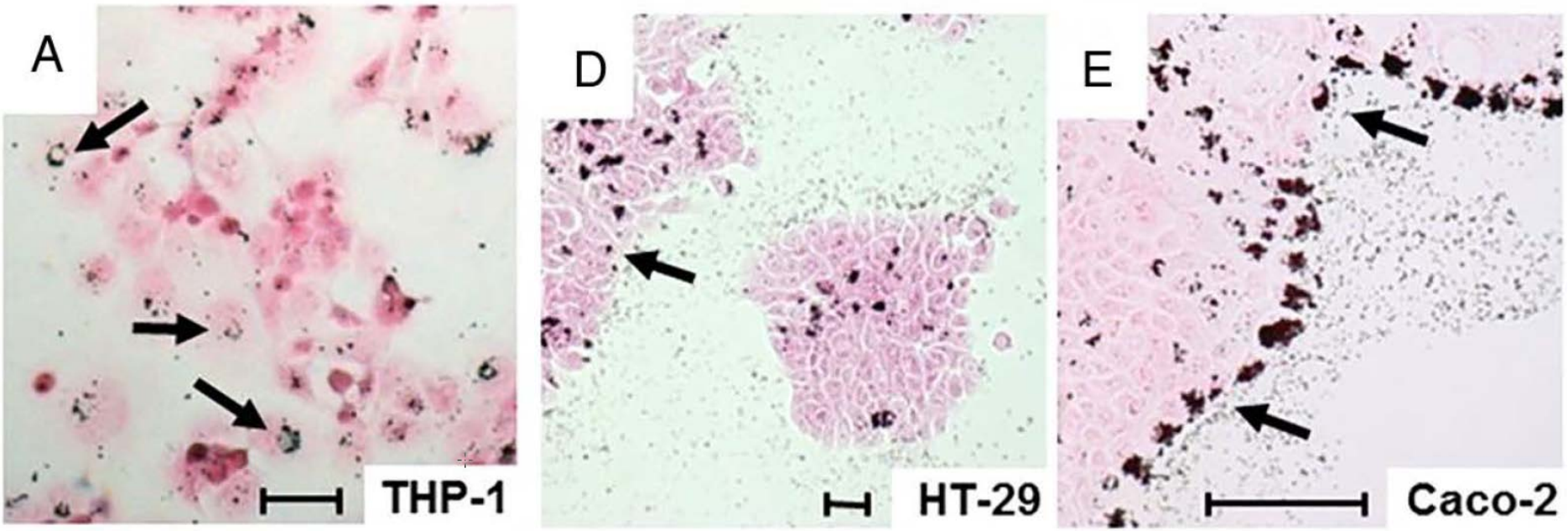
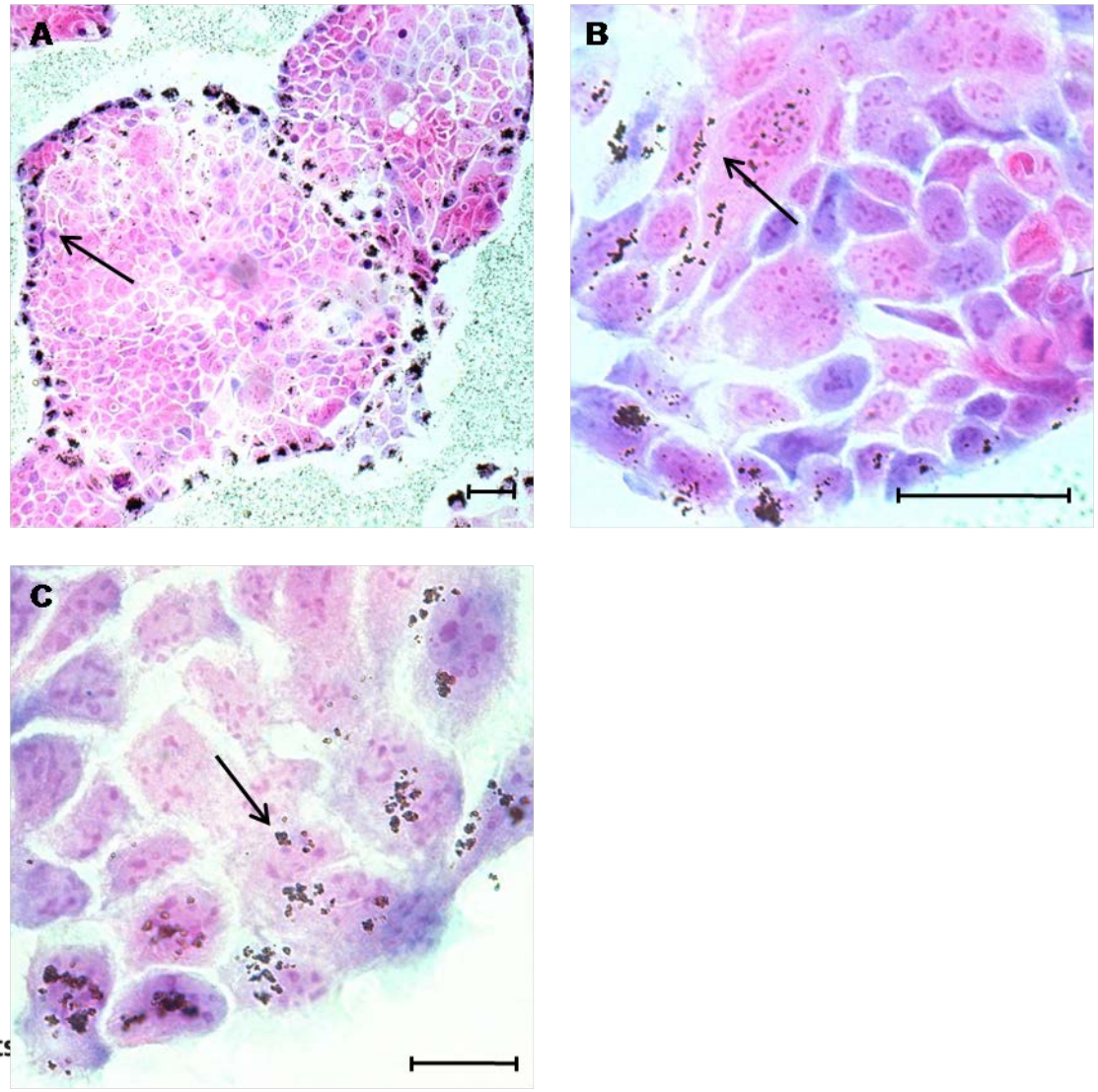
Ruiz PA, et al. Gut 2017;**66**:1216–1224

Aggregates of TiO2 accumulate in macrophages and intestinal epithelial cells (IECs).



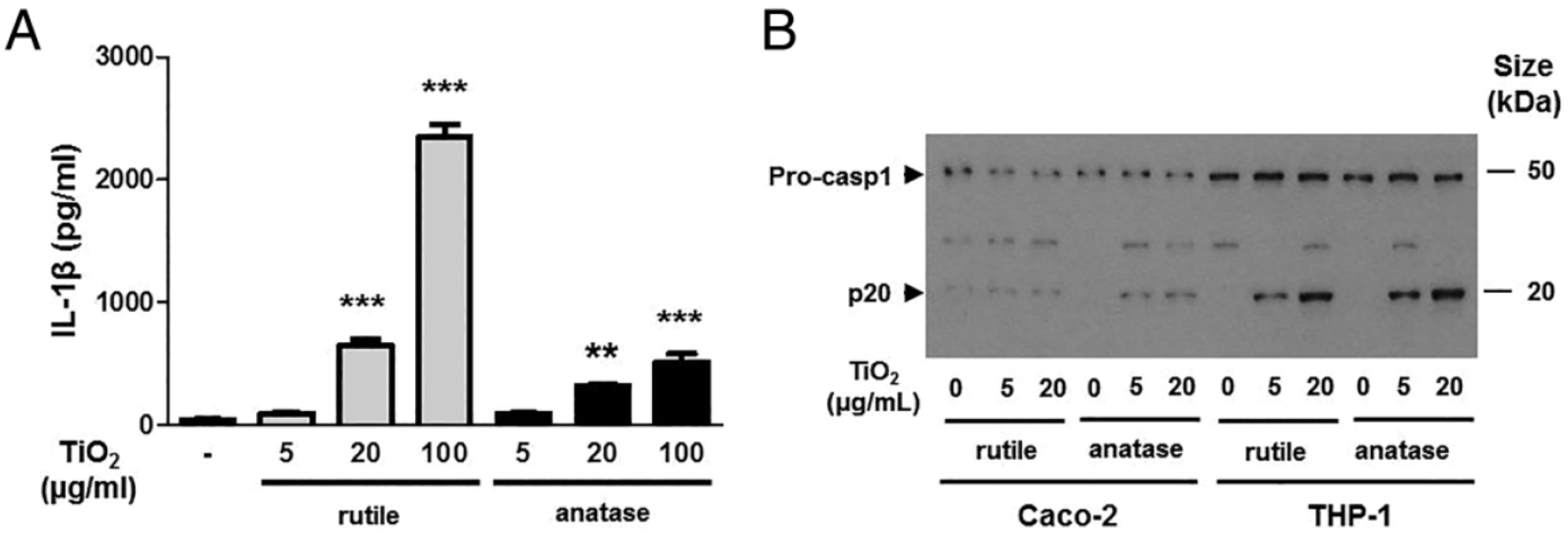
Ruiz PA, et al. Gut 2017;**66**:1216–1224

Aggregates of TiO2 accumulate in IECs



unpublished

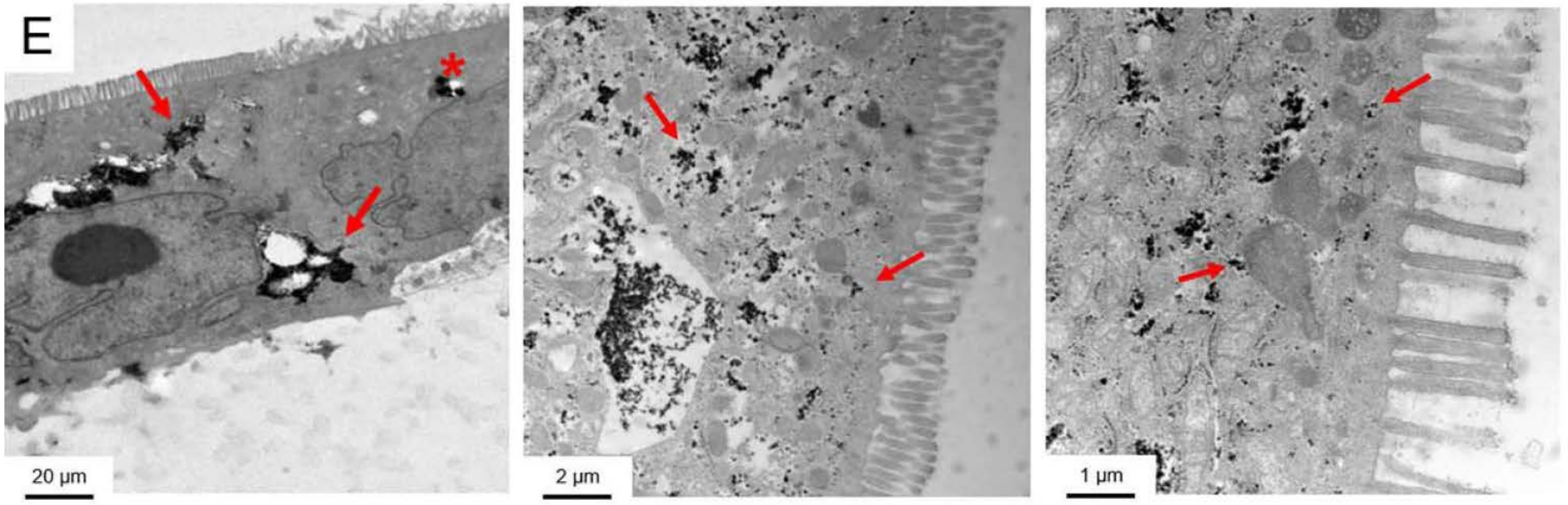
Caspase-1 downstream effectors are activated in macrophages and intestinal epithelial cells (IECs) on exposure to TiO2

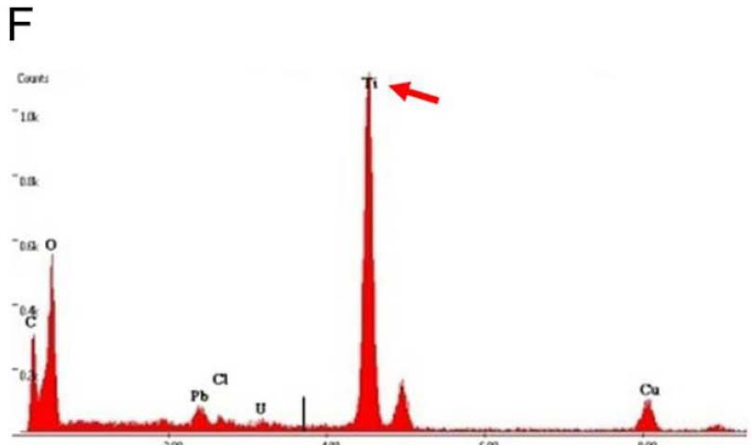


UniversitätsSpital Zürich

Ruiz PA, et al. Gut 2017;**66**:1216–1224

Aggregates of TiO2 accumulate in IECs





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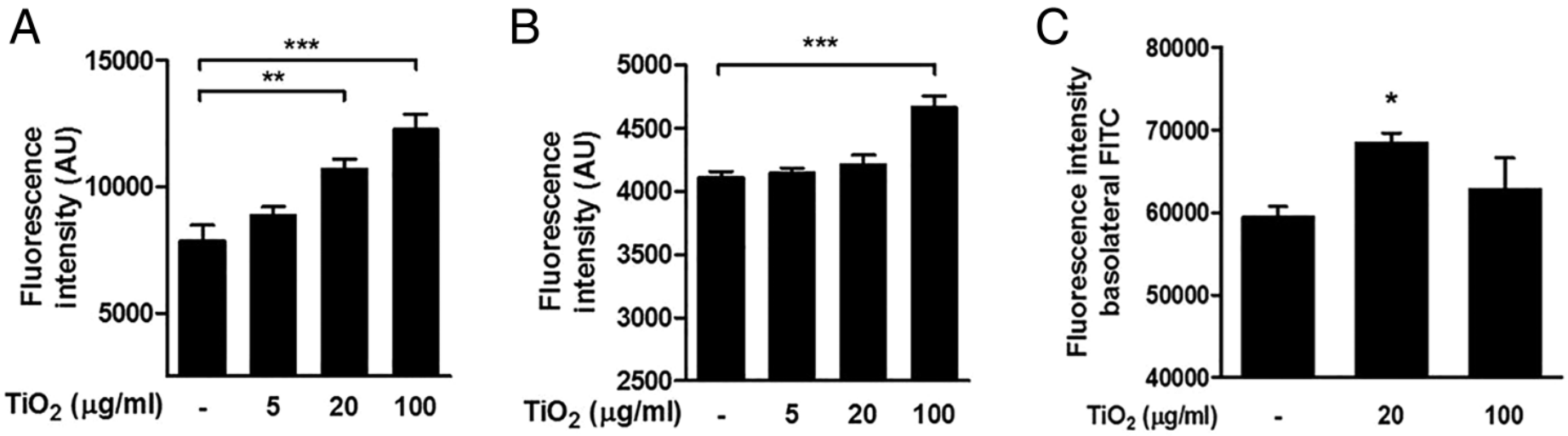
Ruiz PA, et al. Gut 2017;**66**:1216–1224

TiO2 triggers production of reactive oxygen species (ROS) and influences epithelial permeability



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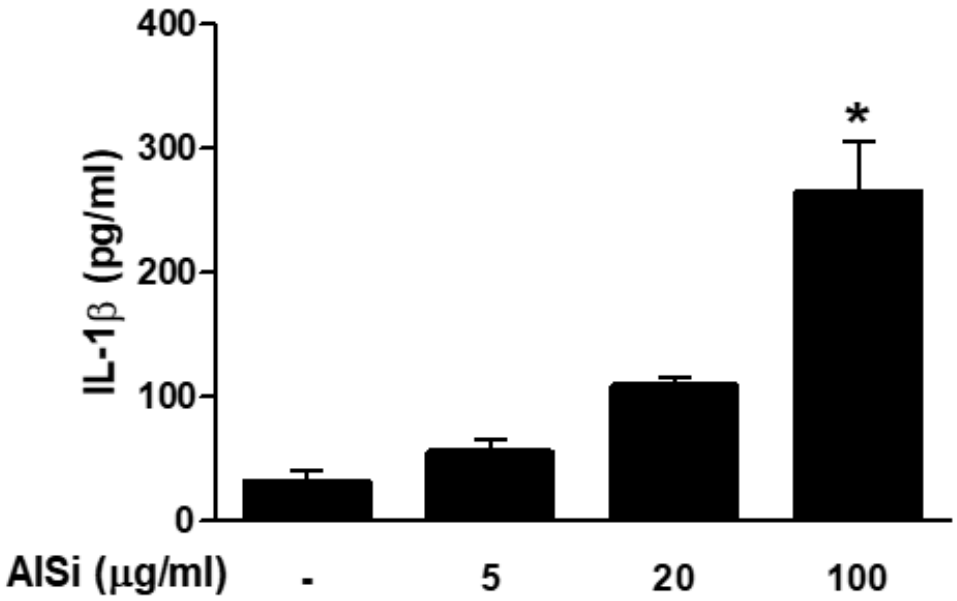
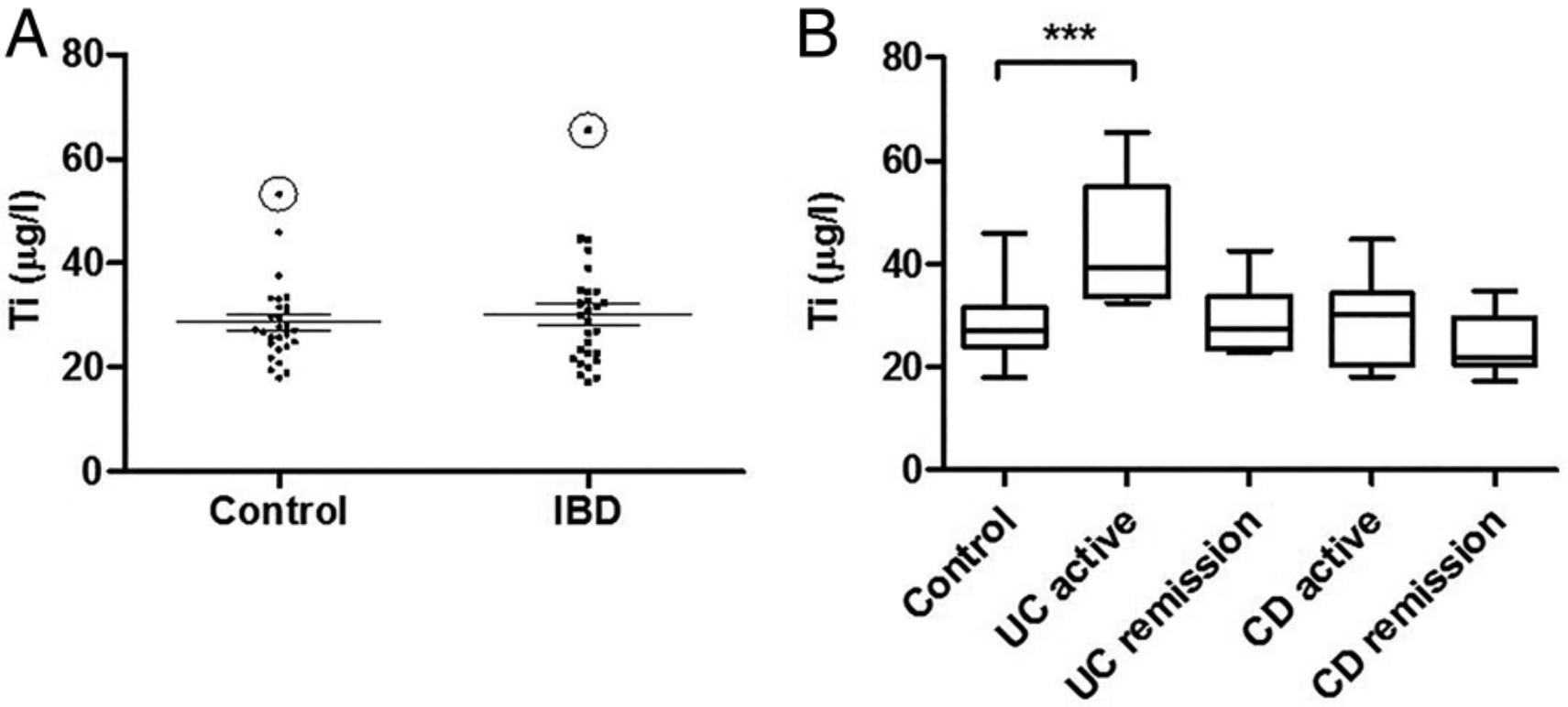
Ruiz PA, et al. Gut 2017;**66**:1216–1224

The immune-stimulating effect is not “specific” for TiO2



unpublished

Patients with IBD present elevated titanium levels in blood



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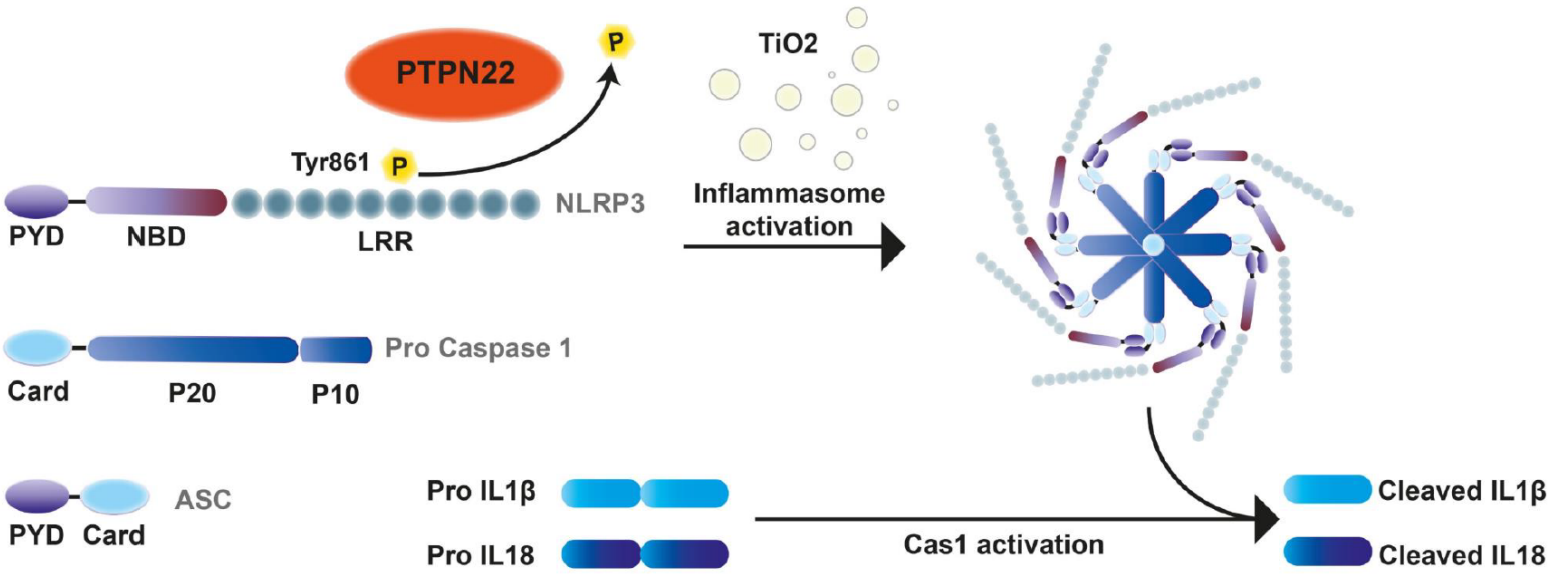
Ruiz PA, et al. Gut 2017;**66**:1216–1224

Inflammasome («TiO2 receptor») and PTPN-22



Men: **CD patients reveal increased IL18 and IL1β beta levels**

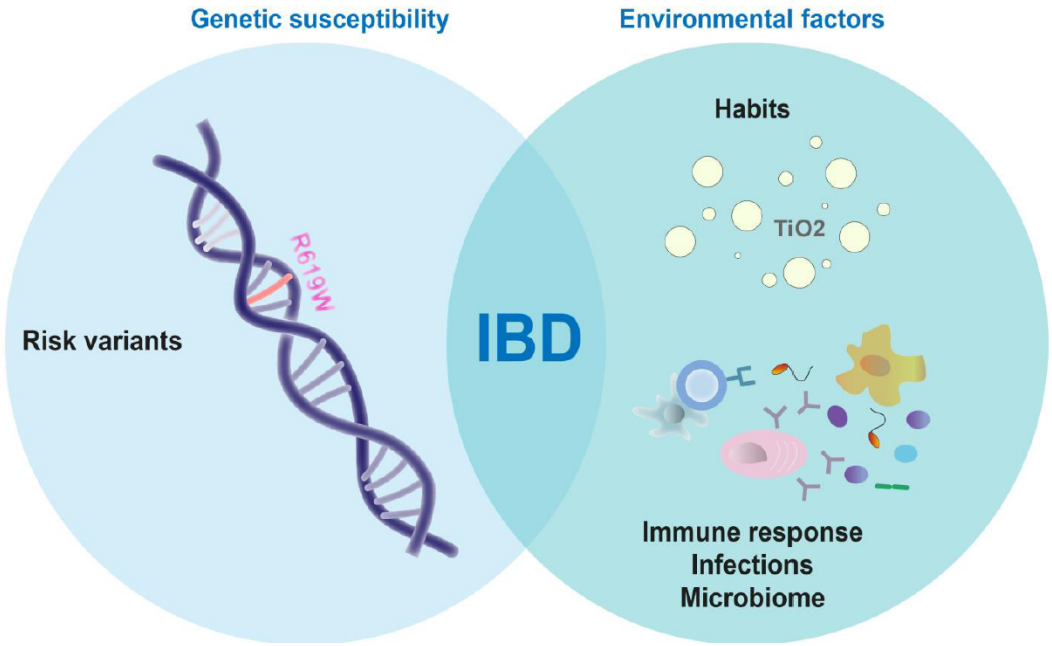
Mice: **PTPN22 KO results in increased NLRP3 phosophorylation and decreased activation**

R619W variant enables faster and more efficient NLRP3 dephoshorylation and subsequent activation

Pizarro et al. J Immunol. 1999, Schmidt et al. Int J Colorectal Dis. 2007, Spalinger et al. JCI. 2016, Spalinger et al. Autophagy 2017, Dai et al. JCI 2013

Present reasearch activities

- Investigate the correlation between environmental factors and genetic risk variants in IBD pathology
- Characterize the role PTPN22 is playing in regulating TiO2 mediated inflammasome activity
- Decode the protective mechanism of the R619W variant and its potential to modulate inflammasome activity
- Depict the systemic effects of TiO2 in different organs, especially in the GIT



What are the new findings?

- Oral administration of titanium dioxide nanoparticles worsens intestinal inflammation in the dextran sodium sulfate (DSS) mouse model of colitis.
- Titanium dioxide crystals accumulate in the spleen of DSS-treated mice following oral gavage.
- Titanium dioxide particles accumulate and activate the NLRP3 inflammasome in human intestinal epithelial cells and macrophages.
- Levels of titanium are increased in the blood of patients with IBD.

Ruiz PA, et al. Gut 2017;**66**:1216–1224

How might it impact on clinical practice in the foreseeable future?

- Components of the inflammasome may represent novel therapeutic targets for the treatment of IBD.
- Our results suggest a cautionary use of titanium dioxide in pharmaceutical formulations and support a therapeutic benefit from low inorganic particle diet in patients with IBD.

Ruiz PA, et al. Gut 2017;**66**:1216–1224

Thank you for your attention!

Prof. Dr. Michael Scharl
Prof. Dr. Dr. Gerhard Rogler
Dr. Marianne Spalinger
Dr. Anja Moncsek
Silvia Lang
Kirstin Atrott
Katharina Bäbler
Philipp Busenhart
Larissa Hering
Egle Katkeviciute
Anna Niechcial

UniversitätsSpital Zürich

imMed

Universität Zürich[UZH]

Workshop on possible adverse effects of food additive TiO_2
Amsterdam, July 5-6th, 2018

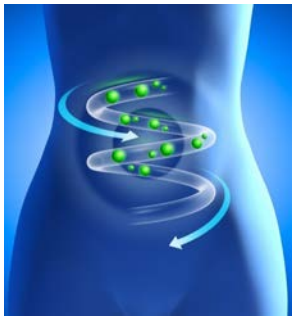
Eric HOUDEAU, PhD (eric.houdeau@inra.fr)
INRA Toxalim, Toulouse, France : www6.toulouse.inra.fr/toxalim

Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

Fate and long-term effects of E171 in the GI tract under « normal » conditions

INRA
SCIENCE & IMPACT

Fate of E171 into the gastrointestinal (GI) tract : the intestine as first target !

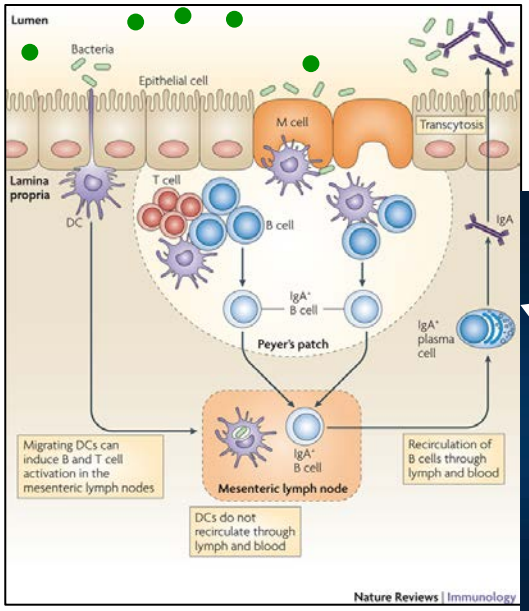


Gut : *an underestimated target in the toxicity assessment of chemicals !*

… often considered as an « entry » for xenobiotics, i.e., the absorption site (**bioavailability**) before distribution in the organism / elimination (**toxicokinetic**), with studies dedicated to systemic effects (**hazard caracterization** for development, reprotox, neurotox […]).

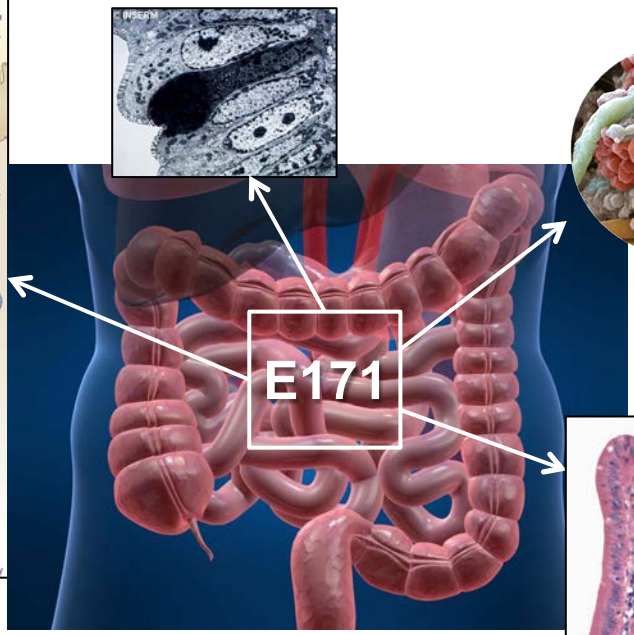
Mucus Goblet cells (mucin secretion)
Mucus layer: 40-300µm thickness in human

Trapping properties *or* local reservoir ?



Immune system
Gut-Associated Lymhoid Tissue (GALT)
Oral tolerance / Host defenses

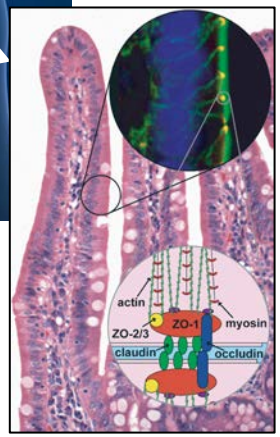
Allergy-Intolerance / Suceptibility to inflammation ?



Microbiota
$10^{12\text{-}14}$ micro-organisms
digestion, immune system homeostasy, barrier to pathogens…

Mainly in the colon !
slow transit = resident bacteria

Dysbiosis (ecology/activity) ?
Immune system dysfunction, metabolic diseases, brain activity….



Epithelium
Gut barrier
towards bacteria, viruses, xenobiotics…

Host homeostasy
nutrient/water absorption, hydro-electrolytic exchanges

NP translocation ?
Barrier dysfunction ?
Colorectal cancer ?

Workshop on possible adverse effects of food additive TiO_2

Amsterdam, July 5-6th, 2018

Data from :

(1) SCIENTIFIC REPORTS

OPEN

Food-grade TiO_2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon

Received: 13 June 2016
Accepted: 06 December 2016
Published: 20 January 2017

Sarah Bettini[1], Elisa Boutet-Robinet[1], Christel Cartier[1], Christine Coméra[1], Eric Gaultier[1], Jacques Dupuy[1], Nathalie Naud[1], Sylviane Taché[1], Patrick Grysan[2], Solenn Reguer[3], Nathalie Thieriet[4], Matthieu Réfrégiers[3], Dominique Thiaudière[3], Jean-Pierre Cravedi[1], Marie Carrière[5,6], Jean-Nicolas Audinot[2], Fabrice H. Pierre[1], Laurence Guzylack-Piriou[1] & Eric Houdeau[1]

(2) frontiers in Microbiology

ORIGINAL RESEARCH

Toxicity of Food-Grade TiO_2 to Commensal Intestinal and Transient Food-Borne Bacteria: New Insights Using Nano-SIMS and Synchrotron UV Fluorescence Imaging

Joanna M. Radziwill-Bienkowska[1], Pauline Talbot[2], Jasper B. J. Kamphuis[3], Véronique Robert[2], Christel Cartier[3], Isabelle Fourquaux[4], Esther Lentzen[5], Jean-Nicolas Audinot[5], Frédéric Jamme[6], Matthieu Réfrégiers[6], Jacek K. Bardowski[1], Philippe Langella[2], Magdalena Kowalczyk[1], Eric Houdeau[3], Muriel Thomas[2] and Muriel Mercier-Bonin[3]

(3)

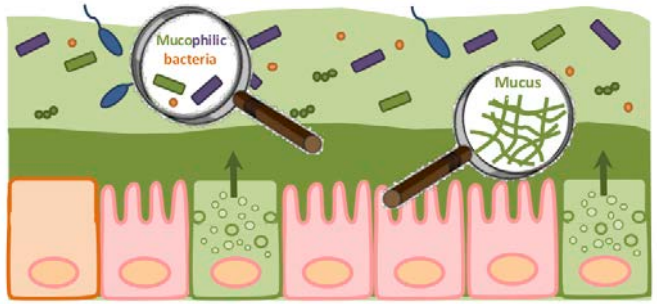
Talbot *et al. J Nanobiotechnol (2018) 16:53*
https://doi.org/10.1186/s12951-018-0379-5

Journal of Nanobiotechnology

RESEARCH Open Access

Food-grade TiO_2 is trapped by intestinal mucus in vitro but does not impair mucin *O*-glycosylation and short-chain fatty acid synthesis in vivo: implications for gut barrier protection

Pauline Talbot[1], Joanna M. Radziwill-Bienkowska[2], Jasper B. J. Kamphuis[3], Karine Steenkeste[4], Sarah Bettini[3], Véronique Robert[1], Marie-Louise Noordine[1], Camille Mayeur[1], Eric Gaultier[3], Philippe Langella[1], Catherine Robbe-Masselot[5], Eric Houdeau[3], Muriel Thomas[1] and Muriel Mercier-Bonin[3*]



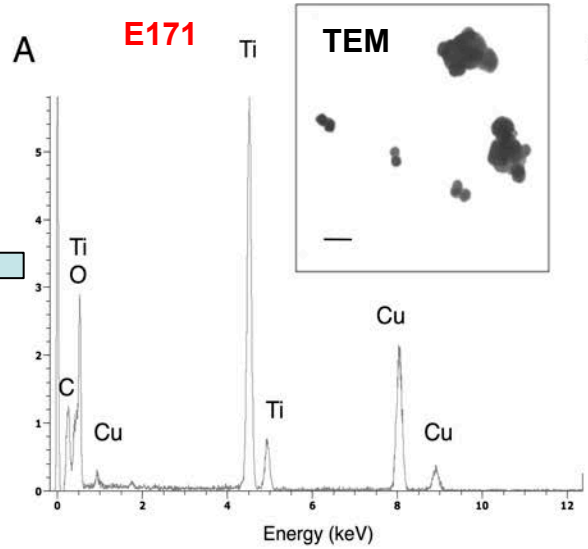
Researchs conducted in the frame of :

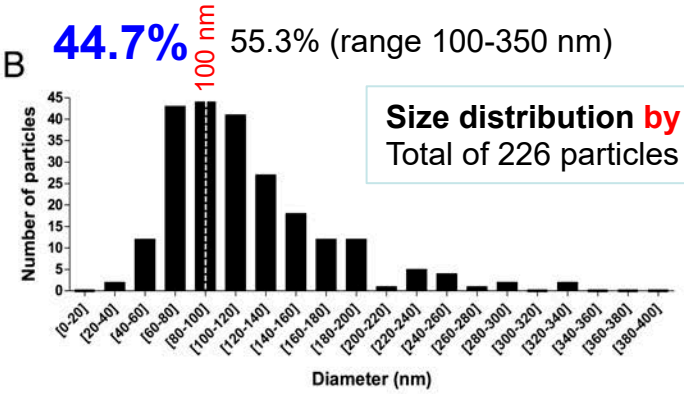
NanoGut anses
PNR-EST-2013-024
and,
Grant from INRA Division
Human Nutrition, Food Safety and Consumer Behaviour

E171 *vs.* ***P25 NM105 :*** *characterization of particle size and crystal form*

Bettini *et al* (2017) **Scientific Reports**

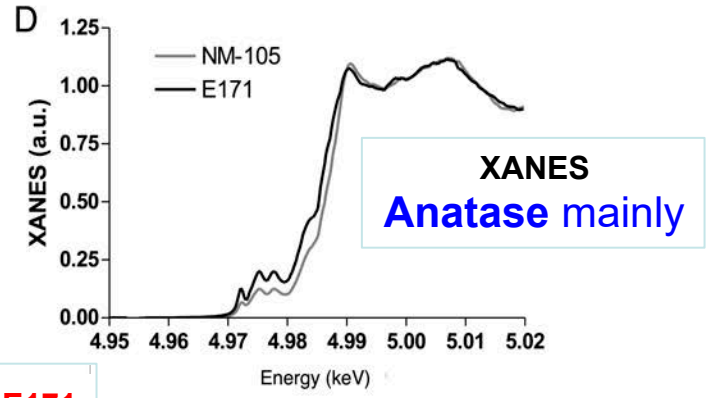
EDX with doubled count time for « trace elements » : **TiO_2** close to purity

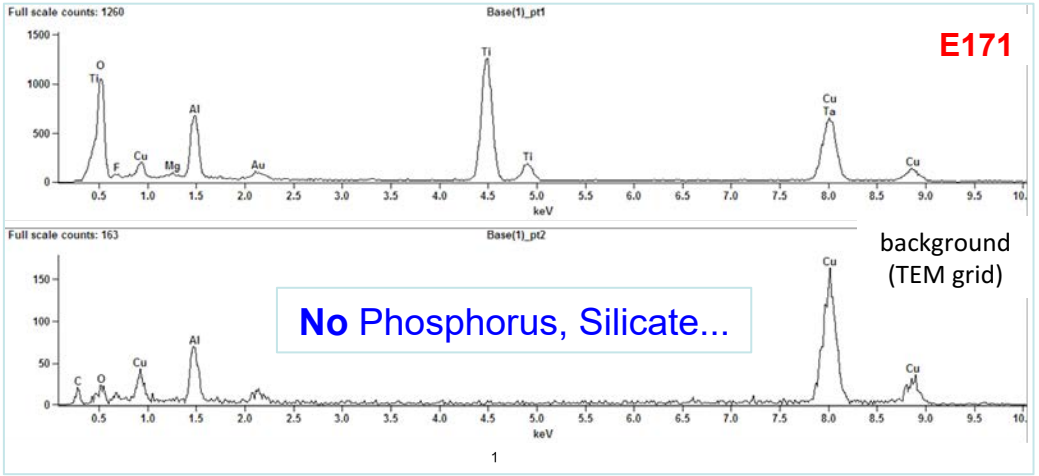




C

	Z average	PdI	ζ (mV)
NM-105	192.23 ± 5.43	0.133 ± 0.014	5.013 ± 0.025
E171	373.5 ± 20.22	0.316±0.048	-23.9 ± 2.36



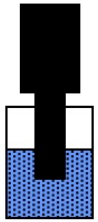


E171 *vs.* ***P25 NM105 :*** *methods used for dispersion and treatments*

Bettini *et al* (2017) **Scientific Reports**

French commercial source

NANOGENOTOX



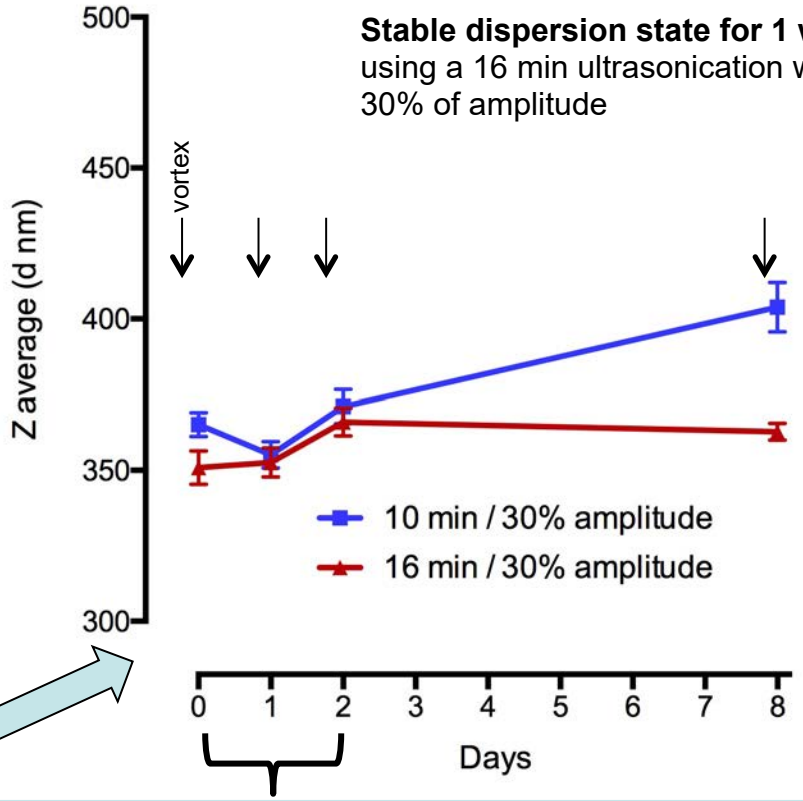
2.56 mg/ml NM Stock Suspension
in EtOH / BSA
(instilled, diluted or dosed into specific test mediums)

Ultrasonication with probe
preliminary tests 10 *vs.*16 min
at 30% amplitude

Hydrodynamic diameter (DLS) with time

(ultrasonicated TiO_2 re-suspended in water)

Stable dispersion state for 1 week
using a 16 min ultrasonication with
30% of amplitude



- **for chronic oral study in rats :** renewal of the E171 suspension in drinking water every 2 days
- **for *in vitro* studies :** stock suspensions always prepared fresh prior to each experiment

Fate of TiO_2 particles along the gut lumen

from interaction with bacteria (food-borne and commensals)
to trapping by the mucus layer
(E171 vs. P25 NM105)

Bettini *et al* (2017) **Scientific Reports**

Radziwill-Bienkowska *et al* (2018) **Frontiers in Microbiology**

Talbot *et al* (2018) **Journal of Nanobiotechnology**

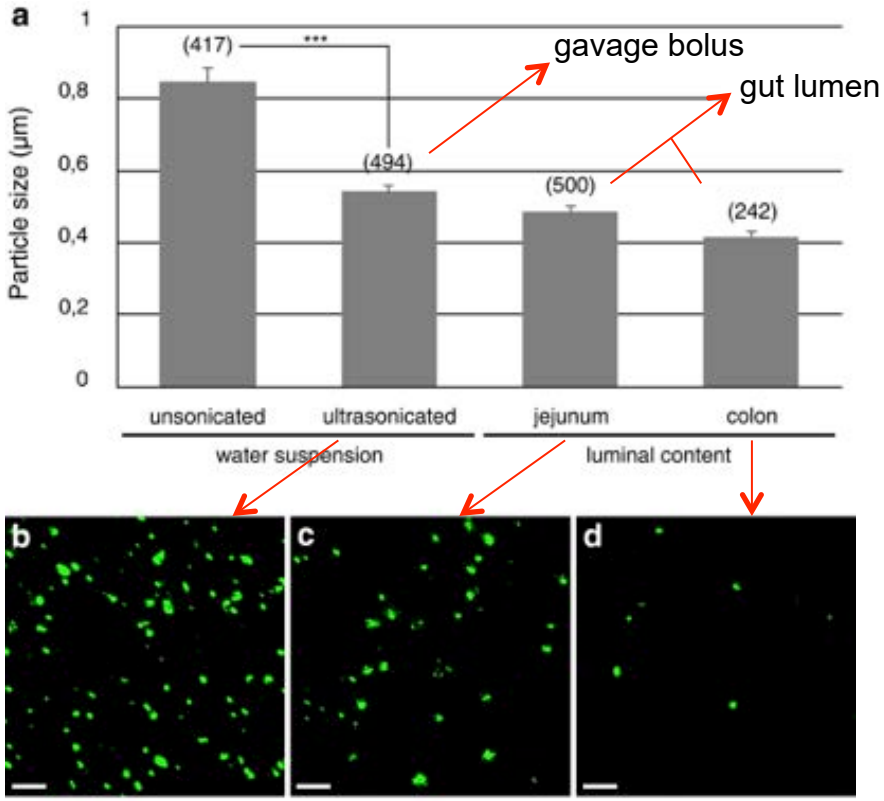
Fate of E171 into the gut lumen : no re-agglomeration in vivo

Bettini *et al* (2017) **Scientific Reports**

In vitro, TiO_2-NP model **re-agglomerate in large clusters** (>1µm) in artificial gastric (pepsin/HCl, pH 2) *and* intestinal (pepsin/HCl, pancreatin and bile extracts, pH 7) phases.

Brun *et al* (2014) Part Fibre Toxicol

In vivo ?



TiO_2 matter size (4h after a single dose in rats) evaluated by confocal microscopy from suspensions spread on glass slides :

- **Similar dispersion state** from the initial water suspensions (**b**) to luminal contents recovered from the small (**c**) and large intestine (**d**).

Laser reflection of TiO_2 was detected with a **magnification given 1 pixel = 50 nm**

Fate of E171 into the gut lumen : *interactions with bacteria*

Bacterial strains

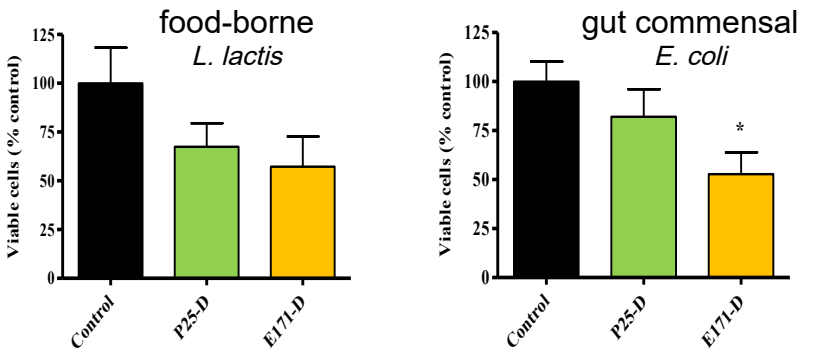
TABLE 1 | List of bacterial strains under study.

commensal intestinal

Species	Strain	Gram characteristics	Origin
Escherichia coli	K12 MG1655	Gram-negative	Laboratory strain
	ATCC8739		Commensal, isolated from infant feces
	CEC15		Commensal, isolated from a conventional suckling rat intestine
Lactobacillus rhamnosus	GG	Gram-positive	Commensal, isolated from a healthy human intestine
Lactobacillus sakei	23K	Gram-positive	Food (meat)
Lactococcus lactis subsp. *cremoris*	IBB477	Gram-positive	Food (raw milk)
Lactococcus lactis subsp. *lactis*	TIL448		Food (peas)
Streptococcus thermophilus	LMD-9	Gram-positive	Food (yogurt)

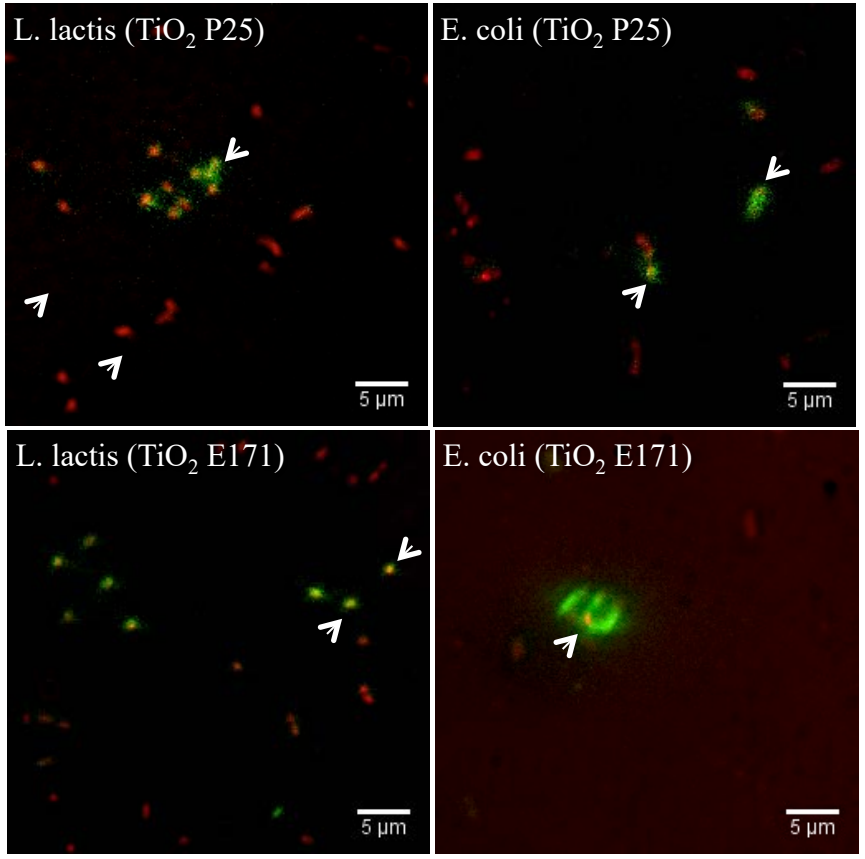
food-borne

Cell viability



Deep UV Synchrotron excitation

TiO_2 (P25 and E171) / bacteria colocalization



Bacteria / Titanium

Yellow (arrowheads) when signals merge

Radziwill-Bienkowska *et al* (2018) **Frontiers in Microbiology**

Fate of E171 into the gut lumen : interactions with bacteria

Radziwill-Bienkowska *et al* (2018) **Frontiers in Microbiology**

Bacterial strains

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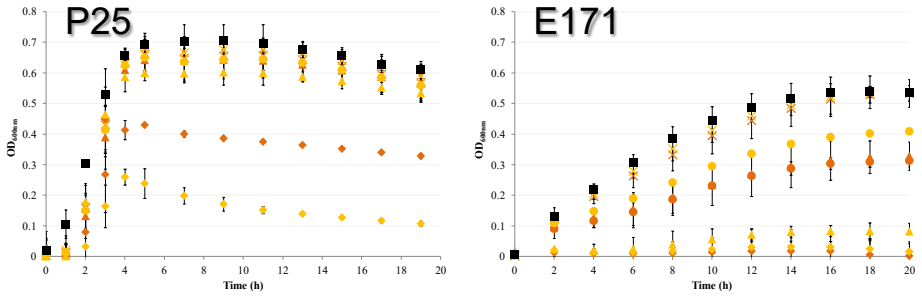
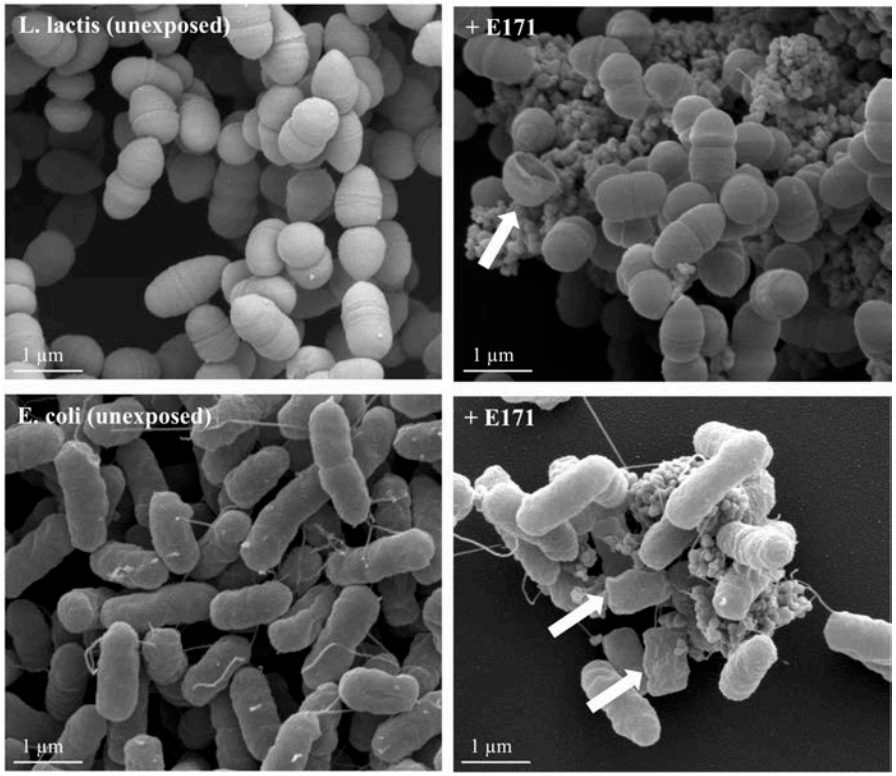
food-borne

Bacterial growth (e.g., *E. coli*)



control, x 32.0, 62.5, 125.0, 320.0 µg/mL
(light colors = dispersed; dark colors = no dispersion)

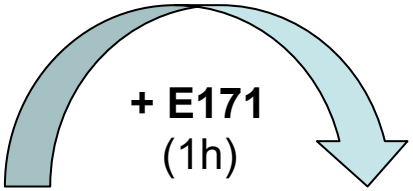
Morphological damages

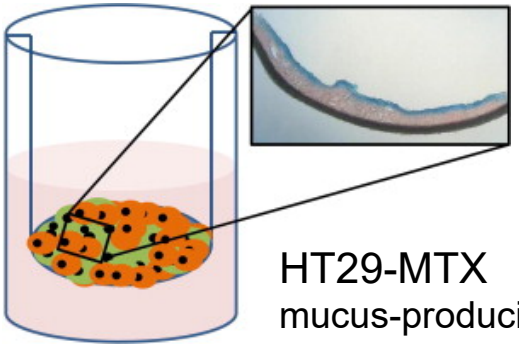


- ✓ Delayed bacterial growth (bacteriostatic) *and* altered cell viability whatever the strain (E171 > P25)
- ✓ *E. coli* exposed to the food additive E171 showed the most striking effects
- ✓ Consequences under chronic conditions (long-term exposure in human)?

Fate of E171 into the gut lumen : *interactions with the mucus layer*

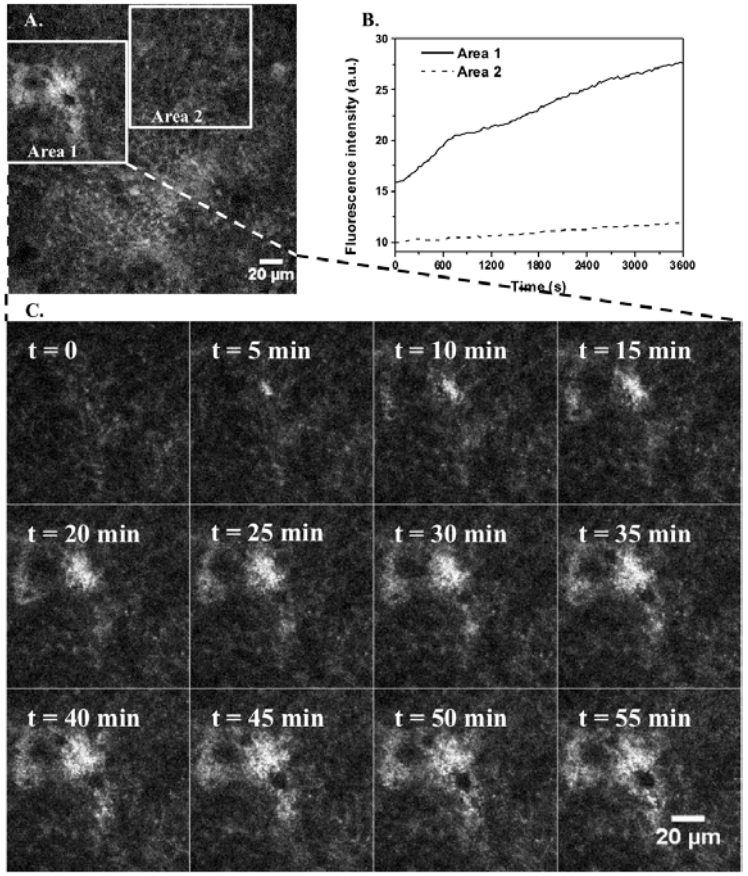
Talbot *et al* (2018) **Journal of Nanobiotechnology**





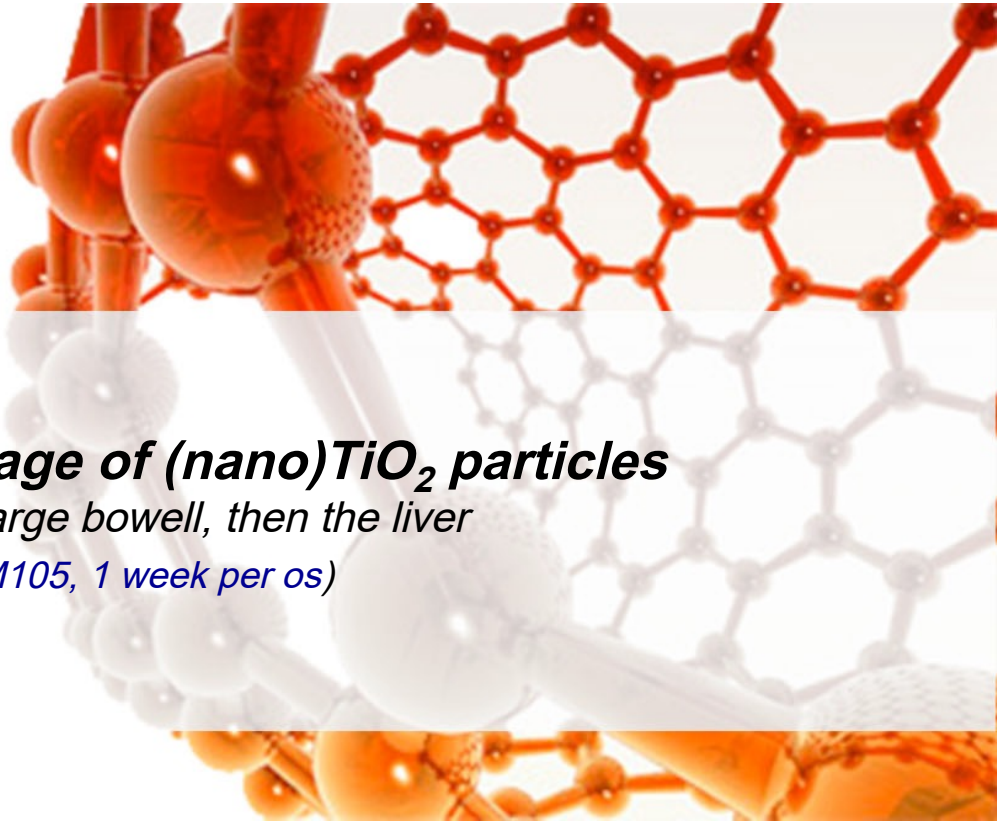
HT29-MTX
mucus-producing cells

(schema from Béduneau *et al* (2014) **Eur J Pharm Biopharm**)



Evolution of TiO_2-fluorescence intensity over time :

- ✓ Accumulation of TiO_2 particles into the mucus layer
- ✓ Region-specific accumulation (e.g., *present* in **Area 1** / *absent* in **Area 2**), where particle matter « sticks on » islets of secreted mucins (18-20 µm above the cell surface)
- ✓ In rats exposed to E171 (60 days) : no effect on mucin *O*-glycosylation *in vivo* (oligosaccharide are « sugars » for commensal bacteria, and adhesion sites for them)
- ✓ No mucus barrier impairment in an healthy gut, but « trapping » properties, possibly increasing TiO_2 particle contact to epihelial surface (with *or* without absorption?)



Lumen-to-mucosal passage of (nano)TiO_2 particles

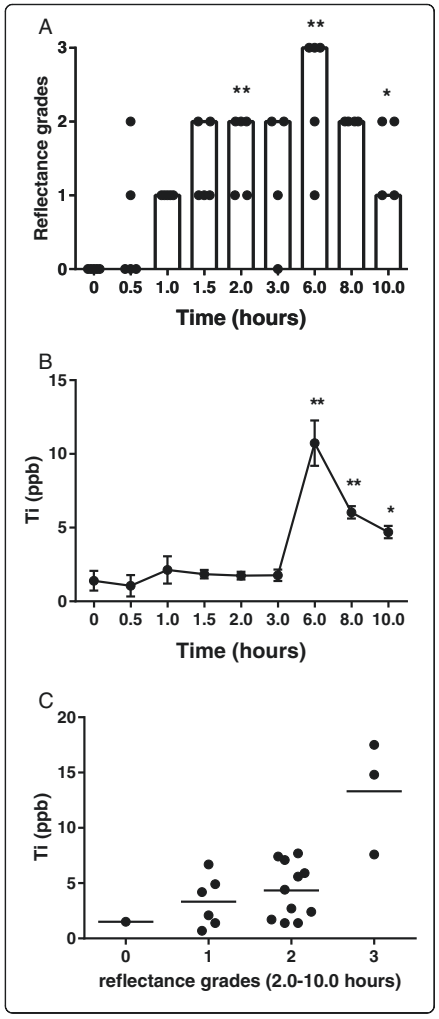
from the small to the large bowell, then the liver

(E171 vs. P25 NM105, 1 week per os)

Bettini *et al* (2017) **Scientific Reports**

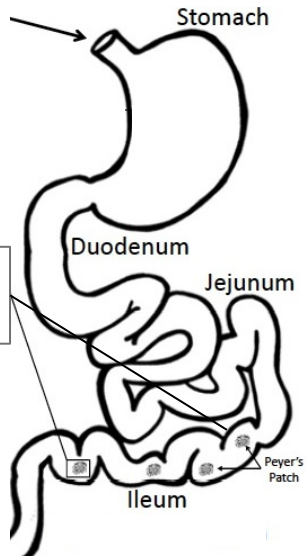
Absorption along the gut : *single dose studies*

Human volonteers
Single oral dose (≈1 mg/kg BW)
TiO_2 detected in blood



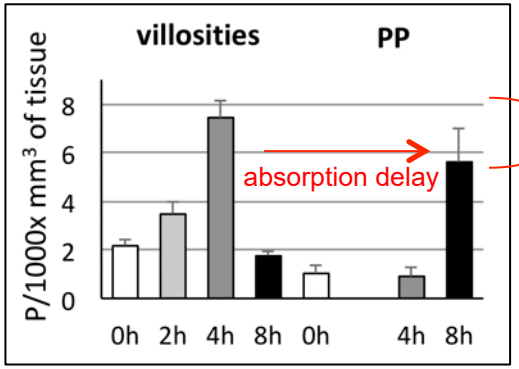
Pele *et al* (2015) **Part Fibre Toxicol**

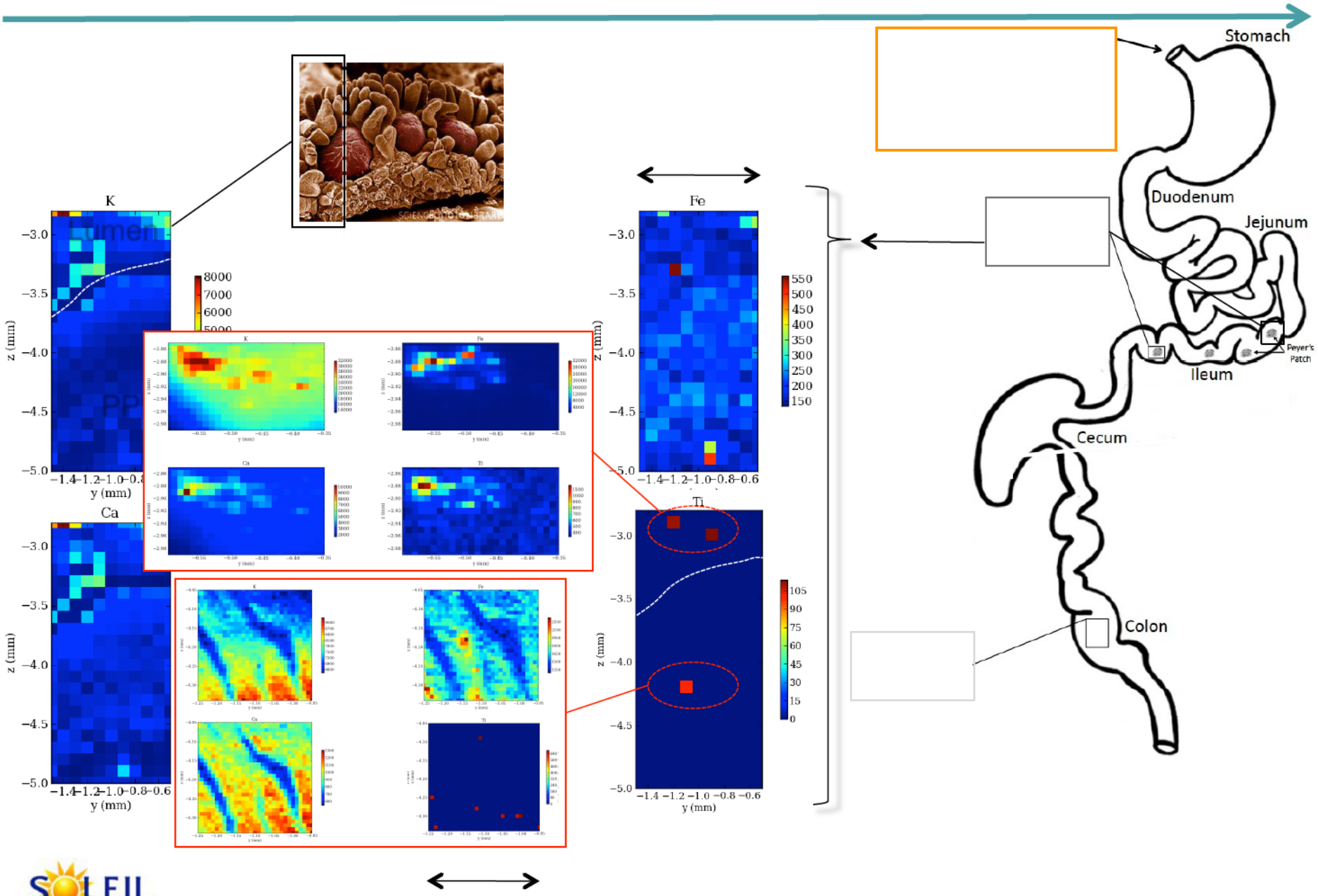
Peyer's patches



- ✓ Rapid passage to the bloodstream (from 1-2h)
- ✓ Bimodal pattern of TiO_2 particle uptake :
  - enterocytes/ villosities (visible by 2h after ingestion)
  - Peyer' patches (retention time / peak at 6h)

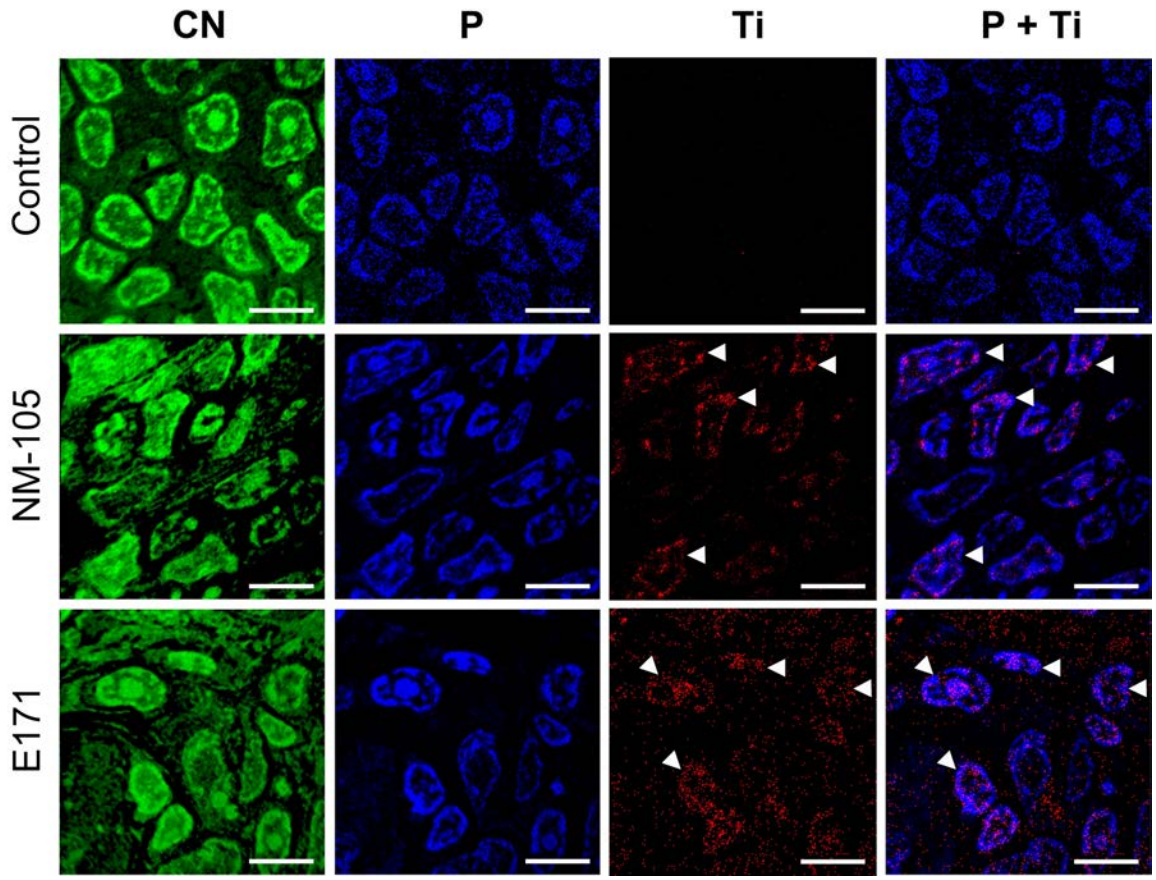
Mice
Single oral dose (40 mg/kg BW)
Comera *et al* (to be published)




Stomach
Duodenum
Jejunum
Peyer's Patch
Ileum
Cecum
Colon
K
Ca
Fe
Ti
z (mm)
y (mm)
lumen
PP

E171 or *TiO_2-NP model in Peyer's patches : nanoSIMS analyses*

Bettini *et al* (2017) **Scientific Reports**



Subcellular Ti distribution into immune cells

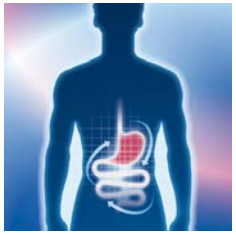
Mass spectrometry detection of $^{48}Ti^{16}O$ clusters

(1 pixel ≈ 80-100 nm / Scale bars 5 μm)

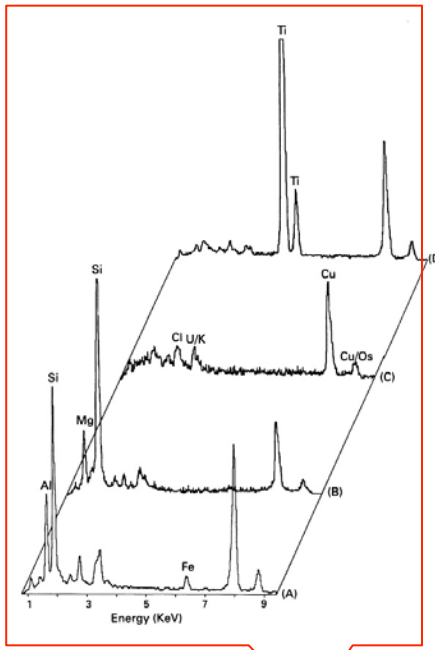
E171 = P25 NM-105

Intestine and mineral particles/additives

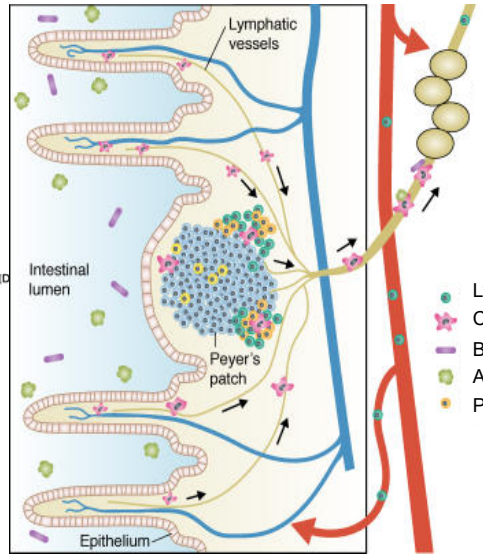
low absorption (<1%) *but* local accumulation with age



Metal oxide particle accumulation in Peyer's patches in Humans



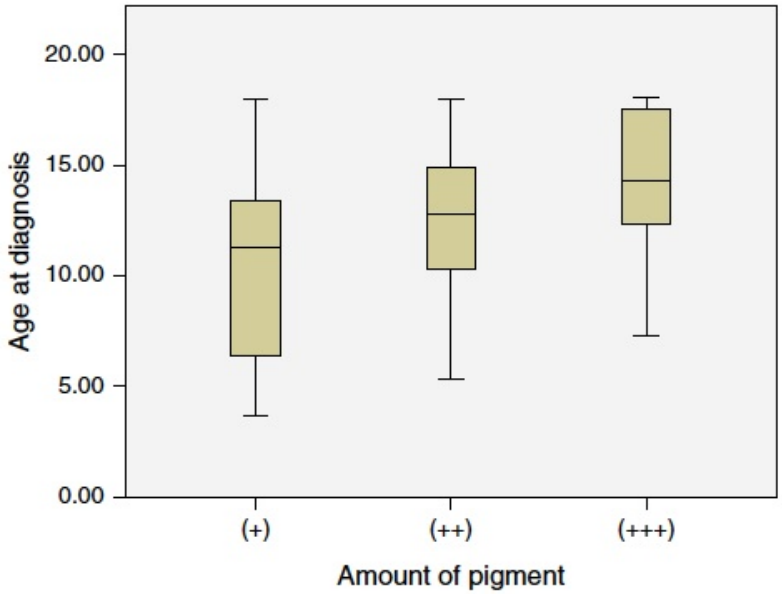
AlSi (E559)
TiO_2 (E171)
SiO_2 (E551)



Mesenteric lymph nodes (MLN)

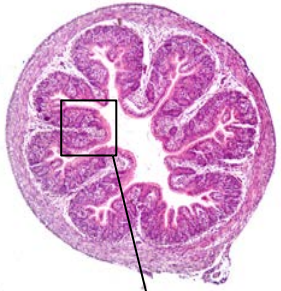
- Lymphocytes T (Tnaïfs Th1, Th2, Th17, Treg)
- Cellules dendritiques (*antigen-presenting cells*)
- Bactéries commensales (microbiote)
- Antigènes alimentaires
- Plasmocytes (lymphocytes B = anticorps)

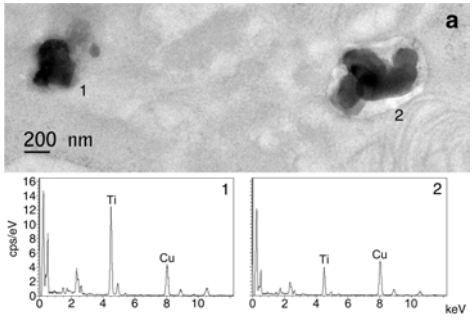
Clinical data



Powell *et al.* Gut, 1996
Powell *et al.* Brit J Nutr, 2007
Butler *et al.* Inflamm Res, 2007
Hummel *et al.* 2014

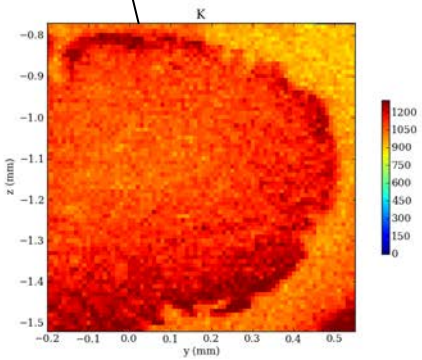
Large intestine : *absorption in the colon after repeated exposure*



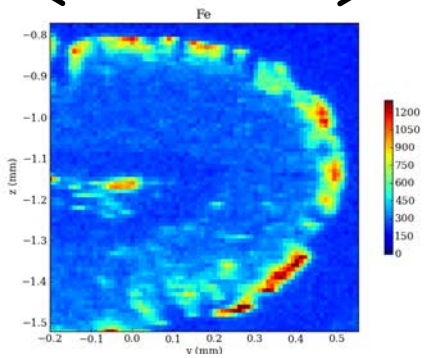


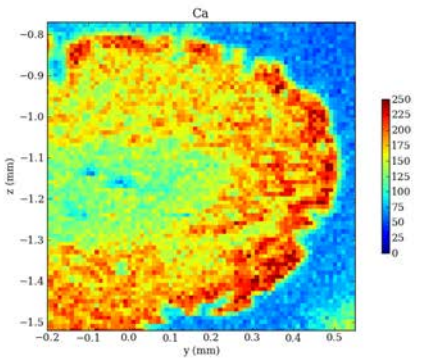
TEM-EDX
TiO_2 particles

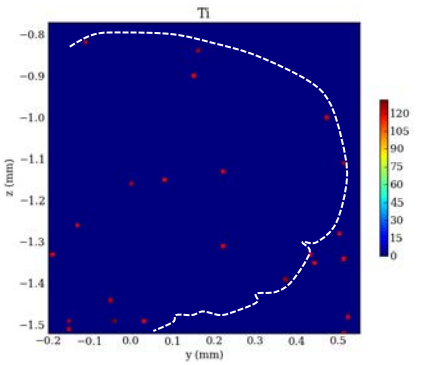
E171 *10mg/kg BW/d*
by gastric gavage
(200 µl in water)
for 7 days



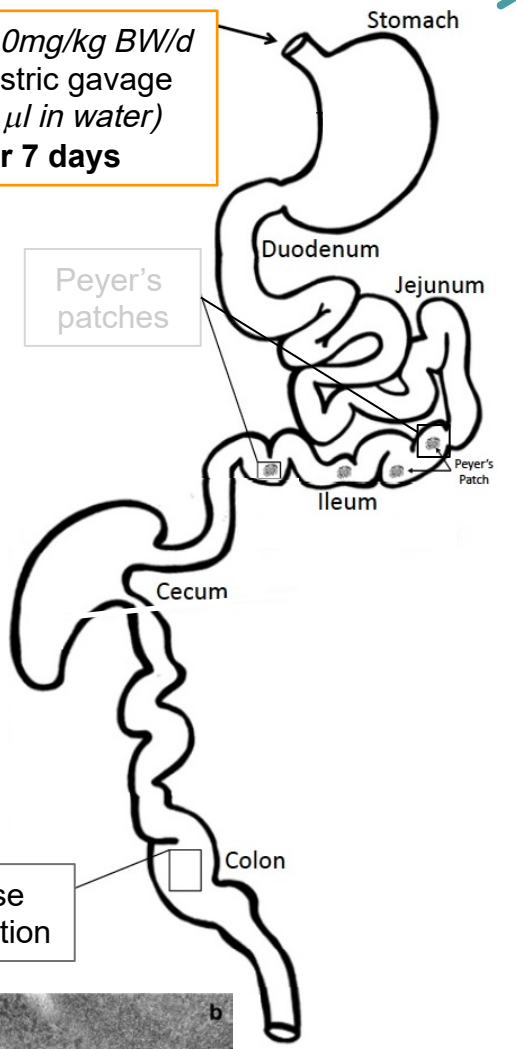
750 µm



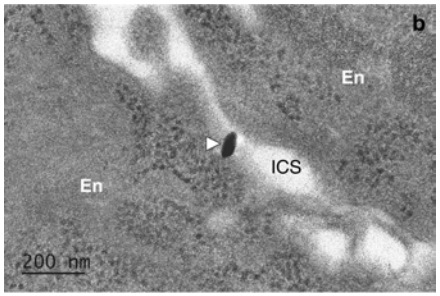




hot points (Ti)
1 pixel = 10 µm



transverse tissue section

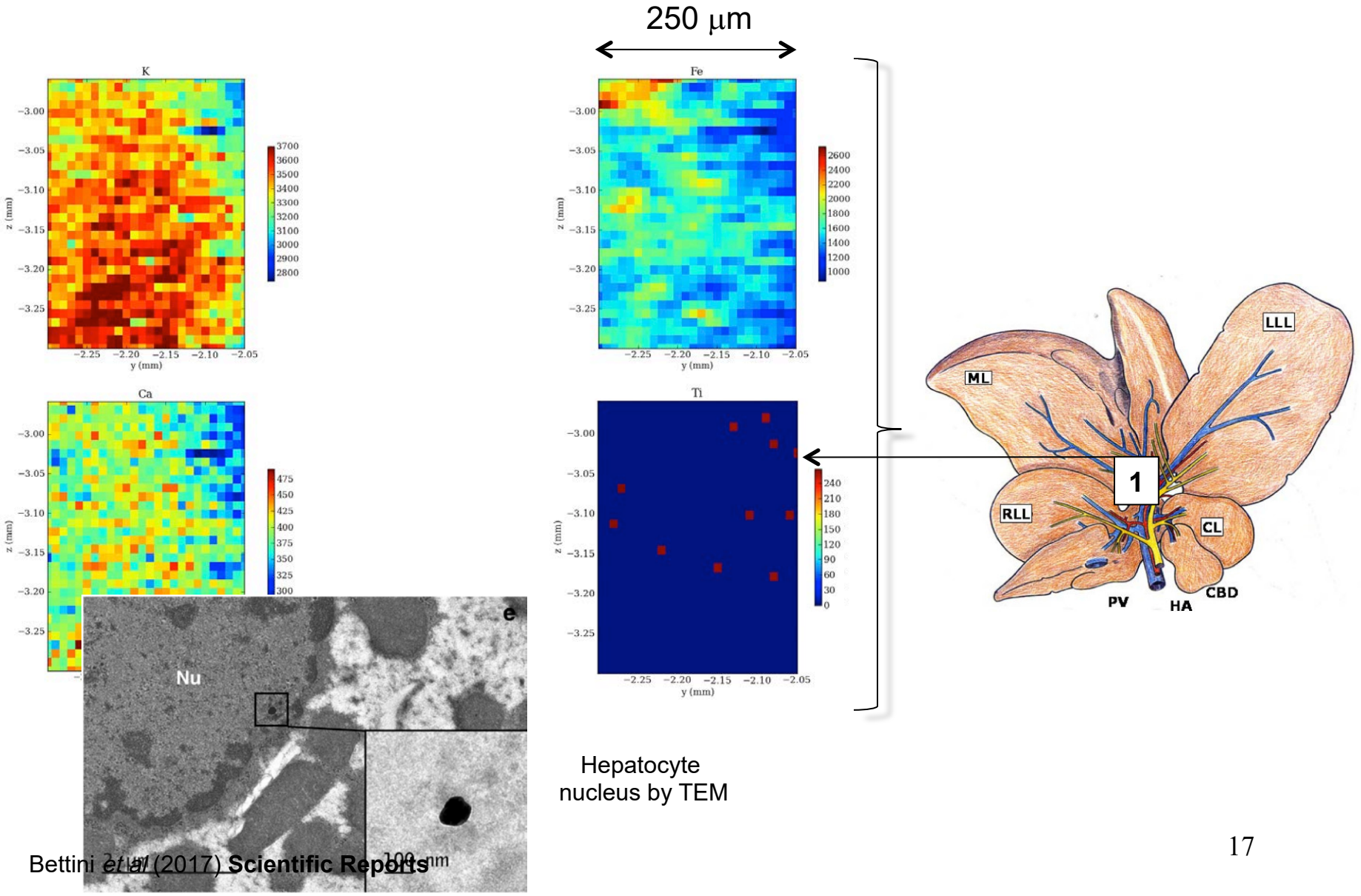


Intercellular space between enteroyctes by TEM

Bettini *et al* (2017) **Scientific Reports**

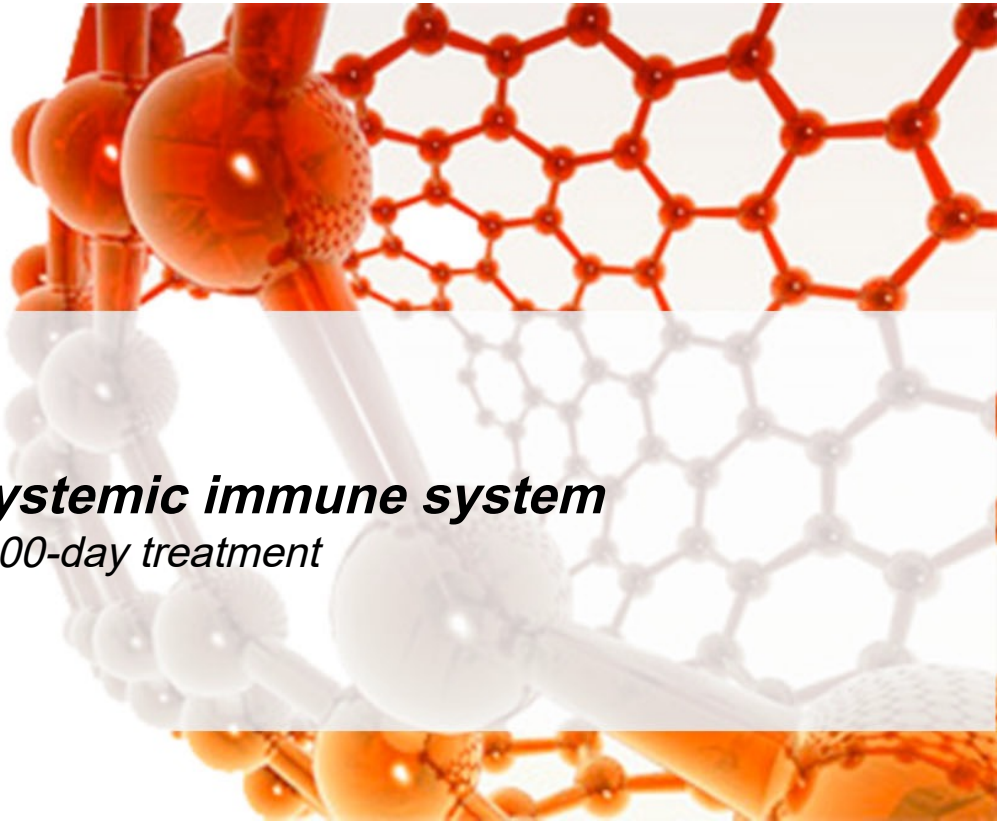
Gut-to-liver passage : *systemic distribution*

Bettini *et al* (2017) **Scientific Reports**



Hepatocyte nucleus by TEM

Bettini *et al* (2017) **Scientific Reports**

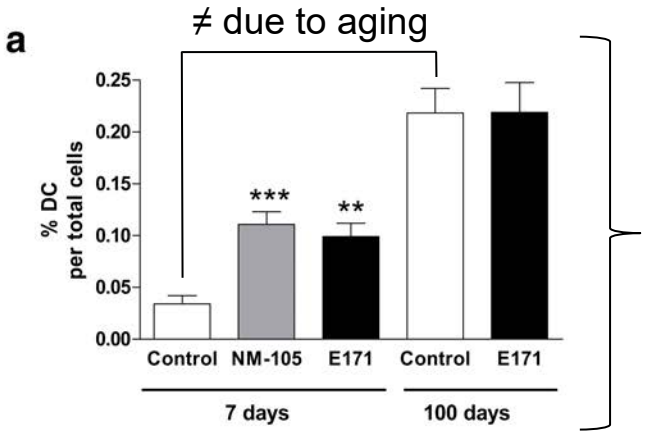


Effects on local and systemic immune system

one week vs.100-day treatment

Bettini *et al* (2017) **Scientific Reports**

Immune cell frequency in PP: *Bettini et al 2017*



Dendritic cells frequency ↗

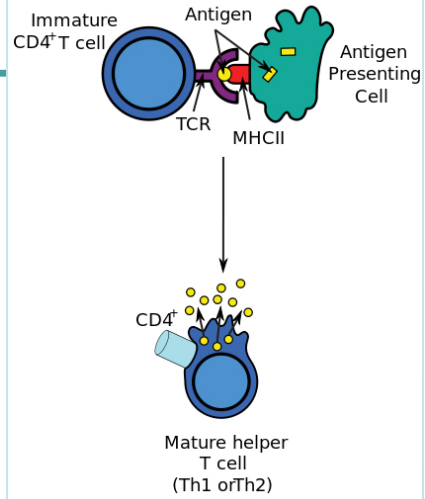
activated antigen-presenting cells

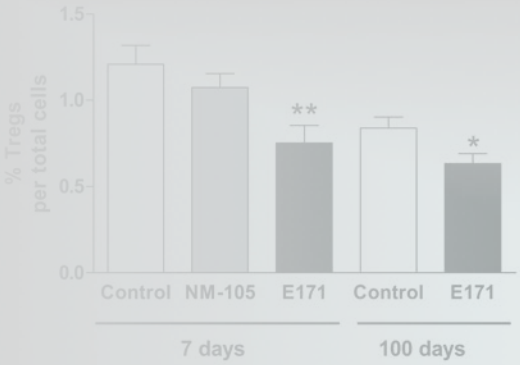
(i.e. CD11b/c⁺ CD103⁺ expressing the major histocompatibility complex MHC-II⁺ on their surfaces for antigen presentation) :

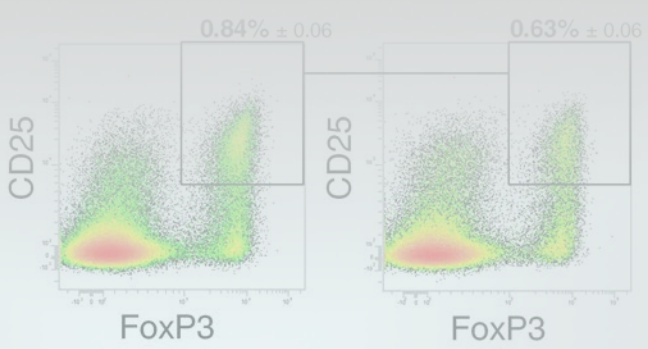
TRANSIENT effect

E171 = P25 NM-105

i.e., return to basal after 100 days of E171 treatment





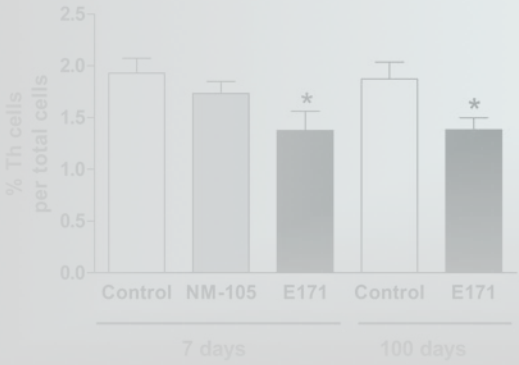


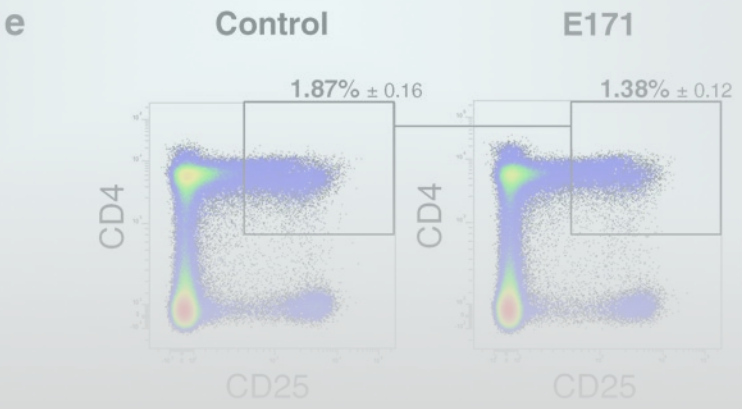
Tregs cells ↘

(CD4⁺ CD25⁺ FoxP3⁺)

immunosuppressive activity (IL-10, TGFβ) :

They organize **oral tolerance** to food antigens, **self-tolerance**, and **regulate inflammatory responses**





CHRONIC effect

E171 >> P25 NM-105

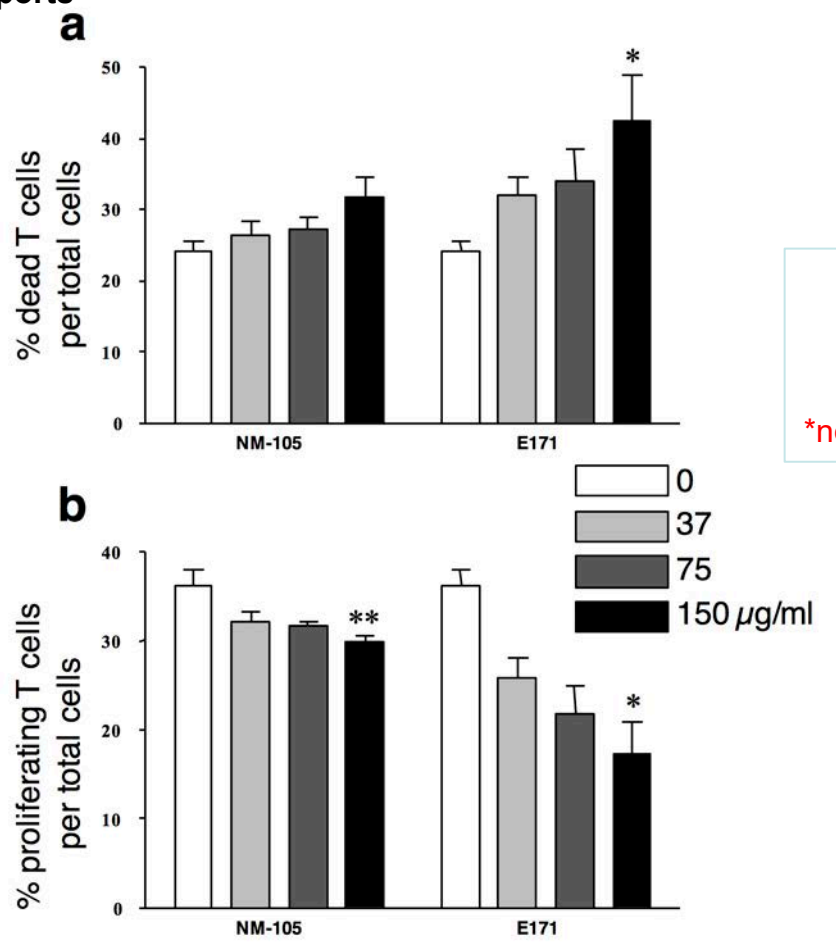
Total T cells ↘

(CD4⁺ CD25⁺)

include T-helper (Th)1 +Th2+Th17+Tregs

Dose-dependent cytotoxicity on T cells : Bettini et al 2017

Bettini *et al* (2017) **Scientific Reports**



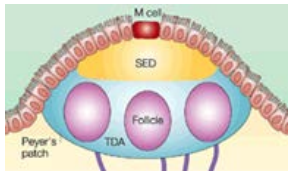
E171 >> P25 NM-105
on T cells isolated from naive* Peyer's patches
*normal rats, i.e., untreated with TO_2

- **Food grade TiO_2** (E171) **more cytotoxic than TiO_2-NP model** (P25 NM-105) **on total T cells** *ex vivo* (Th1, Th2, Th17, Tregs)
- **Data in support of** *in vivo* **observations**

Immune cell response : Bettini et al 2017

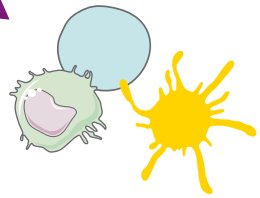
E171
vs. P25
NM-105

Peyer's patches
*(**initiator site** of proinflammatory processes)*

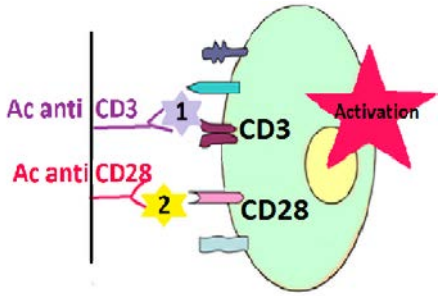


Spleen
(systemic level)

total cell isolation



Ex vivo re-stimulation
with anti-CD3/CD28 antibodies



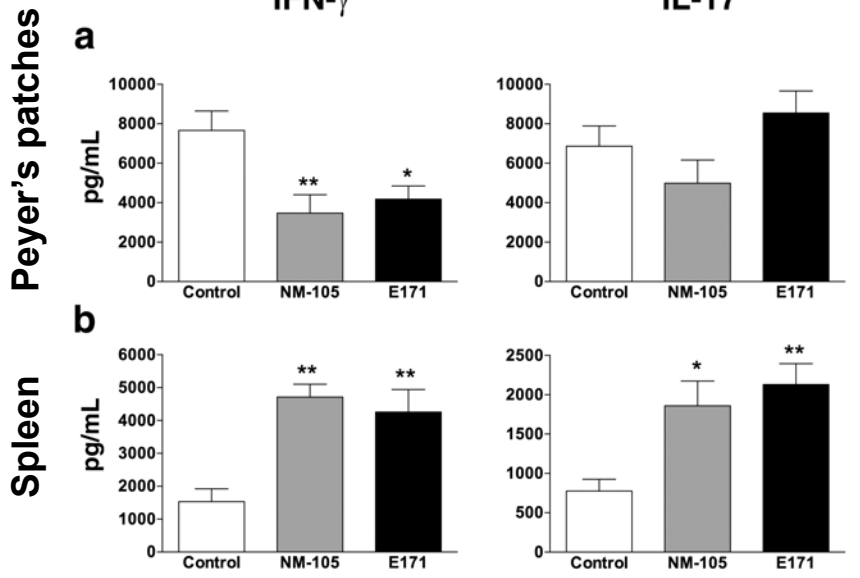
Direct T cell activation

Th1 cell response

Th17 cell response

Cytokine IFN-gamma

Cytokine IL-17



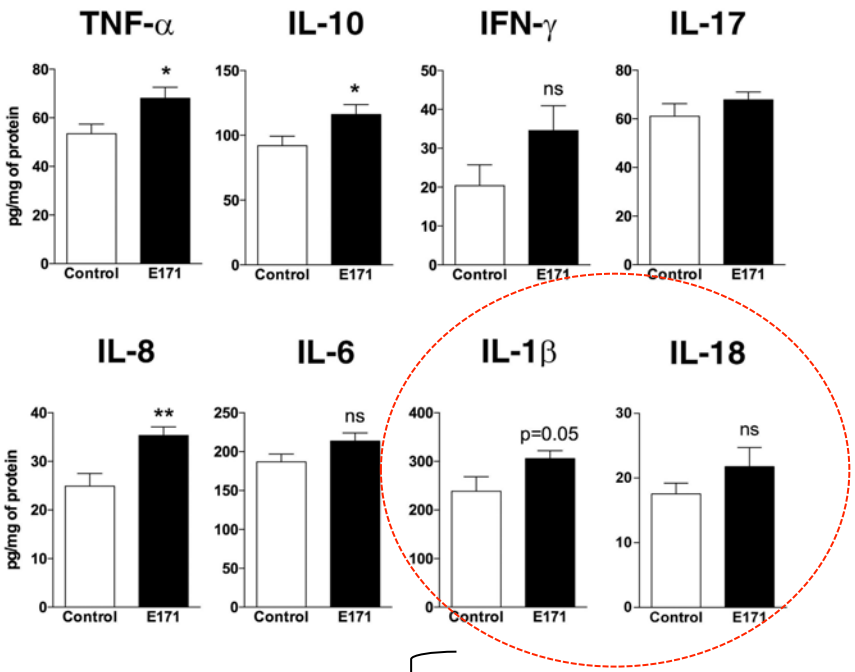
- **Local immunosuppression of Th1 response** in the gut : **E171 = P25 NM-105**
- **Th1/Th17 immune deviation at systemic level** (spleen) : **E171 = P25 NM-105**
- Heringa *et al* Part Fibre Toxicol 2018 : TiO_2 particles accumulate **in human spleen** (≥ 24% nanosized).

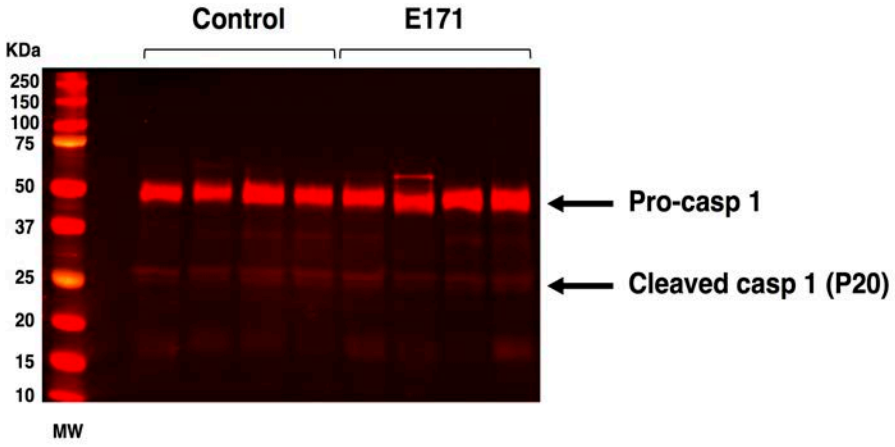
Mucosal inflammation after 100 days : no IBD-like conditions

E171 *only*

after 100 days

Lamina propria in the colon (***effector site*** *of proinflammatory processes)*





- **LOW GRADE inflammation in the colon** (i.e., **NO SIGNIFICANT CHANGES** in IFN-γ, IL-6, IL-18, IL-1β cytokine levels)

- **NO inflammasome activation** (i.e., caspase clivage) **at this level of dose/in the absence of other inflammatory conditions** (**CORRELATES NO CHANGES** in the downstream production of IL-18 and IL-1β cytokines)

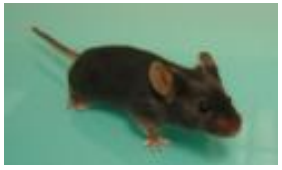
Conclusion (1)

- **Food-grade TiO_2 *per os*** in rats at a human relevant dietary level,
  - A source of TiO_2-NPs for the intestinal mucosa (and beyond for the remaining organism)
  - Translocate to immune cells in Peyer's patches (similarly to NP model).

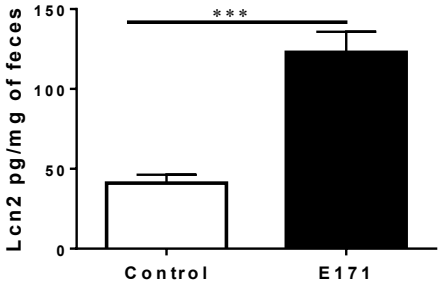
- **E171 absorption is not "neutral"** for the organism through,
  - Imbalance of immune homeostasis in the gut (drop in Treg frequency and Th1 responses),
  - Th1/Th17 immune deviation in splenocytes, suggesting susceptibility to proinflammatory responses at the systemic level (e.g., when face with inflammatory stimuli).

- **Low-grade inflammation** in the colon mucosa after chronic exposure,
  - increase in basal cytokine levels / no sign of IBD-like (acute) inflammation,
  - no inflammasome activation at 10 mg/kg BW/day.

Similar observation in a mouse model orally exposed to E171 (same batch *and* dose) for 60 days by gastric gavage
unpublished



Colon
Fecal lipocaline (LCN2)



Effects on the initiation and promotion of preneoploastic lesions in the colon

oral treatment for 100 days in rats (E171 only)
in vitro studies (E171 vs. P25 NM105)

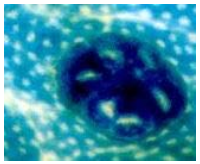
Bettini *et al* (2017) **Scientific Reports**

***Models** and **strategy used :** a strategy trying to « link » size-to-effects*

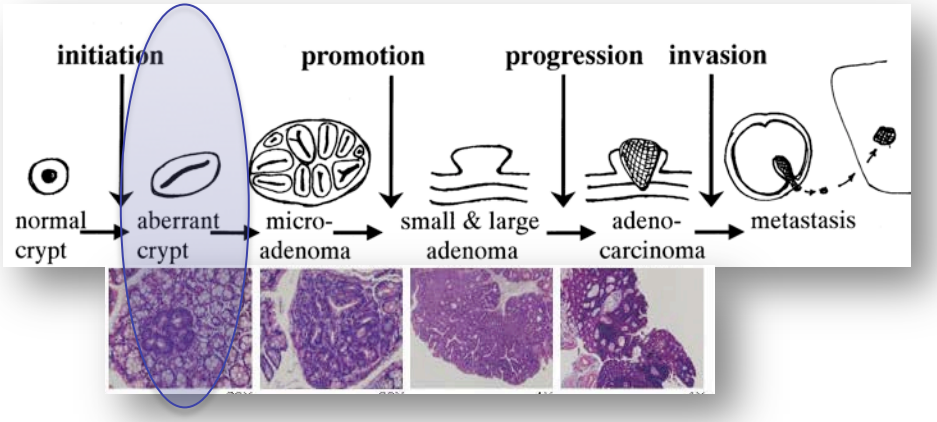
GOAL : to assess whether E171 is a **risk factor** in the **initiation** and/or **promotion of colonic preneoplastic lesions** (i.e., the first stages of colorectal cancer development, ***not tumor formation***).

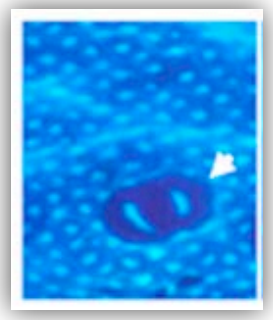
How ? E171 orally administered to rats **for 100 days at 2 doses** (200 µg and/or 10 mg/kg of BW per day) through drinking water,

ACF



- *In vivo* assessment of **spontaneous development** and/or **promotion of preneoplastic lesions** (Aberrant Crypt Foci, ACF) in the rat colon
  - Initiation : without dimethylhydrazine (DMH)-induced carcinogenesis
  - Promotion: with DMH induction





ACF of 2 crypts at the colonic mucosal surface stained with methylene blue (40X)

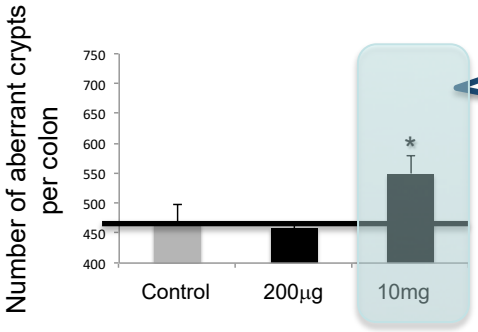
- **ACF** were counted under light microscope (fixation with 10% buffered formalin + methylene blue tissue staining), **in duplicate by two independent readers, blinded for the origin of the colon.**
  - Number of lesions per colon : ACF / colon
  - Size of lesions : Crypts / Foci
  - Number of aberrant crypts per colon : i.e., preneoplastic load = AC/colon
- **Statistics**: t-test *and* contengency test for 2 comparison groups (**initiation**) and ANOVA for 3 comparison groups (**promotion**)

***Promotion* of preneoplastic lesions :** *effect of dose exposure*

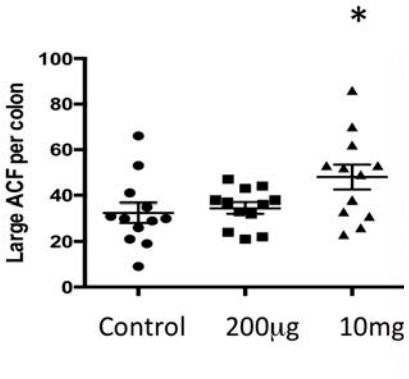
Bettini *et al* (2017) **Scientific Reports**

- Effect on the number of ACF per colon : no significant difference between groups

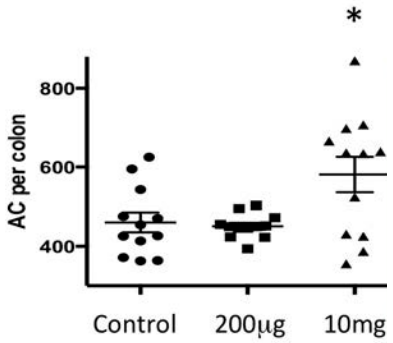
- Effect on the preneoplastic load (AC per colon)



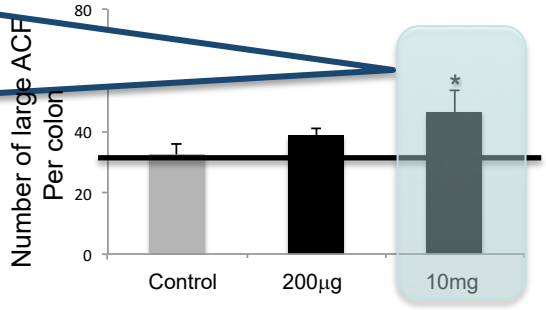
***Promotion of the lesional load** by E171*
-
effect not observed at the lower dose



- Effect on the number of large ACF (≥ 4 crypts)



***Promotion of number of large ACF** by E171*
-
effect not observed at the lower dose

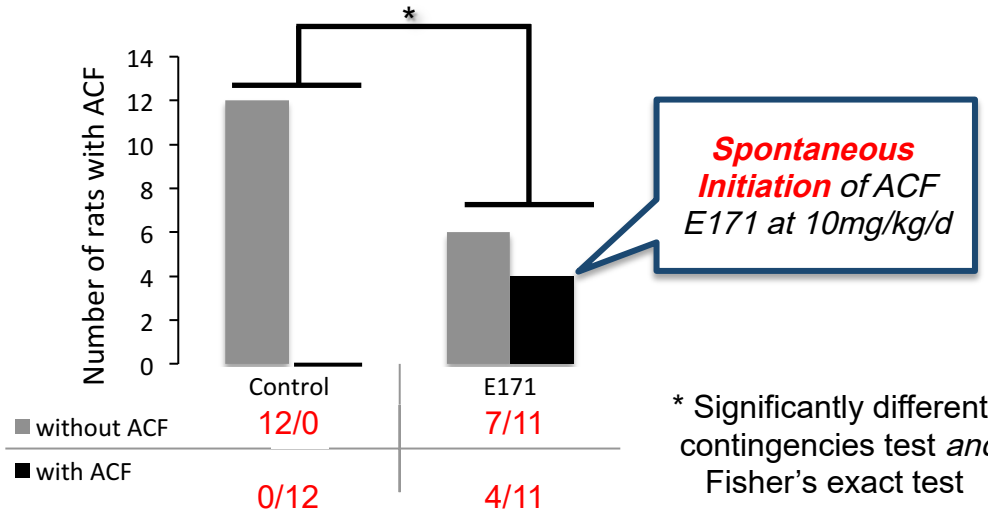


* Significantly different of controls : ANOVA and Fisher's Least-Significant-Difference Test

- **The food grade** TiO_2 (E171, ultrasonicated OR NOT) **PROMOTES preneoplastic lesions in the rat colon,** at 10 mg/kg BW/d for 100 days.
- Not observed at a lower dose (200 µg/kg BW/d).

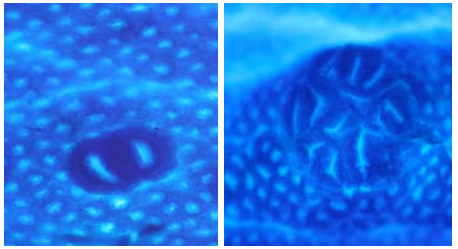
Initiation of preneoplastic lesions

Number of rats with or without preneoplastic lesions after 100 days of E171 exposure



* Significantly different : contingencies test *and* Fisher's exact test

ACF of 2 and **12 crypts**



ACF at the colonic mucosal surface stained with methylene blue (40X)

- **The food grade** TiO_2 (ultrasonicated E171) **INDUCES preneoplastic lesions in the rat colon,** at 10 mg/kg BW/d for 100 days.

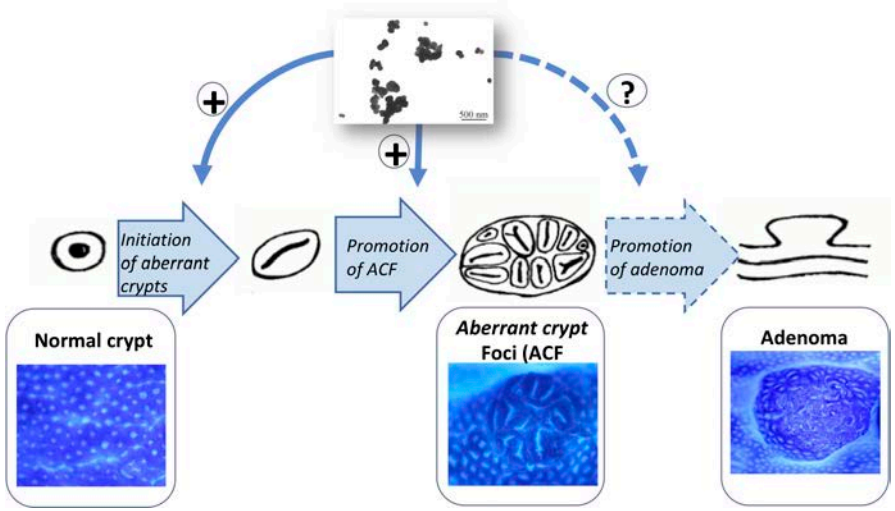
Conclusion (2)

- **Oral exposure to food-grade TiO_2**, at a representative dose of human exposure, **induces** *and* **promotes preneoplastic lesions** in the colon after chronic exposure (100 days)

- These effects were observed on :
  - the **lesional load** (total number of aberrant crypts per colon)**,**
  - the **number of large preneoplastic lesions** (size of ACF)

- Effect correlated with (*but* no causal demonstration) :
  - a **developing "low-grade inflammation"** into the colonic mucosa,
  - a **positive selection of pre-neoplastic cells** *and* **genotoxicity on normal cells** *in vitro* (please see Bettini *et al* suppl data - effect similar to TiO_2-NP model)

- Insufficient to conclude on a risk at the tumor stage :
  - need **Carcinogenecity studies** according to OECD guidelines #451,
  - **focus** on E171 displaying a large fraction of nanoparticulate matter (i.e., ≥ 40%)



Eric Houdeau
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Natalia Breyner
Laurence Guzylack
Sarah Bettini, PhD
Eric Gaultier
Christel Cartier

Muriel Thomas
Muriel Mercier-Bonin
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Pauline Talbot, Master

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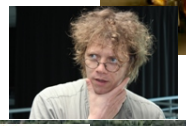

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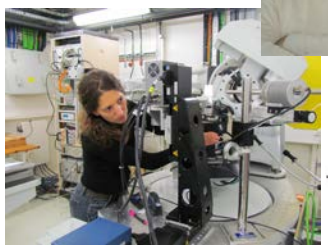
Matthieu Réfrégiers
Dominique Thiaudière
Solenn Reguer

Fabrice Pierre
Jacques Dupuy
Sylviane Taché
Nathalie Naud

Elisa Boutet

Christine Comera
Jean-Pierre Cravedi



DIFFABS

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Promotion of colon cancer by E171

Dra. Yolanda Irasema Chirino

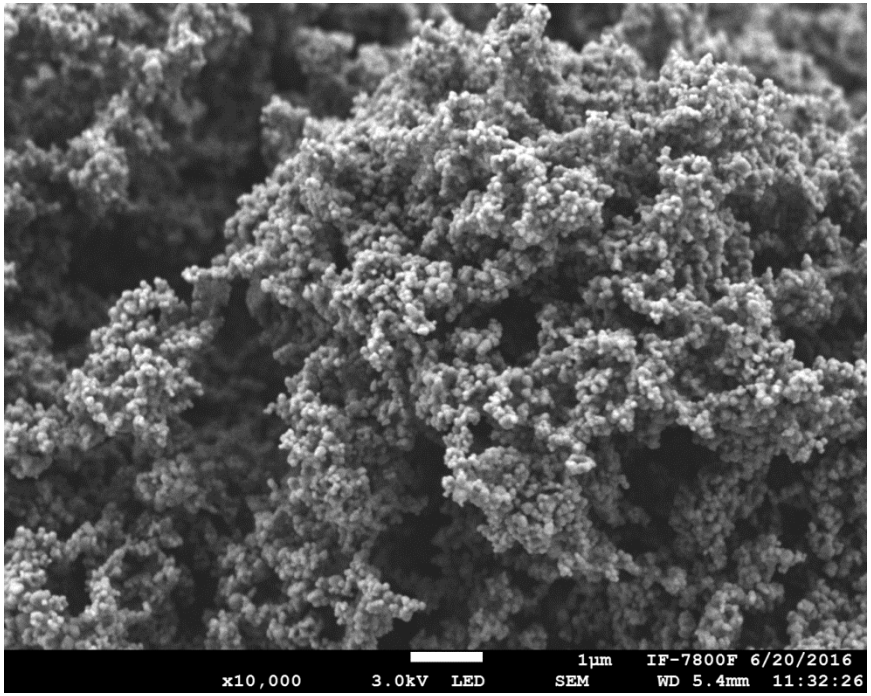
Workshop on Possible adverse
effects of food additive E171 (titanium dioxide)
Amsterdam, the Netherlands
July 5-6, 2018

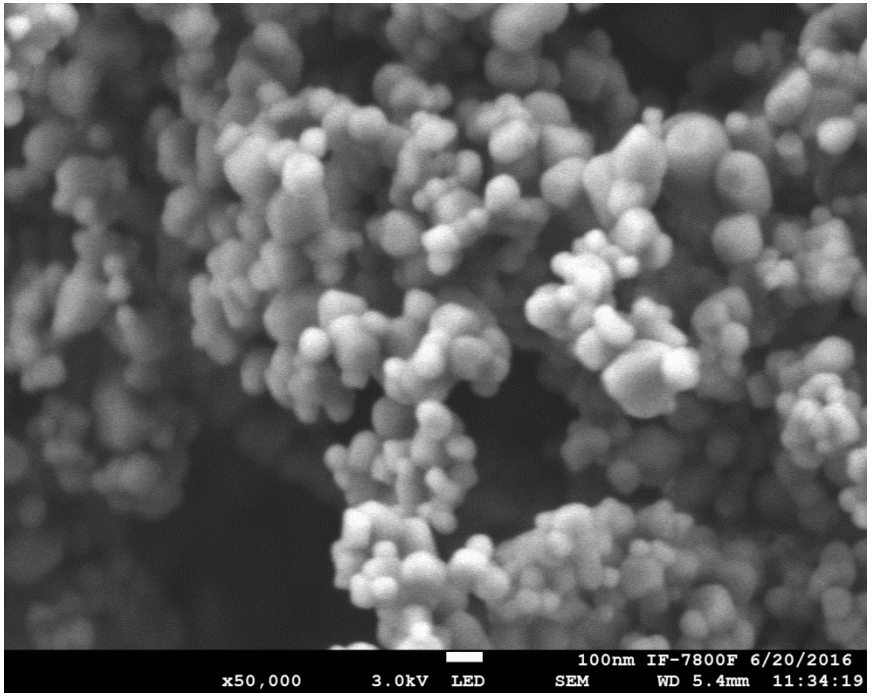
Universidad Nacional Autónoma de México

Outline

1. Introduction
2. Hypothesis & Aim of our first study
3. Results
4. Conclusions
5. Research ongoing (solid vs liquid E171 administration)

1. Introduction





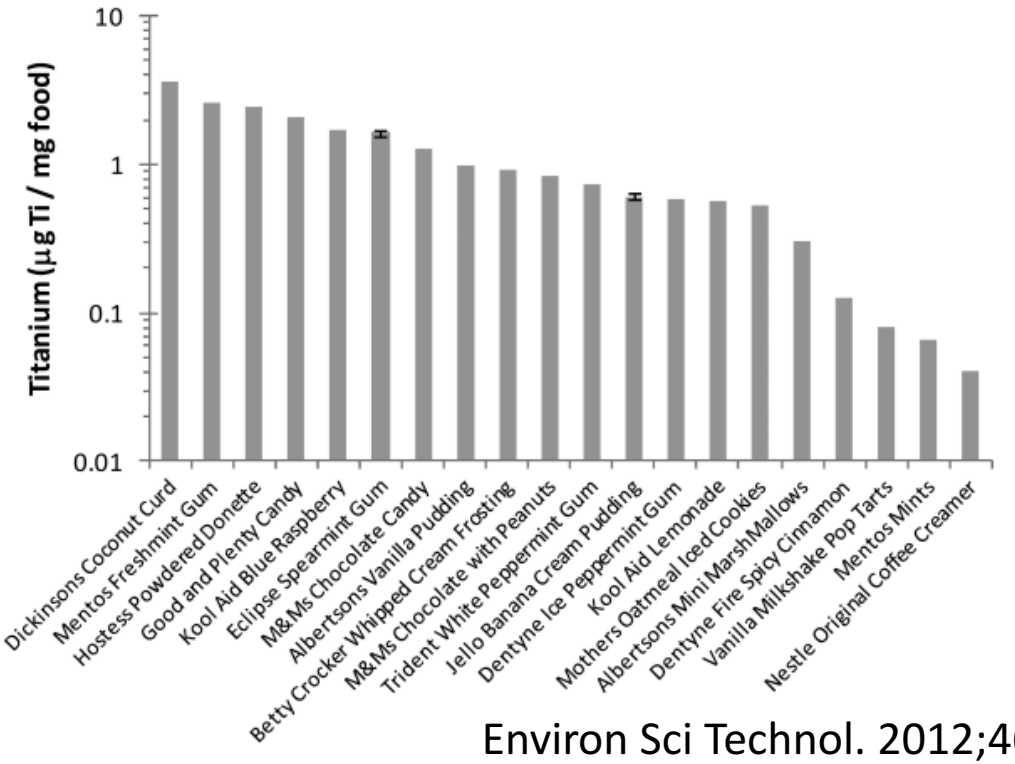
Evidence of TiO_2 in food products

NIH Public Access
Author Manuscript
Environ Sci Technol. Author manuscript; available in PMC 2013 February 21.

Published in final edited form as:
Environ Sci Technol. 2012 February 21; 46(4): 2242–2250. doi:10.1021/es204168d.

Titanium Dioxide Nanoparticles in Food and Personal Care Products

Alex Weir[1], **Paul Westerhoff**[1,*], **Lars Fabricius**[2,3], and **Natalie von Goetz**[2]



Environ Sci Technol. 2012;46(4):2242-50.

Evidence of TiO_2 deposits in humans

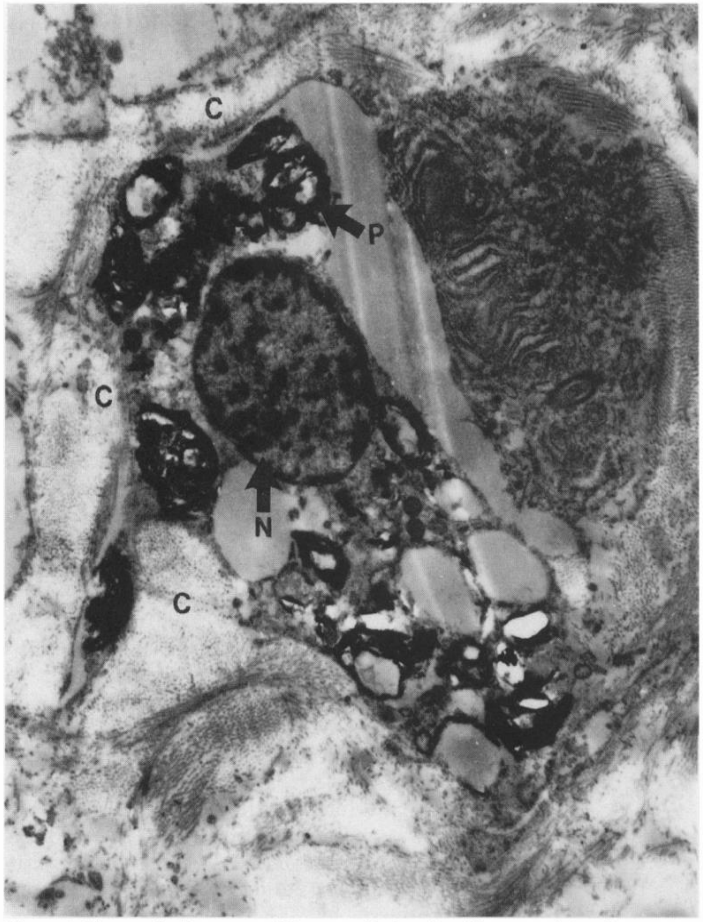


Figure 4: Pigmented cell, reprocessed from wax blocks, viewed by transmission electron microscopy (original ×8200). The cell nucleus (N) and distinct intravesicular areas of microparticles (for example P) are apparent. The extracellular material appears to be mainly collagen (C) and also shown is probably a plasma cell (top right).

Department of Histopathology, Royal Free Hospital School of Medicine, London, England presented this medical findings in 1996.

10 patients with Crohn's disease
5 patients with ulcerative colitis
5 patients with colonic carcinoma

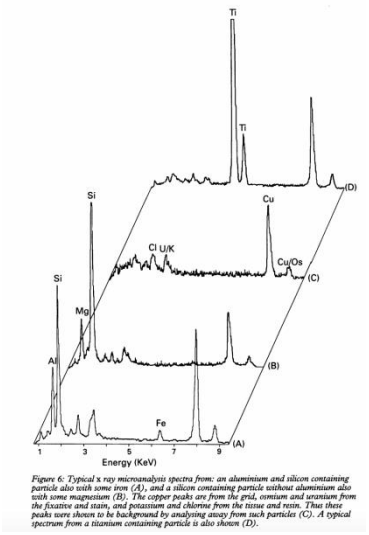
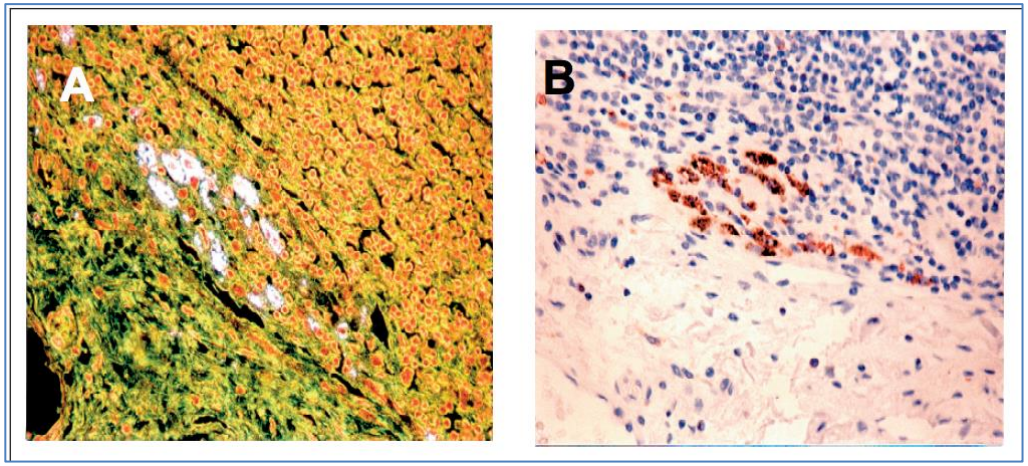


Figure 6: Typical x ray microanalysis spectra from: an aluminium and silicon containing particle also with some iron (A), and a silicon containing particle without aluminium also with some magnesium (B). The copper peaks are from the grid, osmium and uranium from the fixative and stain, and potassium and chlorine from the tissue and resin. Thus these peaks were shown to be background by analysing away from such particles (C). A typical spectrum from a titanium containing particle is also shown (D).

X ray analysis showed the presence of titanium.

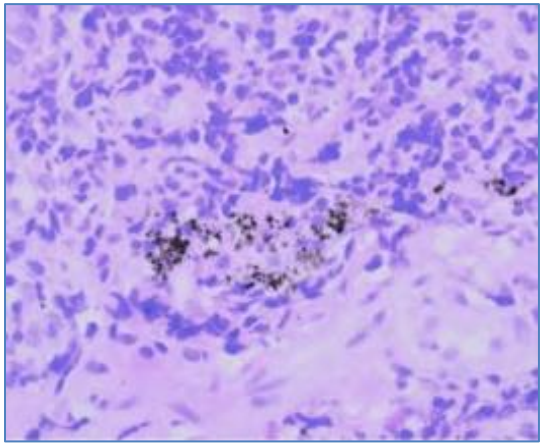
Evidence of TiO_2 deposits in humans



Pigment deposits in Peyer's Patches of patients with IBD (Thoree et al., 2008)

Department of Histopathology, St Thomas' Hospital, London, SE1 7EH, UK

16 patients with adenocarcinoma
23 with Crohn's disease
10 non-colitis



TiO_2 and alluminum deposits in Peyer's Patches in children with IBD (Hummel et al., 2014)

Department of Pediatric Gastroenterology and Nutrition, Academic Medical Center, Amsterdam, The Netherlands

62 with Crohn disease (CD)
26 with ulcerative colitis
63 with non-IBD
In 63 children (42%), deposits of black pigment were found only in biopsies from the terminal ileum, located in Peyer patches.

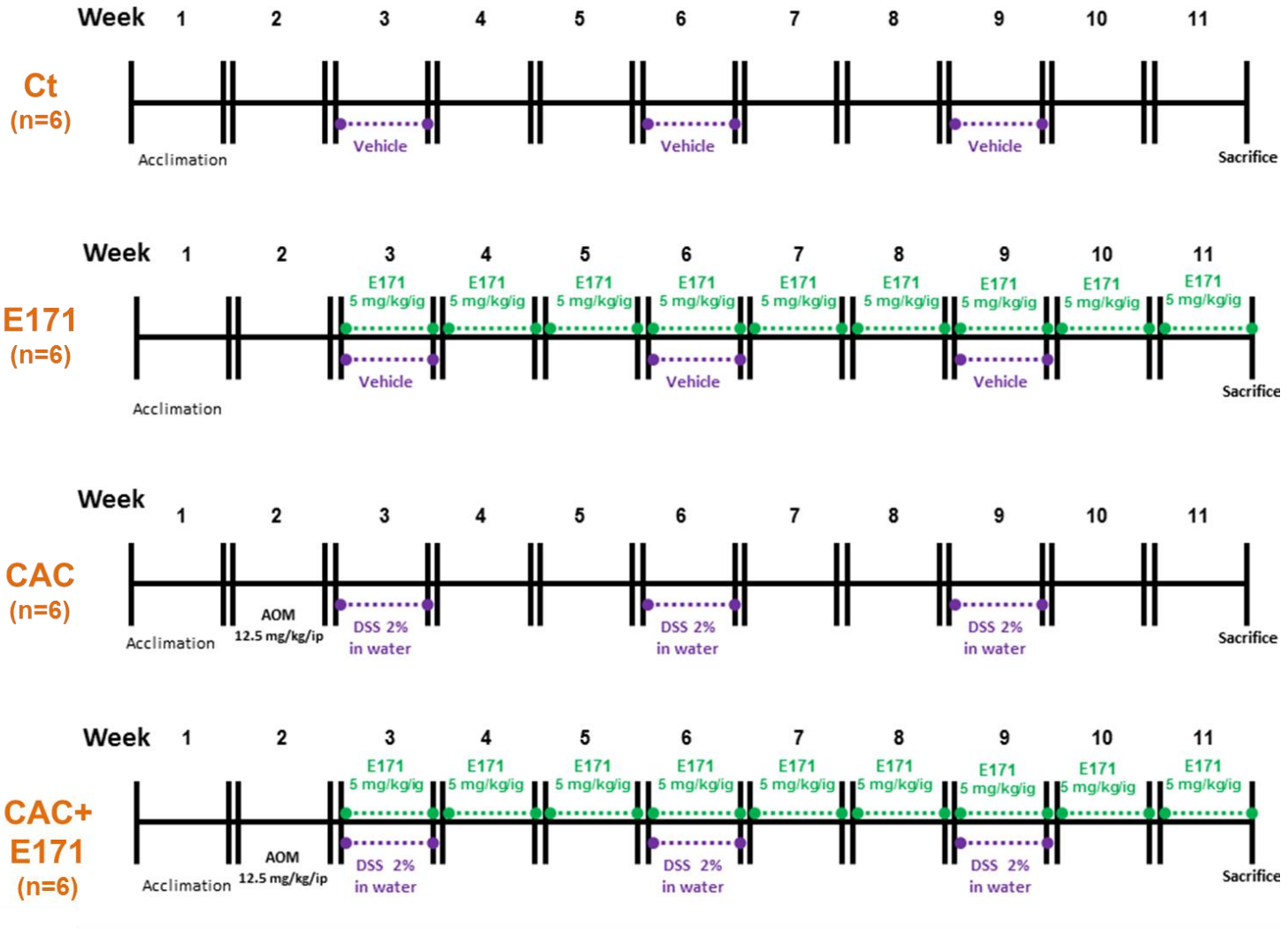
2A. HYPOTHESIS

Administration of E171 enhanced the tumors formation in colon in a colorectal cancer model in mice after 10 weeks of exposure.

2B. AIM

To evaluate the E171 effect by intragastric administration dosed at 5mg/bw/10w in a colorectal cancer mice model induced chemically by axoxymethane/DSS.

Experimental design



3. RESULTS

Food and Chemical Toxicology 93 (2016) 20–31

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Food-grade titanium dioxide exposure exacerbates tumor formation in colitis associated cancer model

Ismael M. Urrutia-Ortega [a, b], Luis G. Garduño-Balderas [a, c], Norma L. Delgado-Buenrostro [a], Verónica Freyre-Fonseca [a, d], José O. Flores-Flores [e], Arturo González-Robles [f], José Pedraza-Chaverri [g], Rogelio Hernández-Pando [h], Miriam Rodríguez-Sosa [a], Sonia León-Cabrera [a, i], Luis I. Terrazas [a], Henk van Loveren [j], Yolanda I. Chirino [a, *]

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[h] *Experimental Pathology Section, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran, Vasco de Quiroga 15, Colonia Sección XVI, Tlalpan, México DF, 14000, Mexico*
[i] *Carrera De Médico Cirujano, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, CP 54059, Estado de México, Mexico*
[j] *Department of Toxicogenomics, Maastricht University, PO Box 616, 6200MD, Maastricht, The Netherlands*

ARTICLE INFO

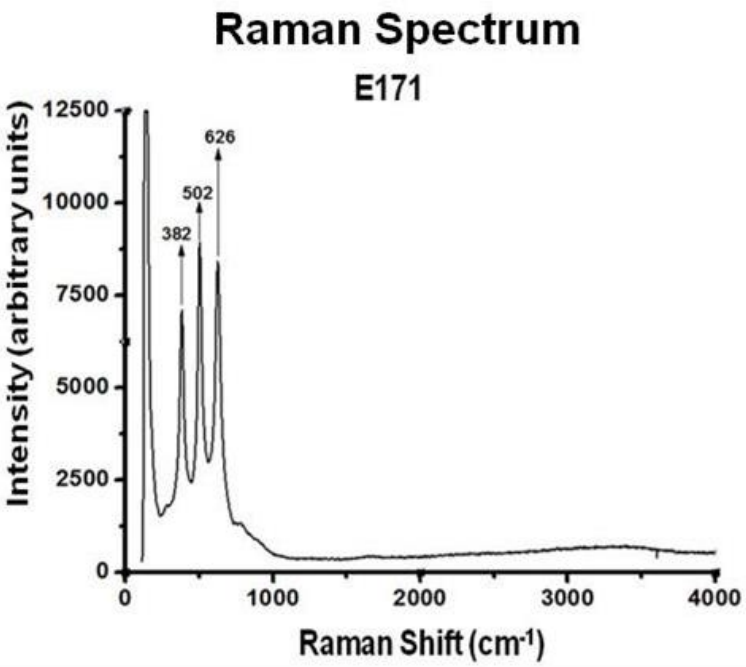
Article history:

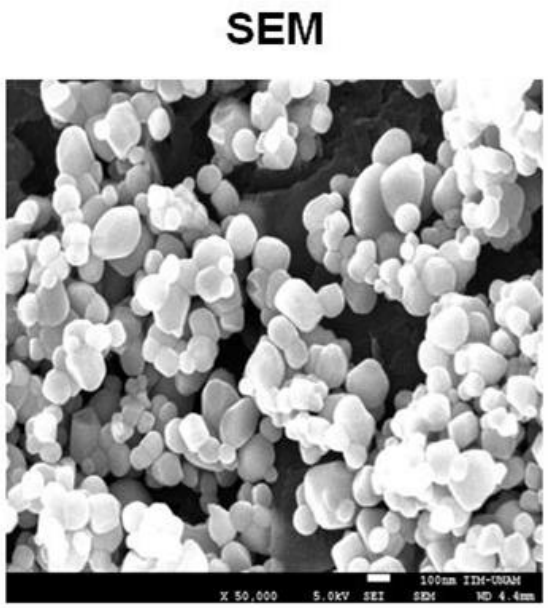
ABSTRACT

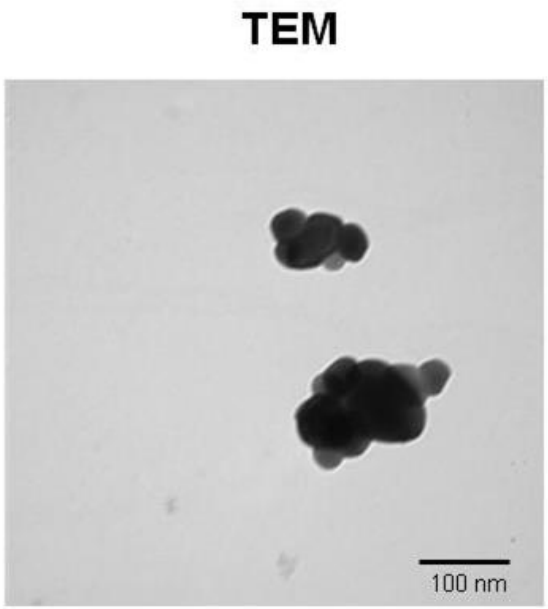
Colorectal cancer is the fourth worldwide cause of death and even if some dietary habits are consider risk

Physicochemical properties of E171 (food grade titanium dioxide)

A

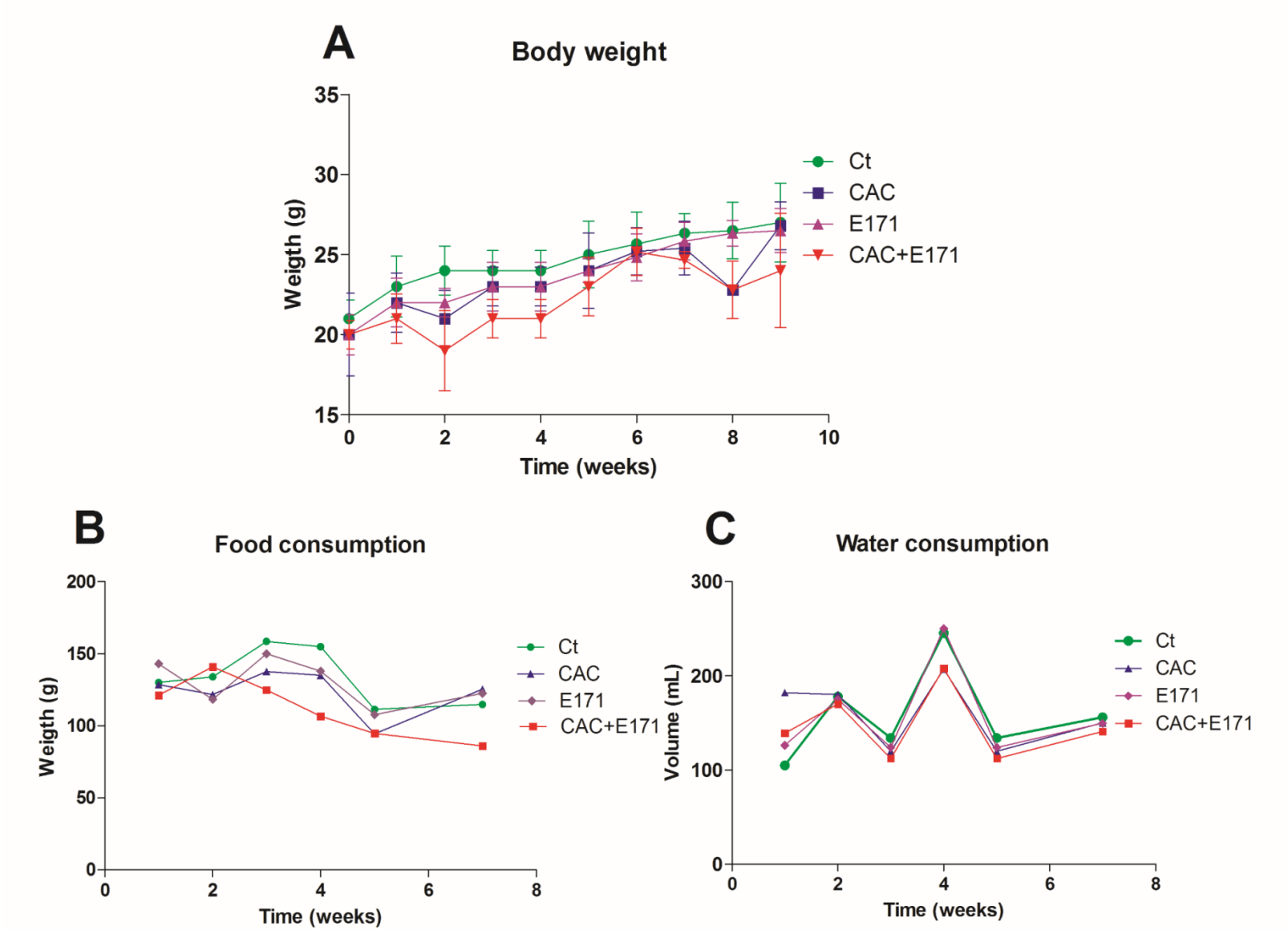




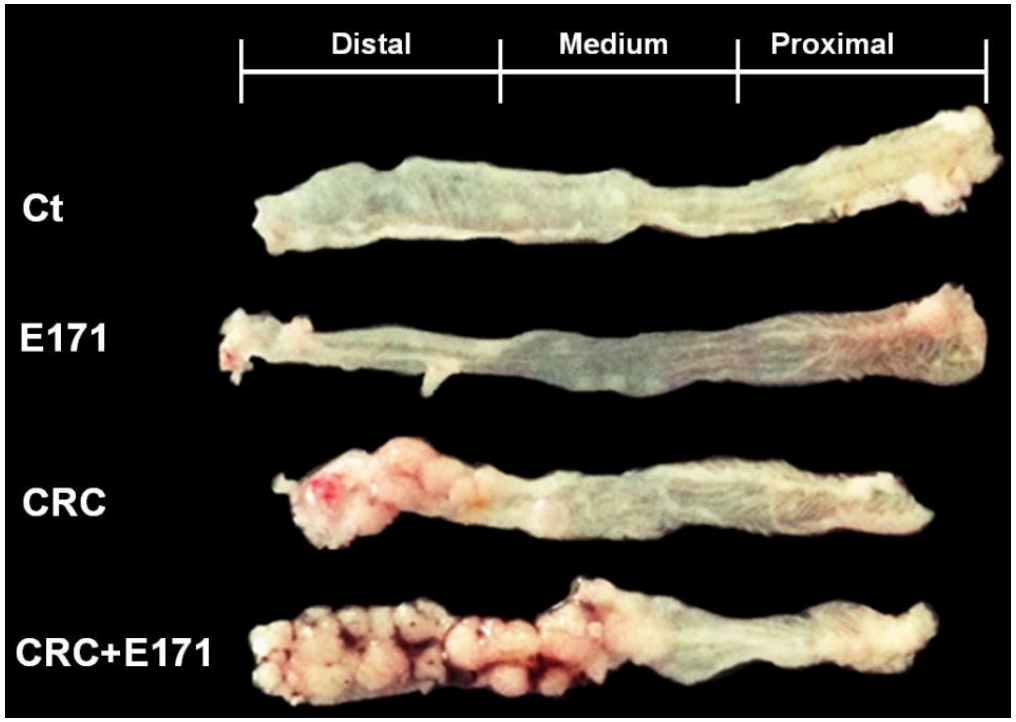


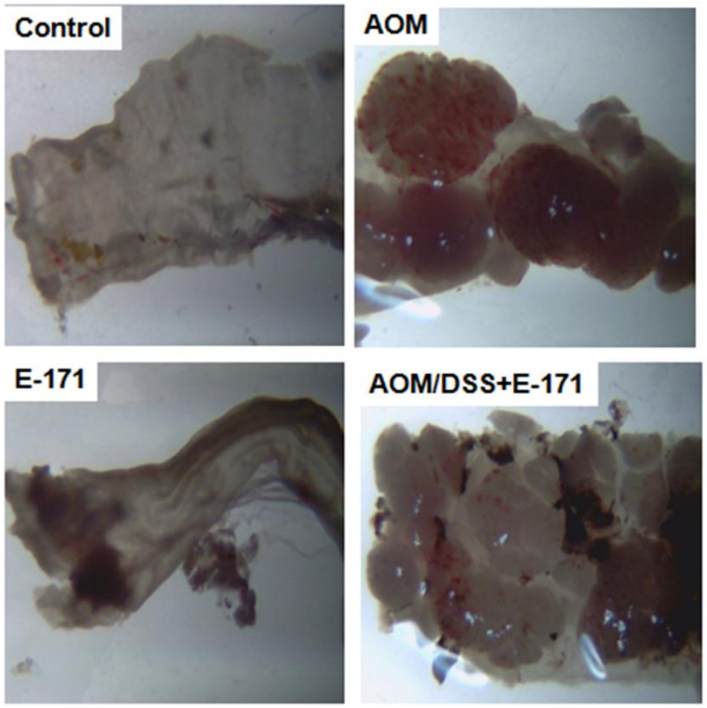
Characterization of E171. A) Representative Raman shift, and scanning electron microscopy (SEM) images of primary particles of E171 and transmission electron microscopy (TEM) of E171 agglomerates dispersed water. B) Polydispersity index of E171 agglomerates shows hydrodynamic diameter distribution by tracking analysis and C) Z potential titration of E171 agglomerates.

E171 had no effects on body weight, food intake nor water consumption



Intragastric E171 administration enhanced the tumor formation in distal colon





Intragastric E171 exposure increased tumor formation. Tumor formation count after 72 days of treatment. CAC+E171 group had increased tumor compared to CAC group (**p≤0.01 vs CAC).

Intragastric E171 administration enhanced tumor formation and induced pre-neoplastic alterations in colon

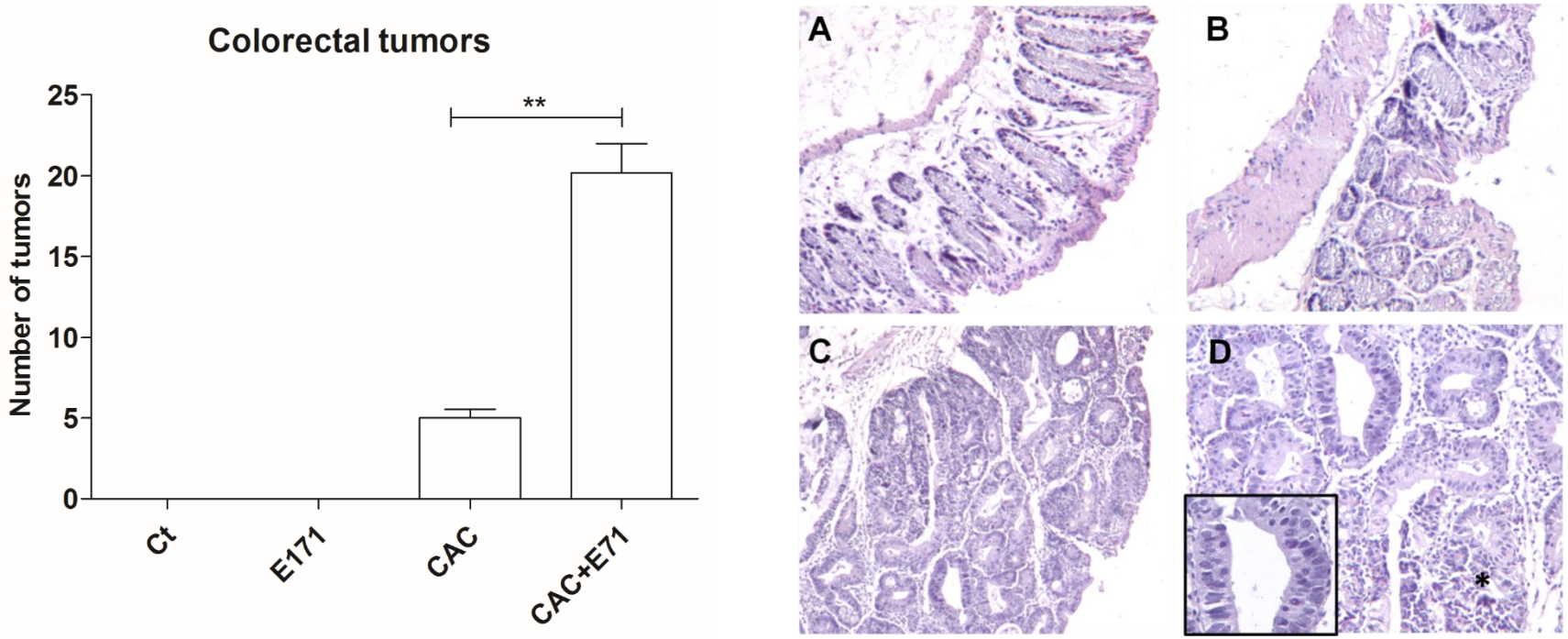
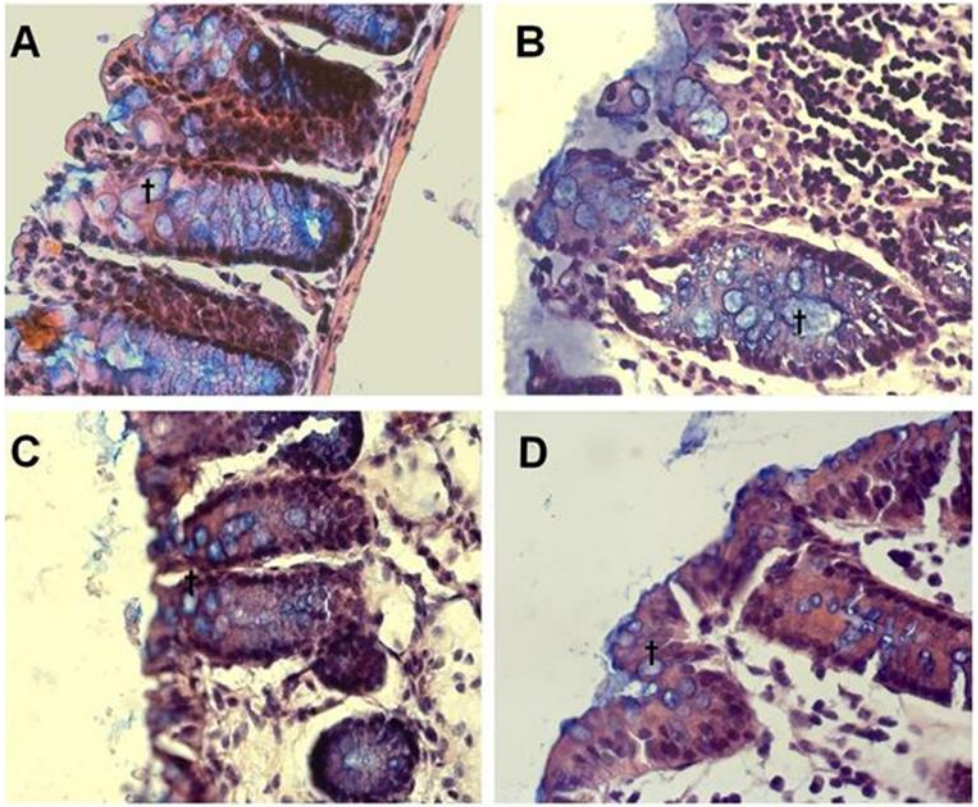
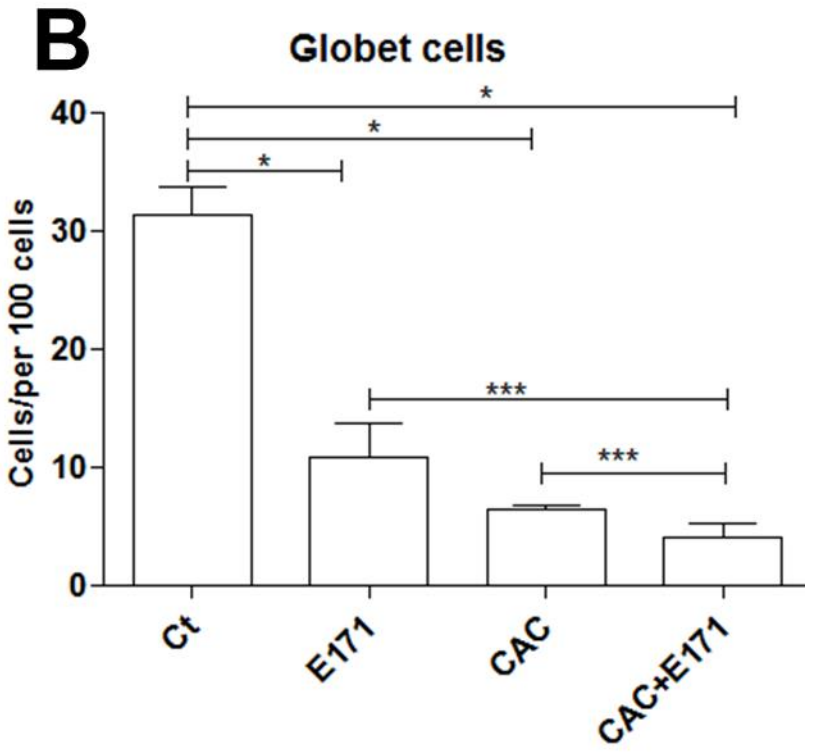


Fig 5. Dysplasia in CAC tumors and colon tissue increased by E171 intragastric exposure. Representative colonic histopathology in experimental groups. A) Control group exhibit normal epithelium and colorectal crypts. B) E171 group show an increase of crypts size and number, revisted with hyperplastic epithelium with slight dysplastic changes. Polypoid well differentiated adenocarcinoma in a mouse from C) CAC group, numerous glands with irregular sizes revisted with dysplastic epithelium. Irregular neoplastic glands revisted with hyperplastic stratified cubic epithelium showing dysplastic changes and solid areas with neoplastic cells (asterisk) is observed in the D) CAC+E171 group. In the inset, a higher power magnification micrograph shows dysplastic epithelium, large cells with hypercromatic nucleous.

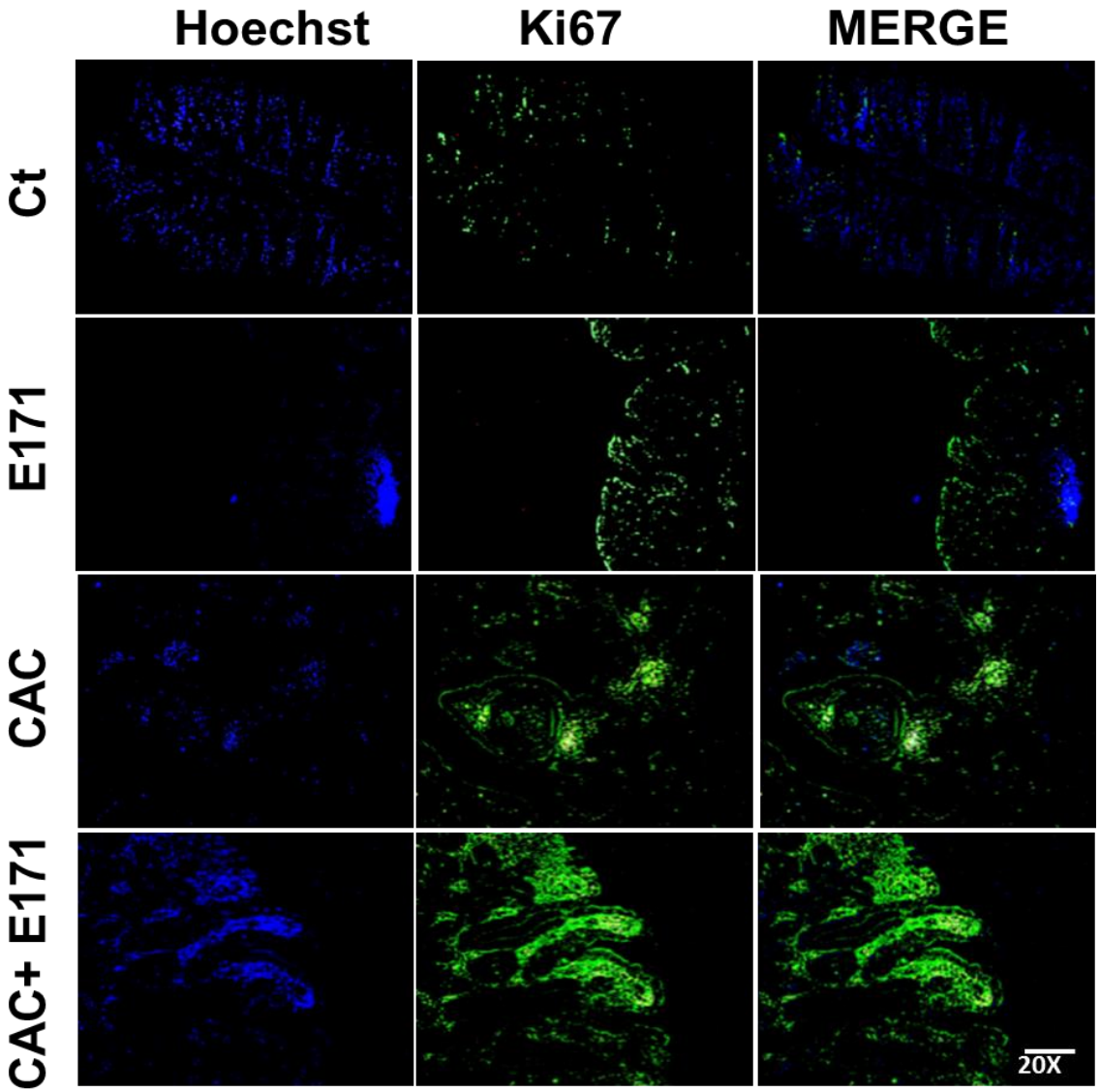
Intragastric E171 exposure reduced the content of goblet cells

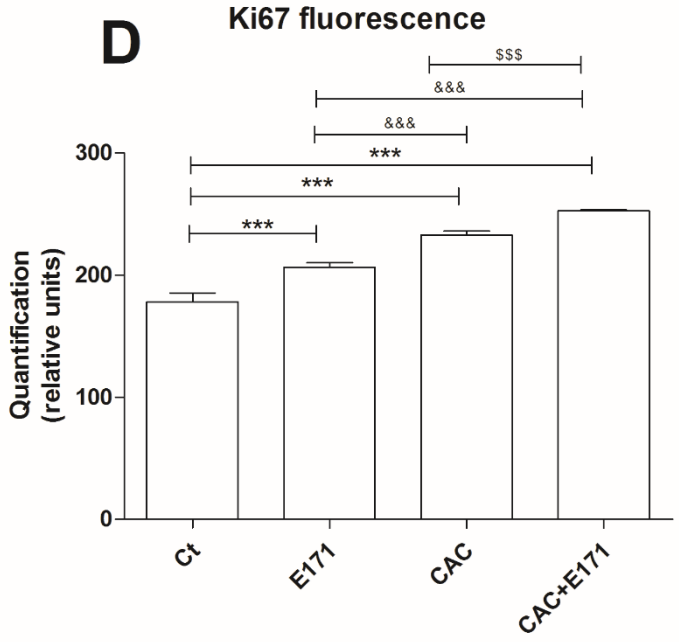




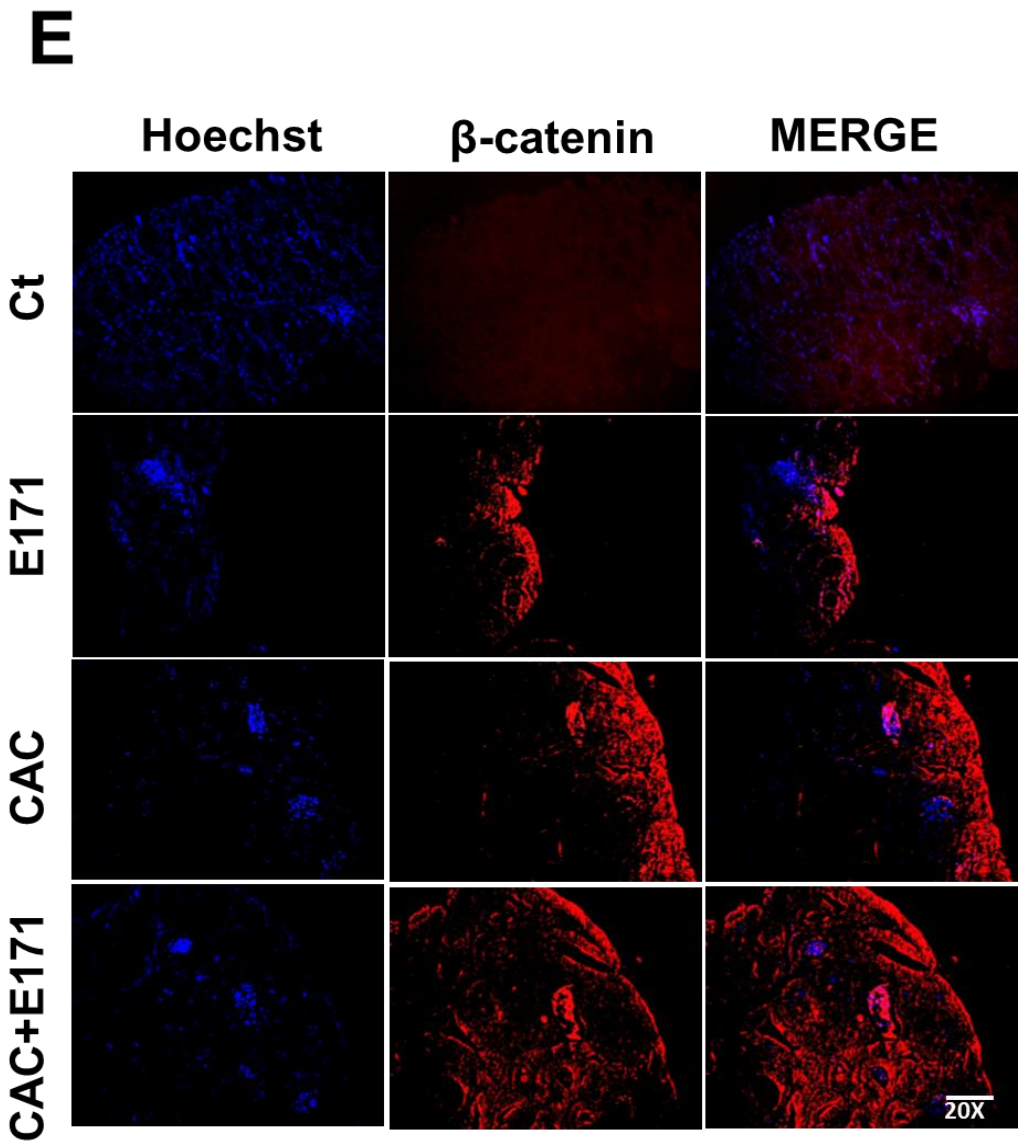
Intragastric E171 exposure reduced number of goblet cells in colon tissue. Goblet cells were stained in blue and marked with a dagger in groups A) Control B) E171, C) CAC and D) CAC+E171. E) Goblet cells in colon decreased with the sole E171 administration, but also in CRC group, however, CAC+E171 treatment had higher decrease in those cells. Detection was performed by Alcian blue staining and blind count was realized in each colon sample. Data are presented as mean±SEM; *$p<0.01$.

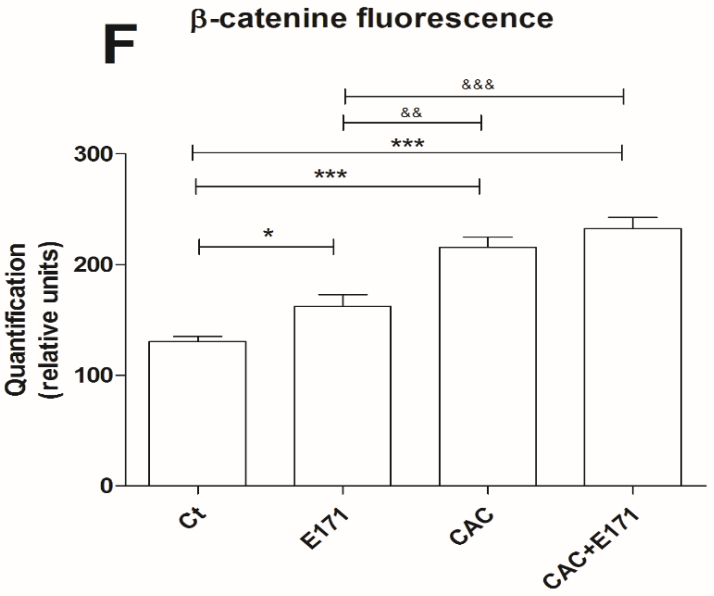
Intragastric E171 exposure enhanced tumor progression markers



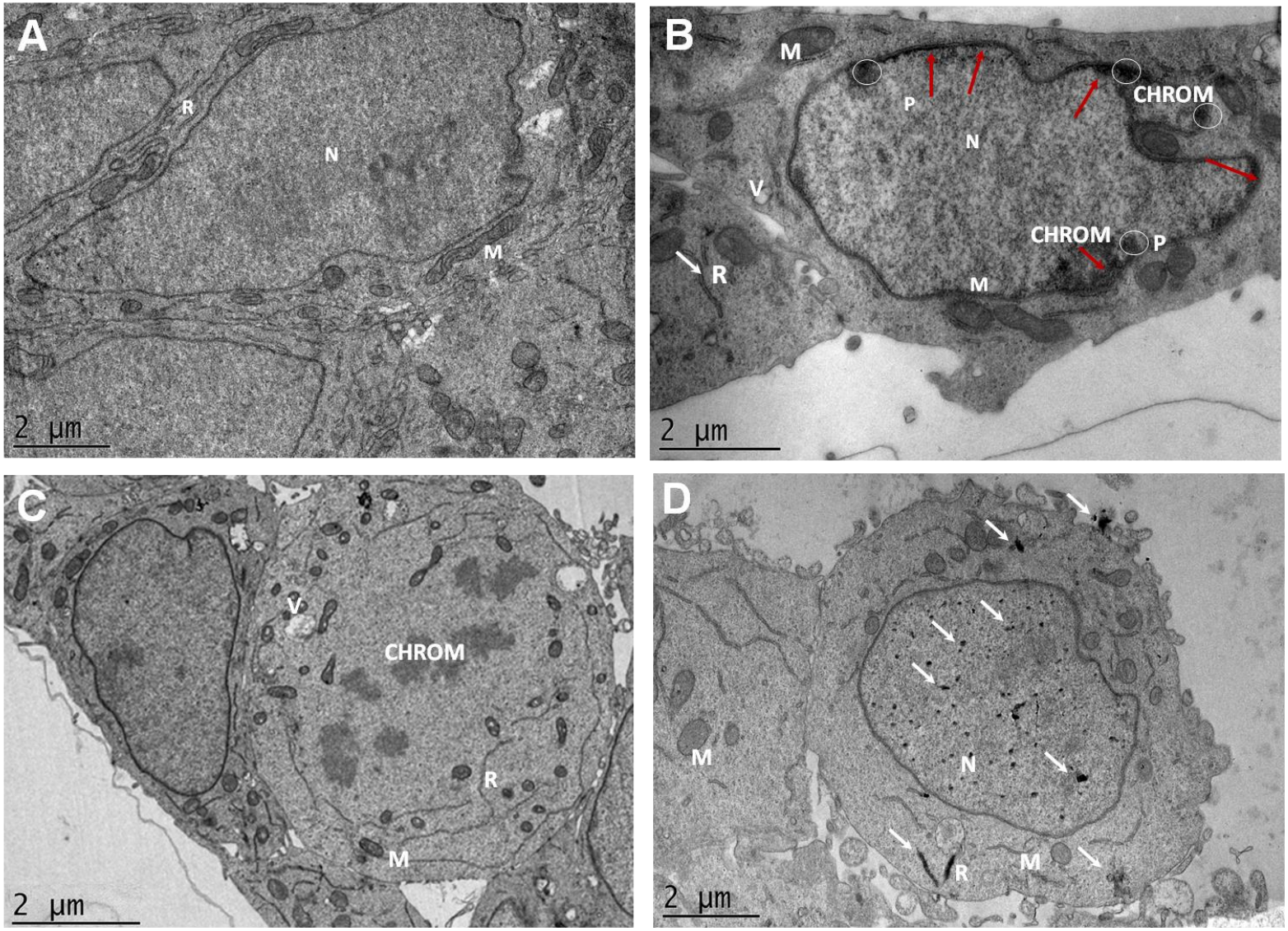


Intragastric E171 exposure enhanced tumor progression markers

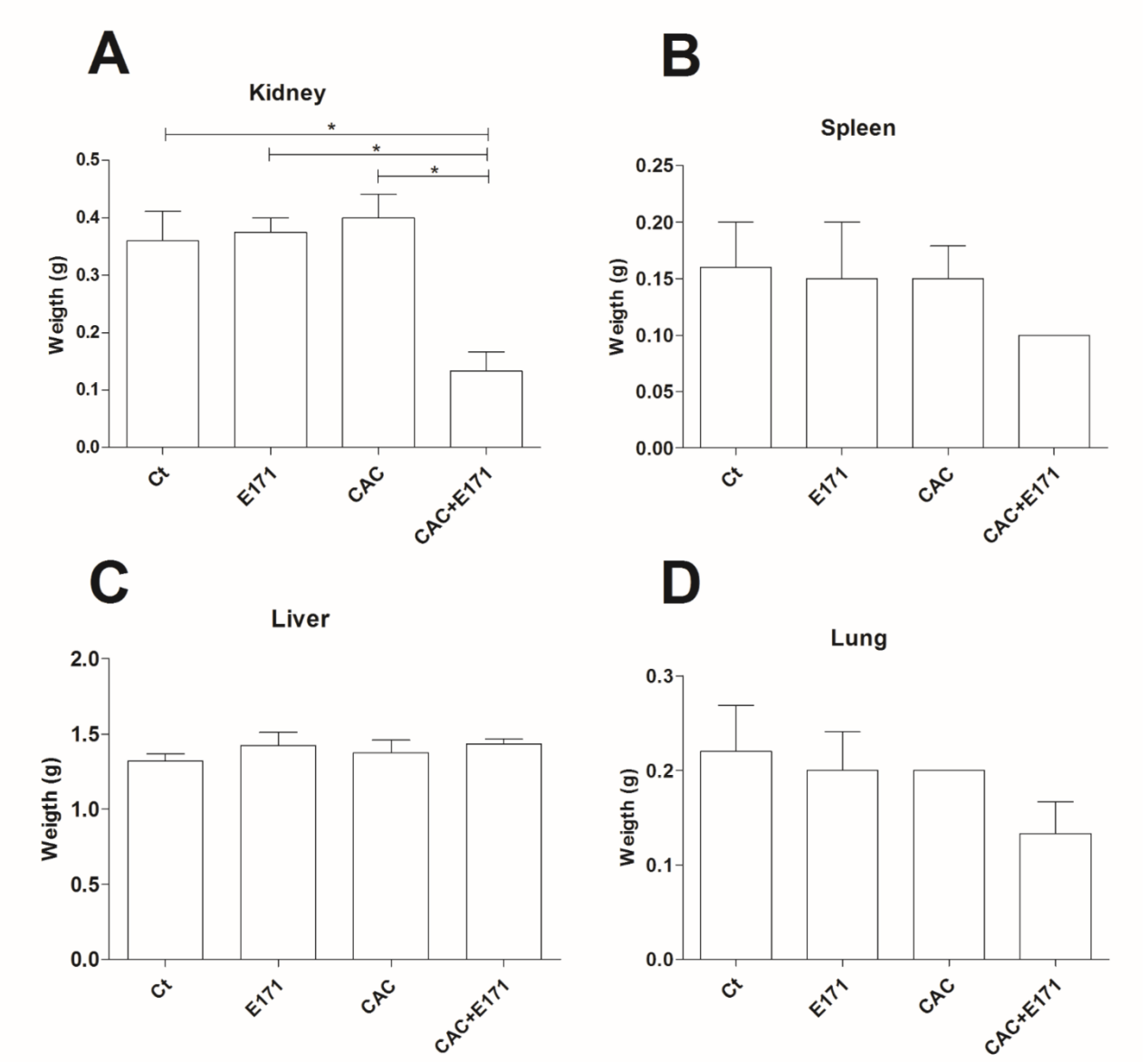




Colonic cells internalized E171 particles and cell retained the particles under ex-vivo cell culture



Intragastric E171 exposure decrease kidney mass in mice with colorectal cancer



4. Conclusions

Intragastric administration of E171 dispersed in water and dosed at 5mg/kgbw/5days/10weeks enhanced tumor formation in a colorectal cancer mice model chemically induced by AOM/DSS. E171 was unable to induce colon tumors itself but dysplastic changes in colonic epithelium and decreased goblet cells.

Maastricht University Leading in Learning!

Mechanisms of E171 induced adverse reactions in the gut

Prof. dr. Theo M. de Kok
Department of Toxicogenomics, Maastricht University, The Netherlands.

NVWA workshop • 5-6 July 2018 • Amsterdam

Outline

- Potential adverse effects of E171
- Suggested mechanisms of action
- Mechanistic studies in vivo
- Mechanistic studies in vitro
- Concluding remarks

Potential adverse effects of E171

Liver toxicity

Endocrine and reproduction toxicity

Cardiotoxicity

Inflammatory responses

Colorectal tumour promotion

Potential adverse effects of E171

Liver toxicity

Endocrine and reproduction toxicity

Cardiotoxicity

Inflammatory responses

Colorectal tumour promotion

Suggested mechanisms of action

Formation of reactive oxygen species

Resulting in oxidative stress

DNA-damage and inflammatory responses

Exacerbation of pre-existing inflammation

Impairment of intestinal and systemic immune homeostasis

Initiation of preneoplastic lesions and promotion aberrant crypt development

Tumour promotion

Mechanistic studies in vivo

Research questions based on the initial paper of Urrutia-Ortega et al (FCT 2016):

1. What histopathological changes and molecular mechanisms are affected after E171 ingestion in normal mice?

2. What molecular mechanisms are affected after E171 ingestion in AOM exposed mice?

3. Are the same molecular mechanisms affected in a transgenic mouse model without chemical induction of tumours?

Mechanistic studies in vivo (normal mice)

Research questions based on the initial paper of Urrutia-Ortega et al (FCT 2016):

1. What histopathological changes and molecular mechanisms are affected after E171 ingestion in normal mice?
2. What molecular mechanisms are affected after E171 ingestion in AOM exposed mice?
3. Are the same molecular mechanisms affected in a transgenic mouse model without chemical induction of tumours?

Food and Chemical Toxicology 111 (2018) 153–165

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Gene expression profiling in colon of mice exposed to food additive titanium dioxide (E171)

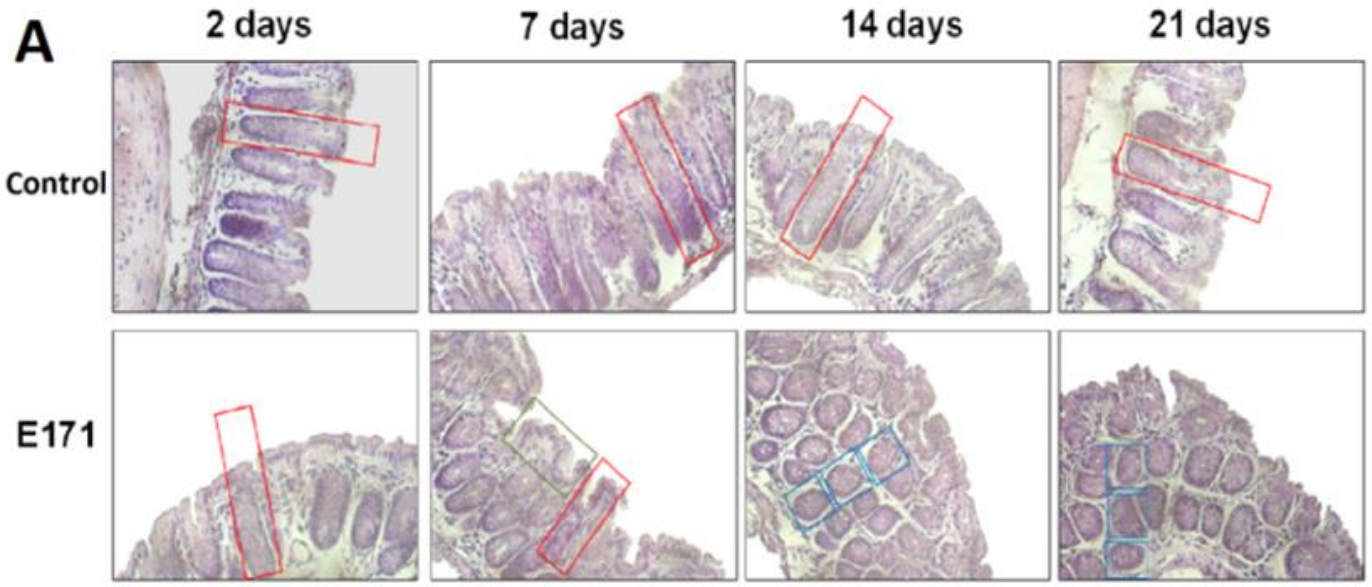
Héloïse Proquin[a,*], Marlon J. Jetten[a], Marloes C.M. Jonkhout[a], Luis G. Garduño-Balderas[b], Jacob J. Briedé[a], Theo M. de Kok[a], Yolanda I. Chirino[b,c], Henk van Loveren[a]

[a] Department of Toxicogenomics, GROW Institute of Oncology and Developmental Biology, Maastricht University, The Netherlands
[b] Laboratorio de Carcinogénesis y Toxicología, Unidad de Biomedicina, FES-Iztacala, UNAM, Estado de México, Mexico
[c] IUF-Leibniz Research Institute for Environmental Medicine, Auf'm Hennekamp 50, 40225 DE Düsseldorf, Germany

- BALB/c mice were exposed by gavage to 5 mg/kg$_{bw}$/day of E171
- for 2, 7, 14, and 21 days;
- Whole genome mRNA microarray analysis on distal colons
- Histopathological changes and proliferation were analysed.

Mechanistic studies in vivo (normal mice)

Histopathological analysis showed alteration and disruption in the normal structure of crypts inducing a hyperplastic epithelium.

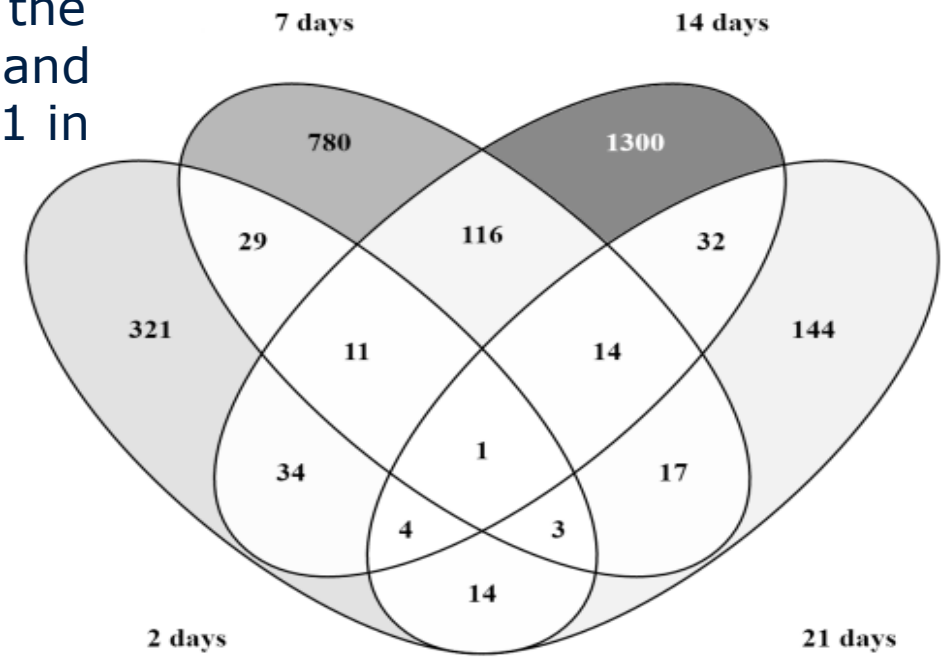


At cell proliferation level (Ki67 expression), no consistent increase over time was observed.

Mechanistic studies in vivo (normal mice)

Gene expression analysis:

Differentially expressed genes ($p<0.05$ and $FC>1.5$) between the different time points (2, 7, 14, and 21 days) after exposure to E171 in colon of mice.



Mechanistic studies in vivo (normal mice)

Pathway analysis per time point:

- E171 regulated GPCR/olfactory receptors, involved in cancer signaling;
- induced oxidative stress and immune response pathways,
- activated genes for DNA repair;
- both up- and down-regulated genes involved in development of colon cancer.

Modulation of genes that thus far have not been defined as being involved in any pathway.

Mechanistic studies in vivo

Research questions based on the initial paper of Urrutia-Ortega et al (FCT 2016):

1. What histopathological changes and molecular mechanisms are affected after E171 ingestion in normal mice?

2. What are the molecular mechanisms behind the tumour stimulatory effects of E171 in combination with azoxymethane (AOM)/dextran sodium sulphate (DSS)?

3. Are the same molecular mechanisms affected in a transgenic mouse model without chemical induction of tumours?

SCIENTIFIC REPORTS

OPEN

Transcriptomics analysis reveals new insights in E171-induced molecular alterations in a mouse model of colon cancer

Received: 18 November 2017
Accepted: 15 June 2018
Published online: 27 June 2018

Héloïse Proquin[1], Marlon J. Jetten[1], Marloes C. M. Jonkhout[1], Luis Guillermo Garduño-Balderas[2], Jacob J. Briedé[1], Theo M. de Kok[1], Henk van Loveren[1] & Yolanda I. Chirino[2,3]

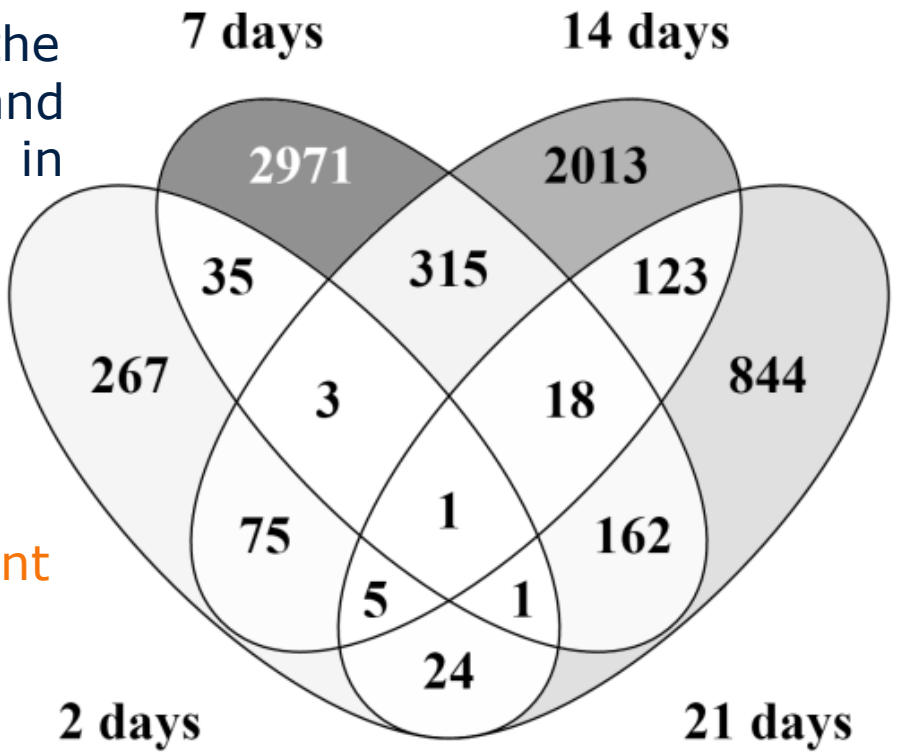
- BALB/c mice were exposed by gavage to 5 mg/kg_{bw}/day of E171
- for 2, 7, 14, and 21 days;
- Whole genome mRNA microarray analysis on distal colons

Hypothesis: oral intake of E171 induces gene expression changes related to inflammation, deregulation of cancer-related genes and impairment of the immune system before tumours are detectable.

Mechanistic studies (AOM/DSS mouse model)

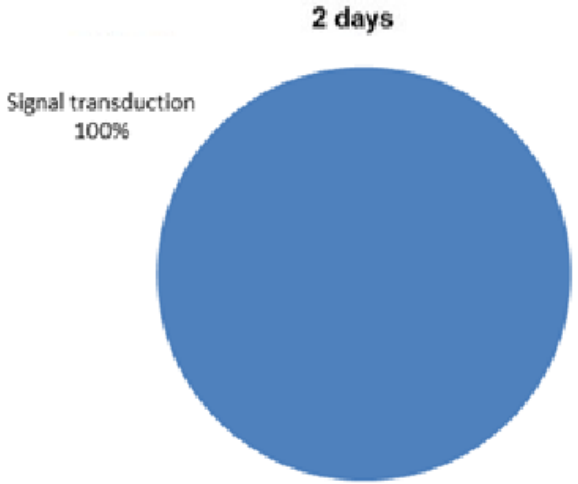
Gene expression analysis:

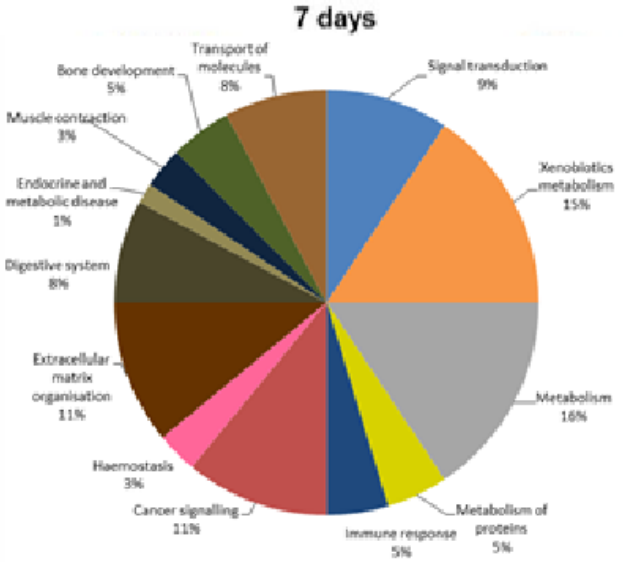
Differentially expressed genes ($p<0.05$ and $FC>1.5$) between the different time points (2, 7, 14, and 21 days) after exposure to E171 in colon of mice.

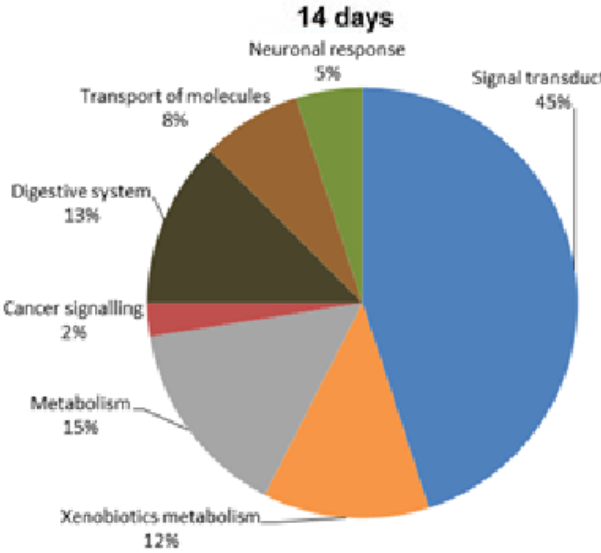


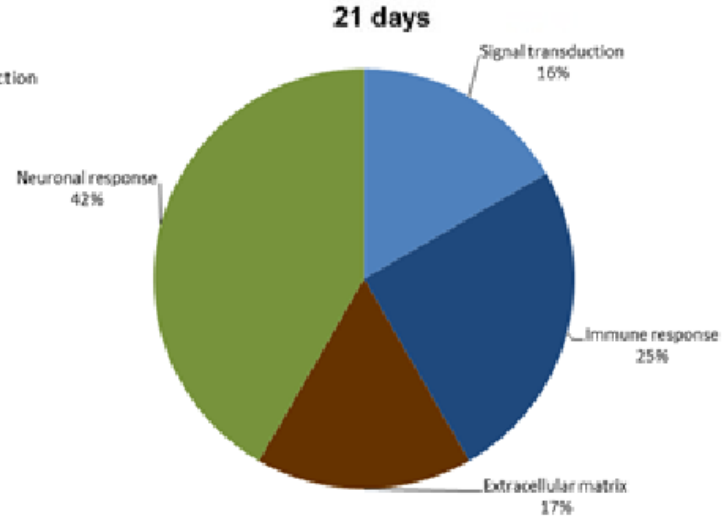
- Pathway analysis per time point
- Time series analyses
- Time point comparisons
- Time course network analysis

Pathways derived from the over-representation analysis (ORA) at different time points of exposure to E171.









Mechanistic studies (AOM/DSS mouse model)

At all time points, E171 affected mRNA levels related to:

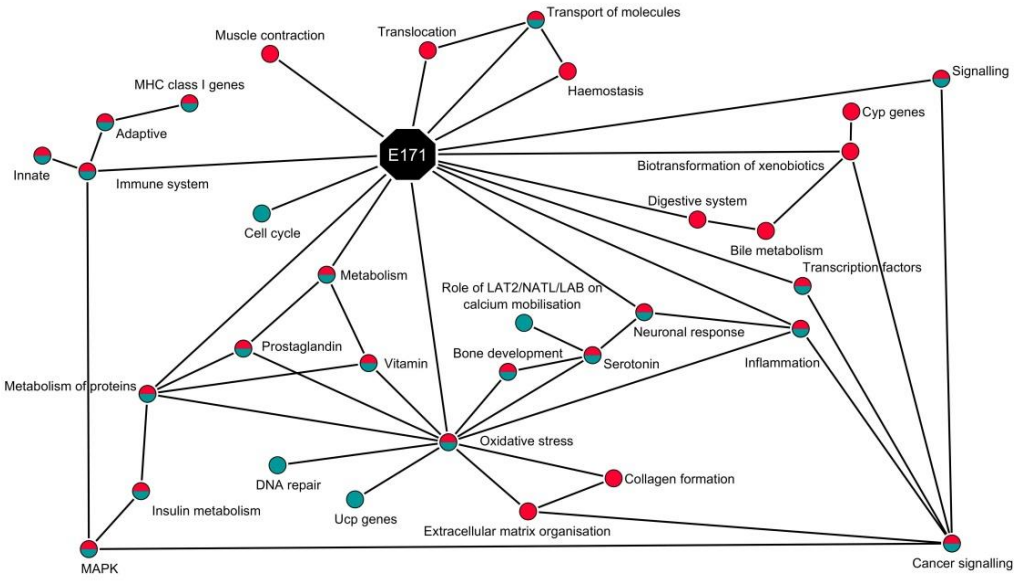
- signaling (GPCR, olfactory, cytokine, cancer signaling)
- immune system (innate and adaptive).

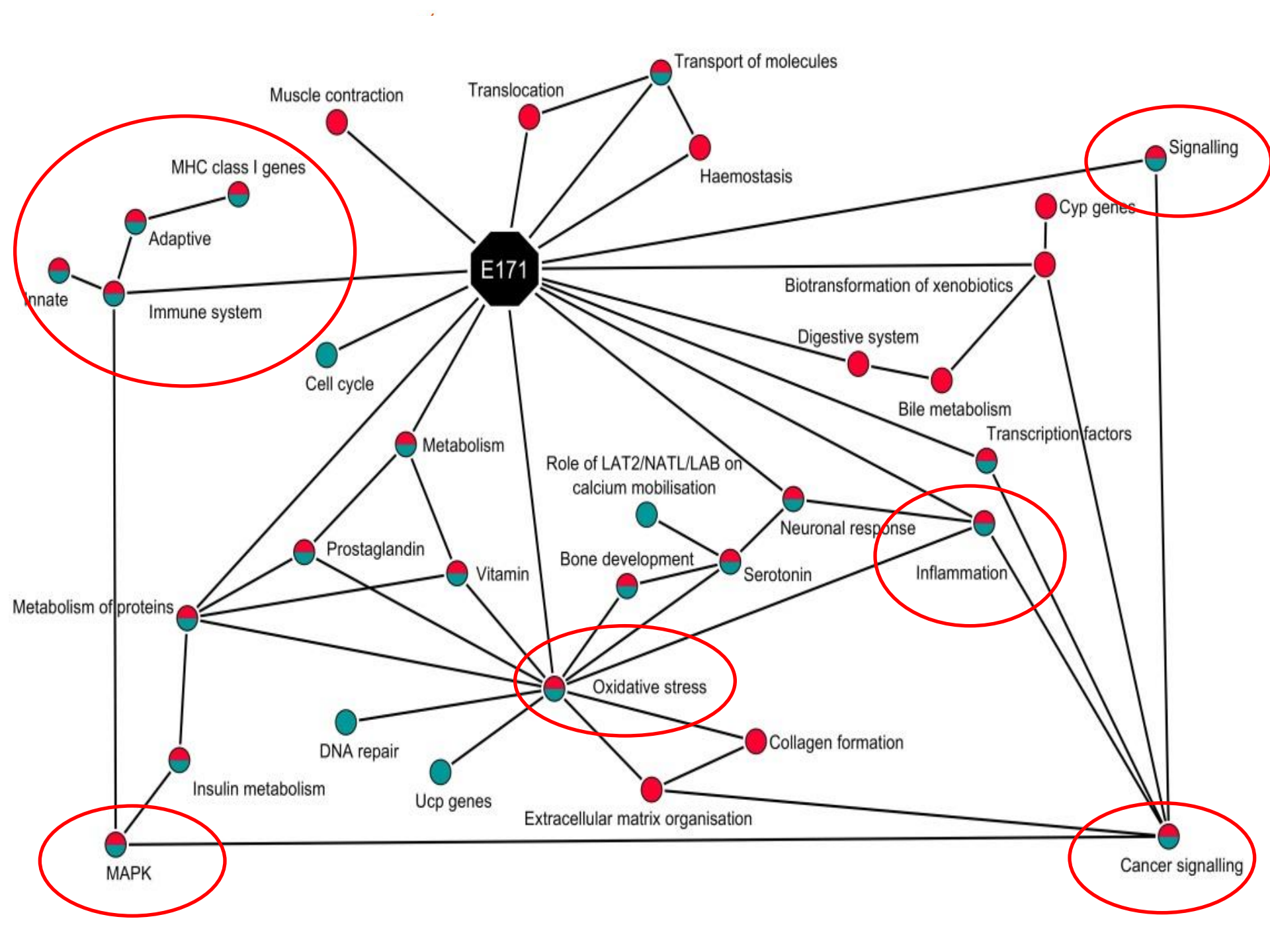
Mechanistic studies (AOM/DSS mouse model)

Time course network analysis based on the pathway analyses as well as relevant DEG over time

→ Comparison between studies with and without AOM induction

- Blue is E171 only
- Red is AOM induced
- Blue/ Red common




Transport of molecules
Muscle contraction
Translocation
Haemostasis
Signalling
MHC class I genes
Adaptive
Cyp genes
E171
Innate
Immune system
Biotransformation of xenobiotics
Digestive system
Cell cycle
Bile metabolism
Transcription factors
Metabolism
Role of LAT2/NATL/LAB on calcium mobilisation
Neuronal response
Prostaglandin
Vitamin
Bone development
Serotonin
Inflammation
Metabolism of proteins
Oxidative stress
DNA repair
Collagen formation
Insulin metabolism
Ucp genes
Extracellular matrix organisation
MAPK
Cancer signalling

Mechanistic studies (AOM/DSS mouse model)

E171 induced a downregulation of genes involved in the innate and adaptive immune system, suggesting impairment of this system.

Effects potentially associated with oxidative stress were observed through modulation of genes related to antioxidant production.

Over time, genes involved in (colorectal) cancer signaling were modulated.

These transcriptomics data reflect the early biological responses induced by E171 which precede tumour formation in an AOM/DSS mouse model.

Mechanistic studies in vivo

Research questions based on the initial paper of Urrutia-Ortega et al (FCT 2016):

1. What histopathological changes and molecular mechanisms are affected after E171 ingestion in normal mice?

2. What are the molecular mechanisms behind the tumour stimulatory effects of E171 in combination with azoxymethane (AOM)/dextran sodium sulphate (DSS)?

3. Are the same molecular mechanisms affected in a transgenic mouse model without chemical induction of tumours?

Mechanistic studies (Transgenic mouse model)

Transgenic mouse model based on the Cre-LoxP system

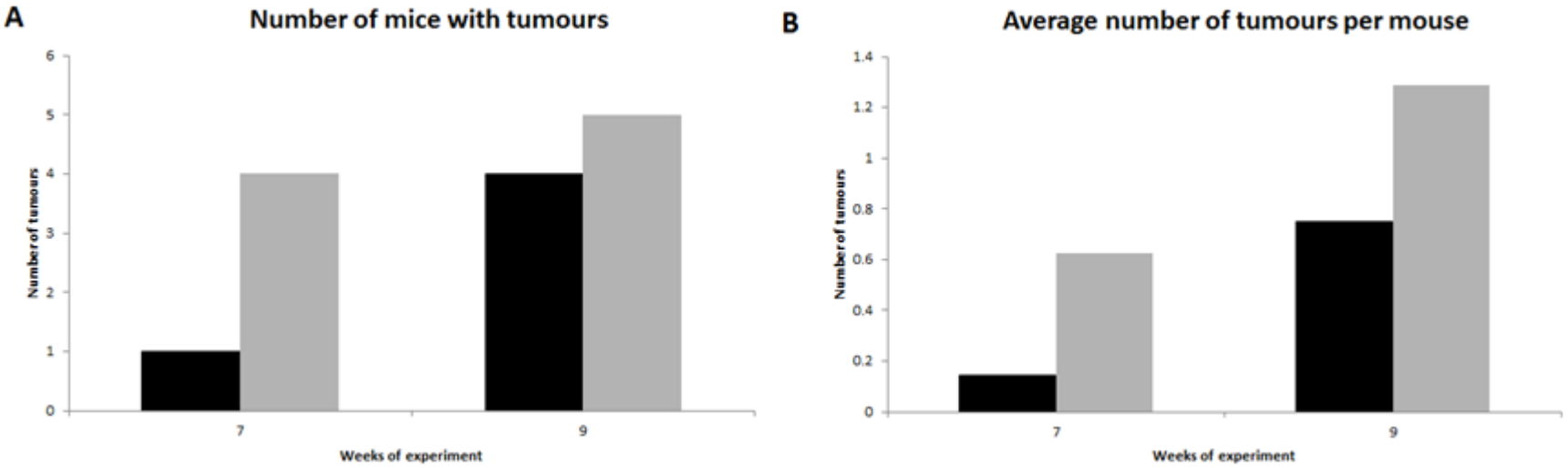
that provides a colon specific knockout model which spontaneously develops colorectal tumours.

From 5 weeks of age, $CAC^{Tg/Tg}$;$APC^{580S/+}$ mice were treated with

- 5 mg/kg_{bw}/day of E171 (n=40) or
- sterile water as control (n=40)
- for 1, 3, 5, 7 or 9 weeks
- To evaluate tumour formation.

Mechanistic studies (Transgenic mouse model)

This study showed that E171 enhanced the number of mice with tumours as well as the average number of tumours in these mice.



Black bars: control mice exposed to sterile water.
Grey bars exposed to 5 mg/kg bw/day of E171.

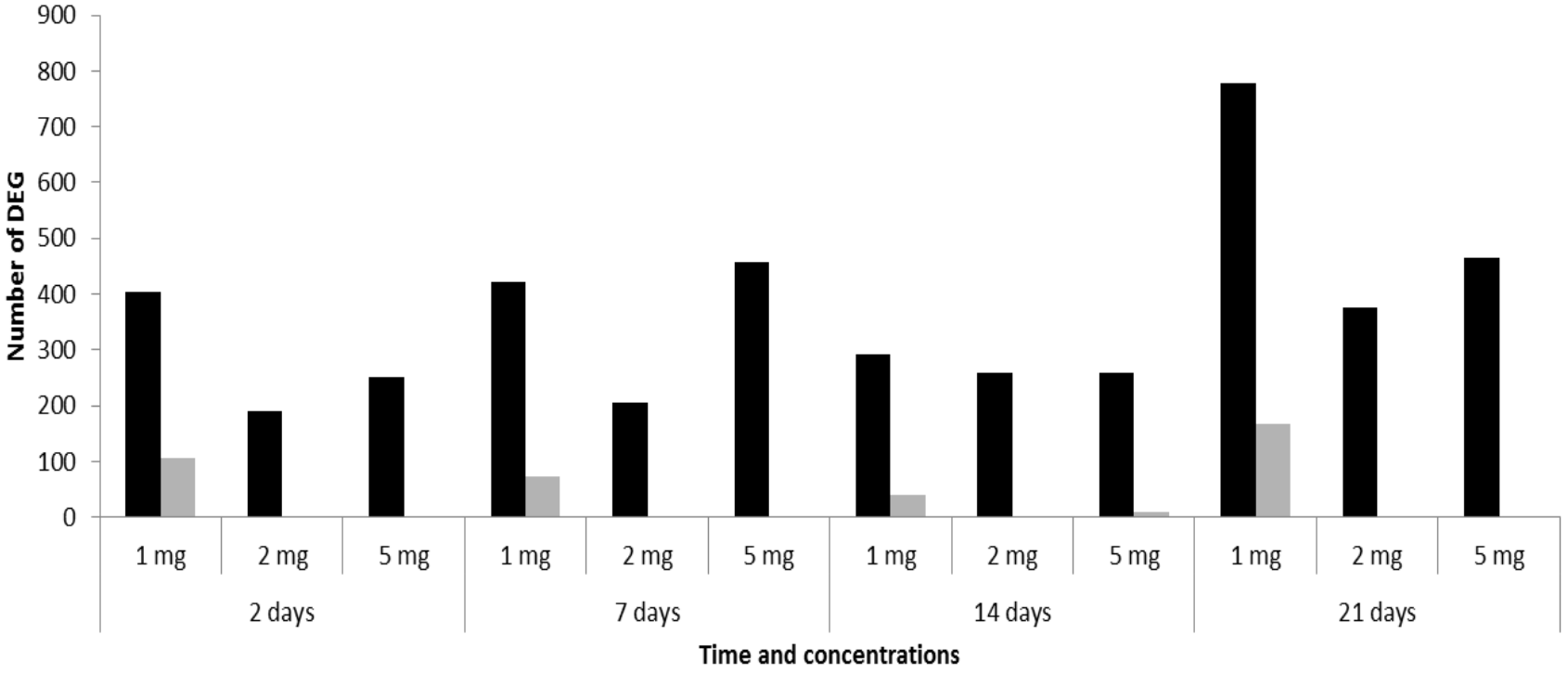
Mechanistic studies (Transgenic mouse model)

For gene expression analysis in the colon:

- 140 mice were divided in 4 groups;
- 3 exposure groups with 3 concentrations of E171 (1, 2, and 5 $mg/kg_{bw}/day$);
- one control group with sterile water;
- Exposure 5 days/week during 2, 7, 14, and 21 days;
- 7 mice per group, 3 females and 4 males.

Mechanistic studies (Transgenic mouse model)

Differentially expressed genes per dose and time point

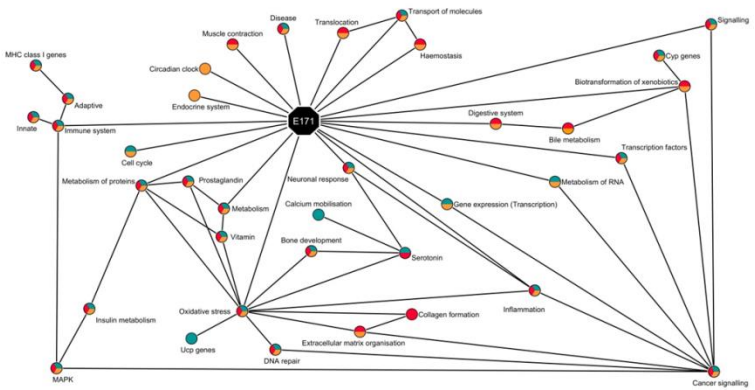


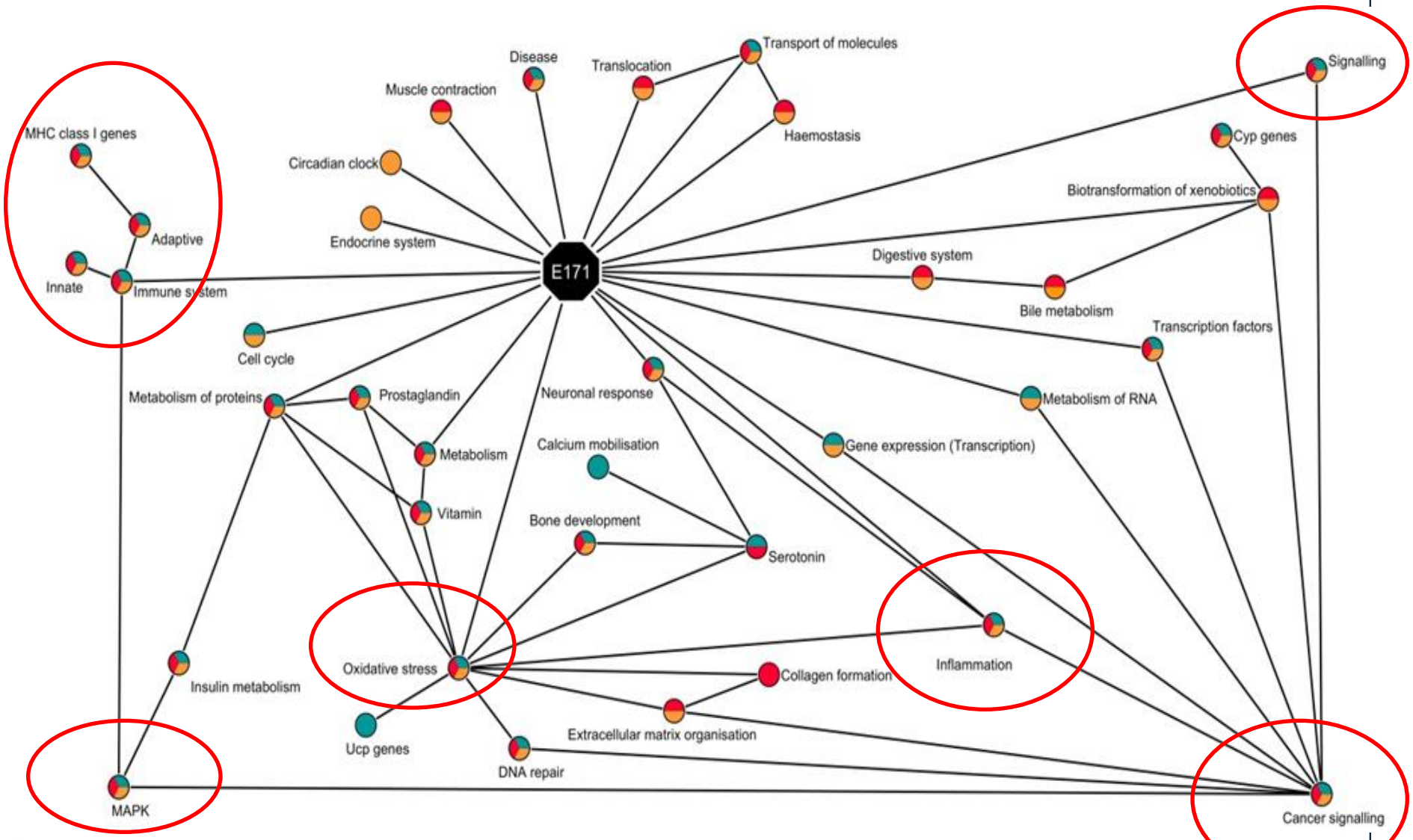
Mechanistic studies (Transgenic mouse model)

Time course network analysis based on the pathway analyses as well as relevant DEG over time

→ Comparison between studies with and without AOM induction and the transgenic mouse model

- Blue is E171 only
- Red is AOM induced
- Orange is transgenic
- Blue/ Red / Orange in common




Transport of molecules
Disease
Translocation
Signalling
Muscle contraction
Haemostasis
MHC class I genes
Cyp genes
Circadian clock
Biotransformation of xenobiotics
Adaptive
Endocrine system
E171
Digestive system
Innate
Immune system
Bile metabolism
Transcription factors
Cell cycle
Neuronal response
Metabolism of proteins
Prostaglandin
Metabolism of RNA
Metabolism
Calcium mobilisation
Gene expression (Transcription)
Vitamin
Bone development
Serotonin
Inflammation
Insulin metabolism
Oxidative stress
Collagen formation
Extracellular matrix organisation
Ucp genes
DNA repair
MAPK
Cancer signalling

Mechanistic studies (Transgenic mouse model)

This model confirms the earlier findings that E171

- downregulates of genes involved in the innate and adaptive immune system, suggesting impairment of this system.
- Induces effects associated with oxidative stress
- Modulates cancer signaling genes.

The combination of these effects may lead to the enhancement of tumour development in transgenic mice.

In vitro mechanistic studies

Findings from only a few previous studies on E171 show:

- Induction of oxidative stress and oxidative damage to DNA in human Caco-2 colon cell culture systems;
- Concomitant downregulation of the expression of the antioxidant enzymes catalase, superoxide dismutase and glutathione reductase.

Dorier-M. et al Nanotoxicology. 2017 Aug;11(6):751-761.

In vitro mechanistic studies

Mutagenesis, 2017, 32, 139–149
doi:10.1093/mutage/gew051
Original Manuscript
Advance Access publication 27 October 2016

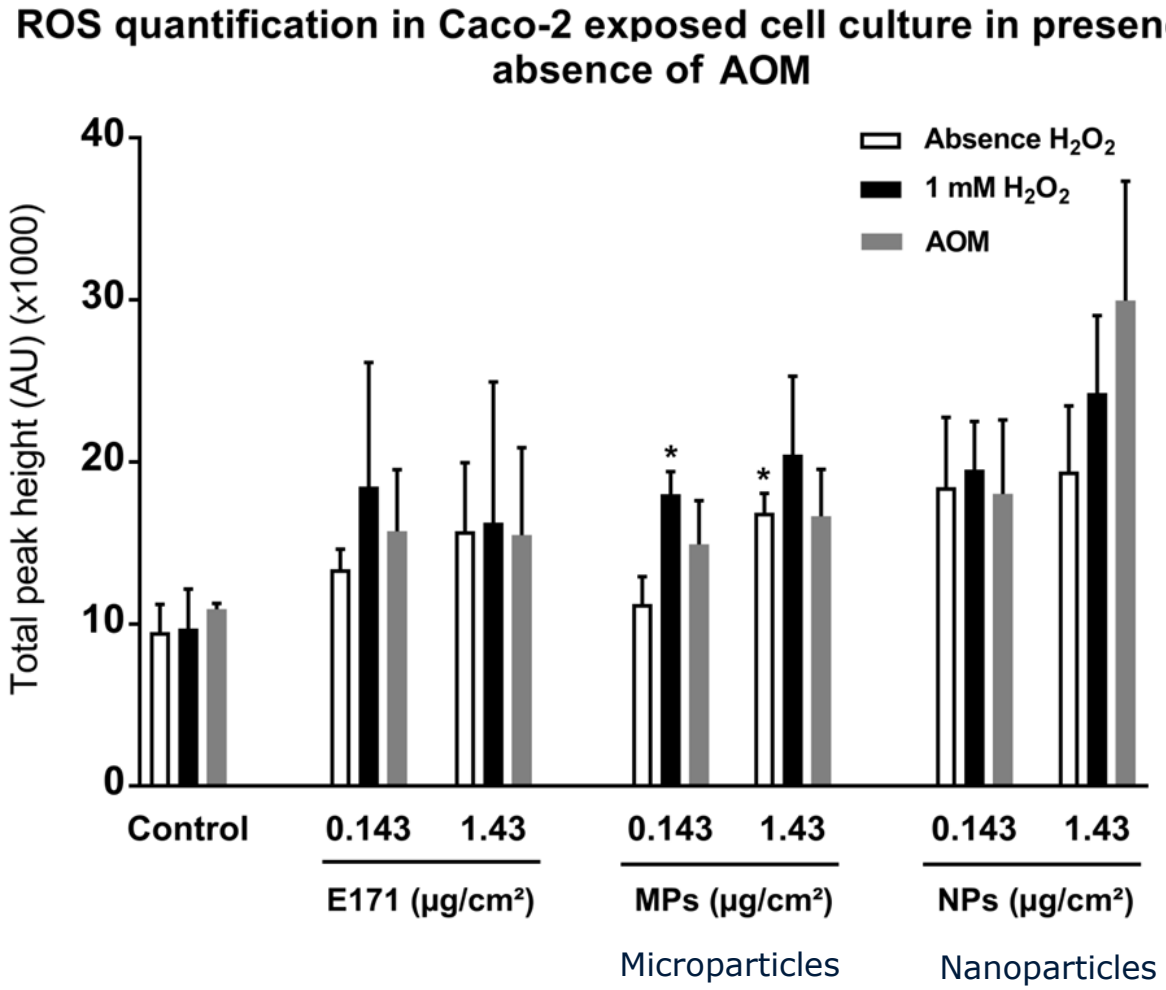
OXFORD

Original Manuscript

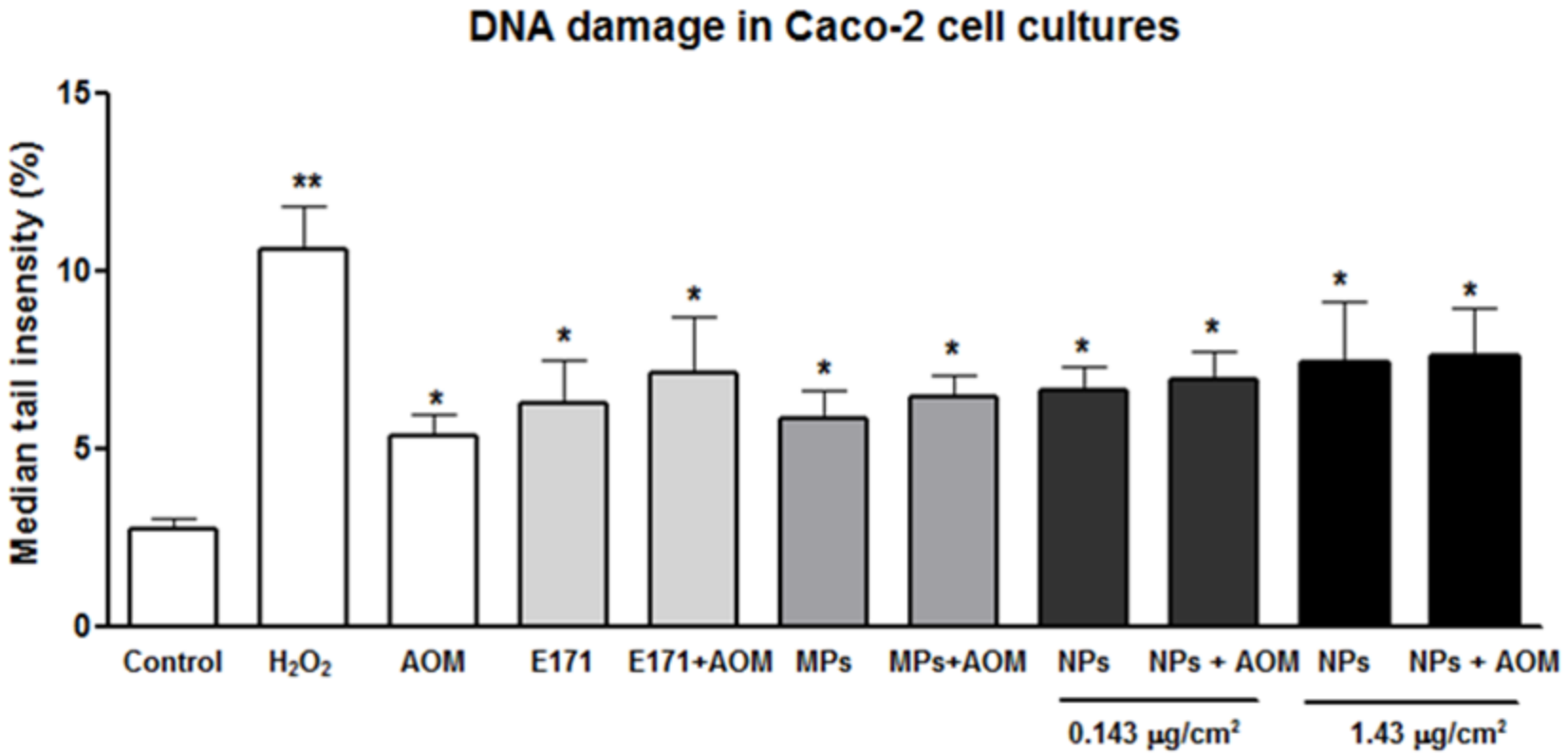
Titanium dioxide food additive (E171) induces ROS formation and genotoxicity: contribution of micro and nano-sized fractions

Héloïse Proquin[1,*], Carolina Rodríguez-Ibarra[2], Carolyn G. J. Moonen[1], Ismael M. Urrutia Ortega[2,3], Jacob J. Briedé[1], Theo M. de Kok[1], Henk van Loveren[1] and Yolanda I. Chirino[2]

In vitro mechanistic studies

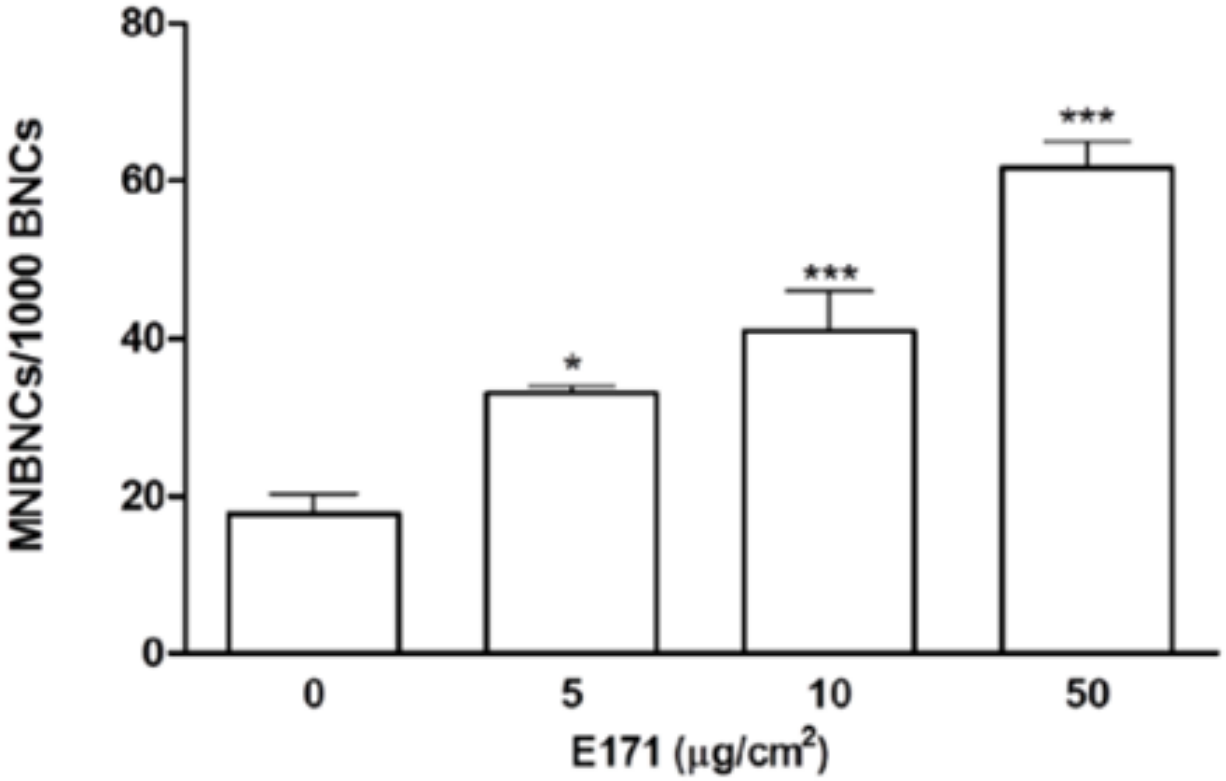


In vitro mechanistic studies



In vitro mechanistic studies (micronuleus test)

E171 induced chromosome damage in HCT116 cell cultures



In vitro mechanistic studies

ROS generation in a cell-free environment was highest for E171 followed by NPs and MPs (not shown).

MPs were capable to induce significant ROS formation in exposed Caco-2 cells.

E171, MPs and NPs all induced single-strand DNA breaks.

Chromosome damage was shown to be induced by E171, as tested with the micronucleus assay in HCT116 cells.

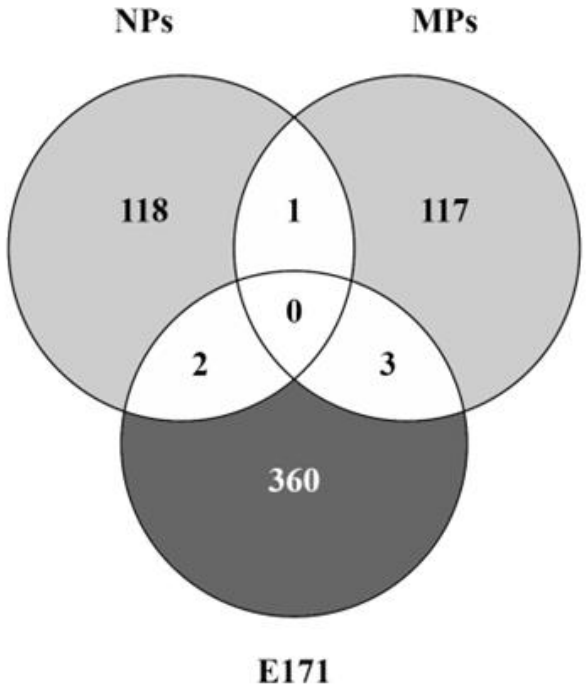
In conclusion, these findings confirm the capacity of E171, MP and NP to induce ROS formation and genotoxic effects.

In vitro transcriptomic responses in Caco-2

Time specific responses to TiO2 NP (2, 4, 24 h)

Most abundant and diverse gene expression and pathway changes after 24h exposure.

Unique and common DEG after exposure of Caco-2 cells to 1.43 µg/cm^2 E171, MPs, and NPs for 24h.



Biological processes modulated:

- Cellular response to (oxidative) stress
- Innate and adaptive immune system
- Inflammation
- Cell signallng
- Cancer

Concluding remarks

- E171 modulates relevant molecular pathways related to cancer development:
  - Oxidative stress
  - Inflammation
  - Immune response
  - Cancer signaling

- These mechanisms are consistently found in different mouse models as well as in human colon cells.

These mechanisms may pay a role in the promotion of tumour development in E171 exposed mice.

Acknowledgement

Maastricht University, The Netherlands

Heloïse Proquin
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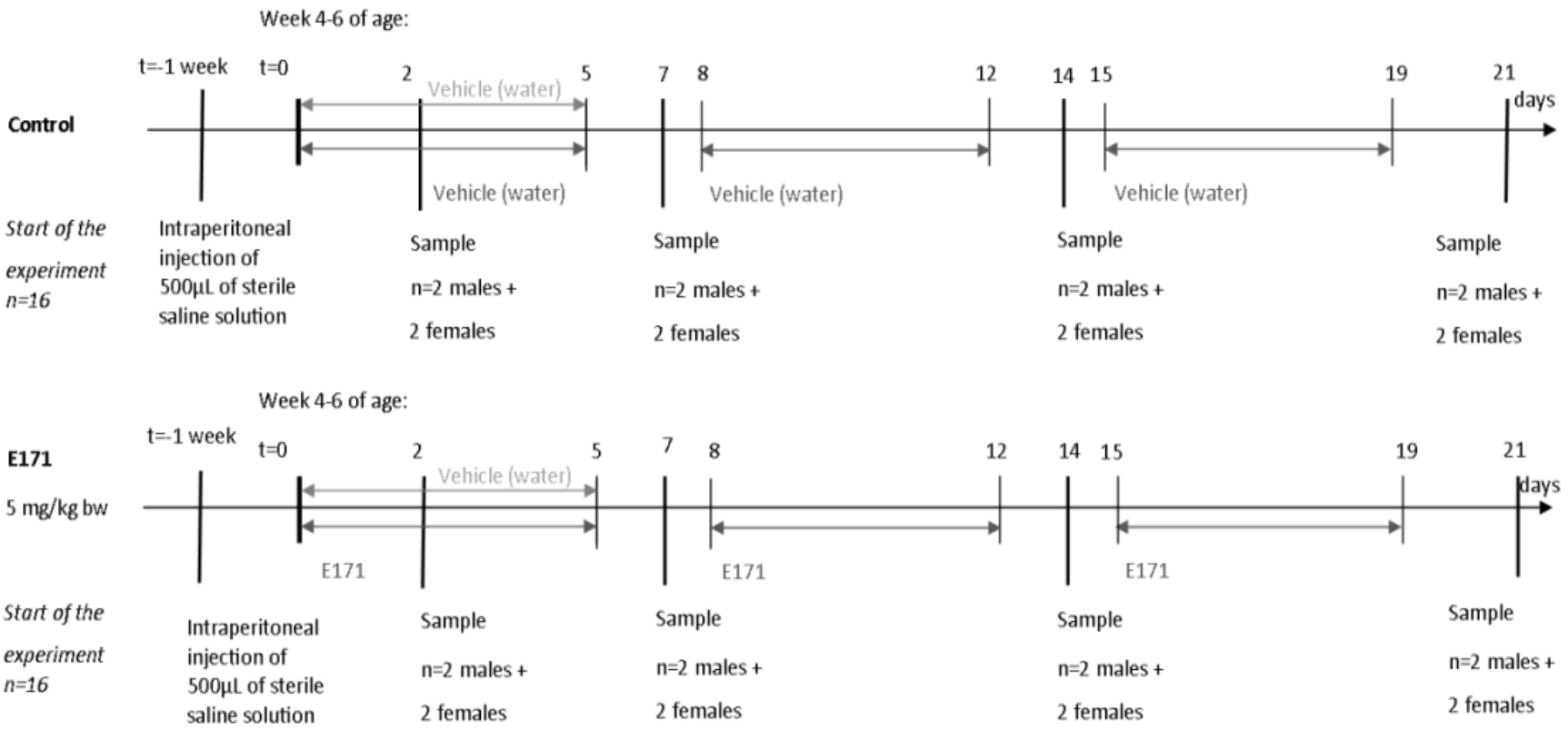
Jacco Briedé
Theo de Kok

Universidad Nacional Autonoma de Mexico, Mexico

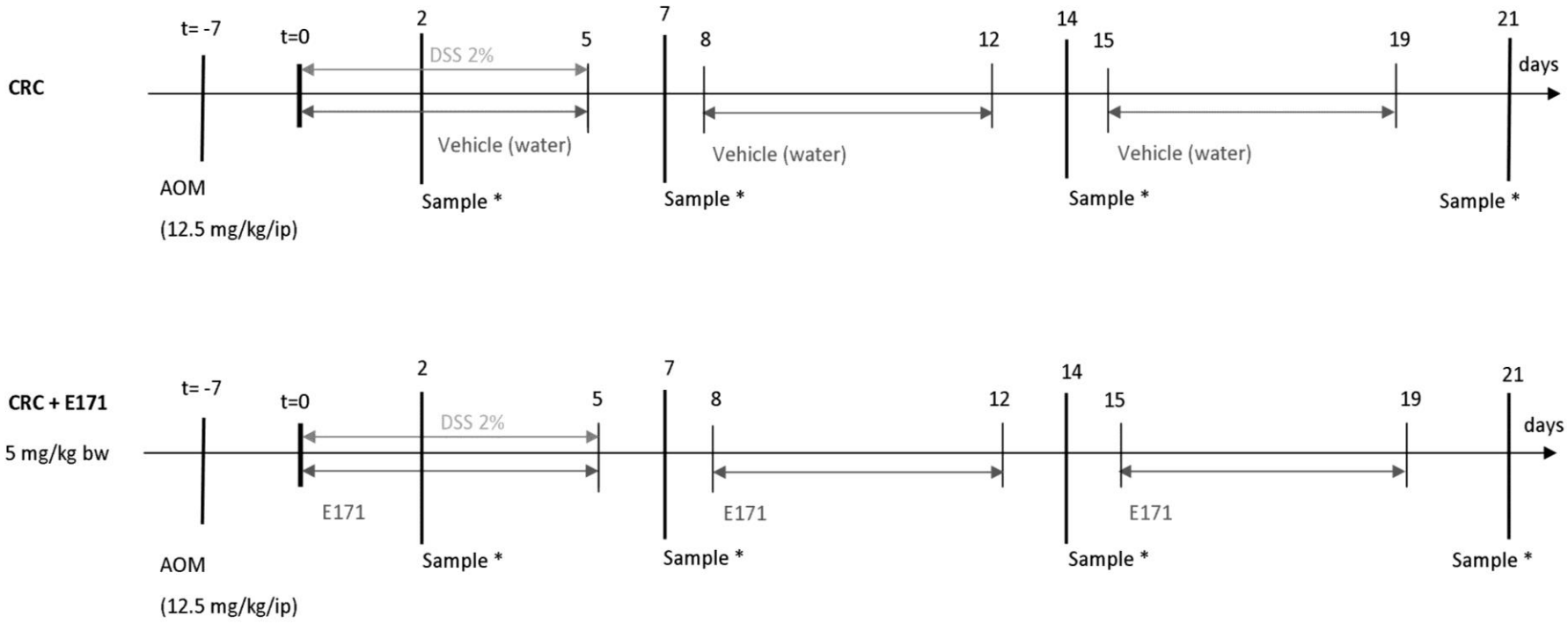
Luis Garduño-Balderas
Ismaël Urrutia Ortega
Carolina Rodríguez-Ibarra
Yolanda Chirino

Additional slides

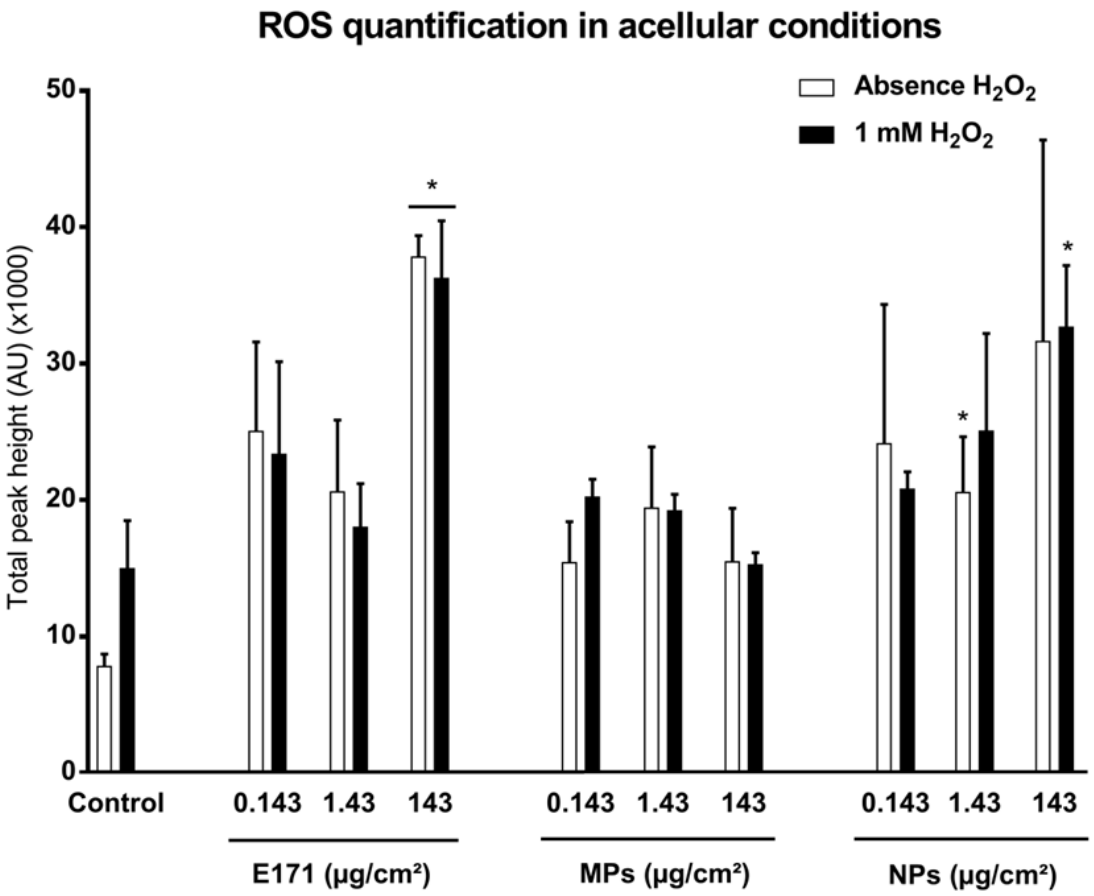
Mechanistic studies (normal mouse model)



Mechanistic studies (AOM/DSS mouse model)



In vitro mechanistic studies



The EFSA re-evaluation of titanium dioxide

Dr David Gott

Head of Toxicology Team, Food Standards Agency and Member of EFSA ANS Panel

Views expressed are those of the author and should not be considered to reflect FSA or EFSA policy

Outline of presentation

- Food additives re-evaluation programme
- EFSA opinion on titanium dioxide (2016)
- Conclusions and uncertainties
- New developments: latest EFSA opinion (June 2018)

The EFSA re-evaluation programme

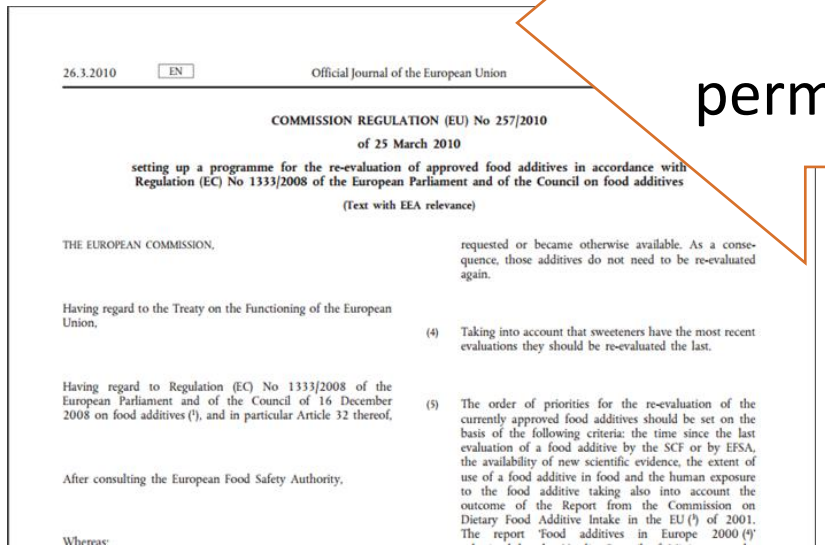
- The following slides are taken from my presentation at the EFSA Stakeholder Workshop in November 2017

- My thanks to the Food Ingredients and Packaging unit for preparing them for that meeting and allowing me to use them today

RE-EVALUATION VS NEW APPLICATIONS

Re-evaluation programme

Reg. 257/2010



26.3.2010 EN Official Journal of the European Union

COMMISSION REGULATION (EU) No 257/2010
of 25 March 2010
setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives
(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (1), and in particular Article 32 thereof,

After consulting the European Food Safety Authority,

Whereas:

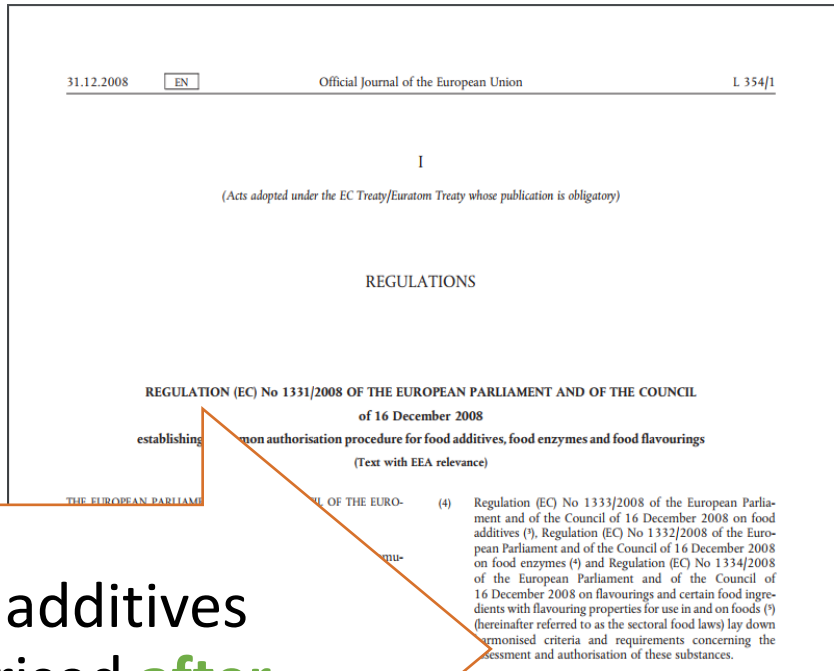
requested or became otherwise available. As a consequence, those additives do not need to be re-evaluated again.

(4) Taking into account that sweeteners have the most recent evaluations they should be re-evaluated the last.

(5) The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the SCF or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU (3) of 2001. The report 'Food additives in Europe 2000 (4)'

Food additives already permitted **before**

Food additives authorised **after**



31.12.2008 EN Official Journal of the European Union L 354/1

I

(Acts adopted under the EC Treaty/Euratom Treaty whose publication is obligatory)

REGULATIONS

REGULATION (EC) No 1331/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 16 December 2008
establishing ...mon authorisation procedure for food additives, food enzymes and food flavourings
(Text with EEA relevance)

THE EUROPEAN PARLIAMENT ...IL OF THE EURO-

...mu-

(4) Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (3), Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes (4) and Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods (5) (hereinafter referred to as the sectoral food laws) lay down ...rmonised criteria and requirements concerning the ...ssessment and authorisation of these substances.

Common authorisation procedure

Reg. 1331/2008

20 Jan 2009

RE-EVALUATION VS NEW APPLICATIONS

Re-evaluation

efsa
European Food Safety Authority

SCIENTIFIC OPINION

Statement on a conceptual framework for the risk assess… food additives re-evaluated under Commission Regulation… 257/2010[1]

EFSA Panel on Food additives and Nutrient Sources added to Food (ANS)[2]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific statement presenting a conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010. This framework will be used in the evaluation made by the Panel, but the expert judgement of the scientific background, on a case-by-case basis, remains essential to reach a final conclusion. The outcome of the re-evaluation of food additives taking into account all available information is presented in the document, as well as the exposure assessment scenarios to be carried out by the Panel considering the use levels set in the legislation and the availability of adequate usage or analytical data.

KEY WORDS

Commission Regulation (EU) No 257/2010, food additives, re-evaluation, risk assessment

Food additives already permitted **before**

Food additives authorised **after**

efsa

EFSA Journal 2012;10(7):2760

SCIENTIFIC OPINION

Guidance for submission for food additive evaluations[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 16 August 2012, replaces the earlier version published on 18 July 2012.[4]

ABSTRACT

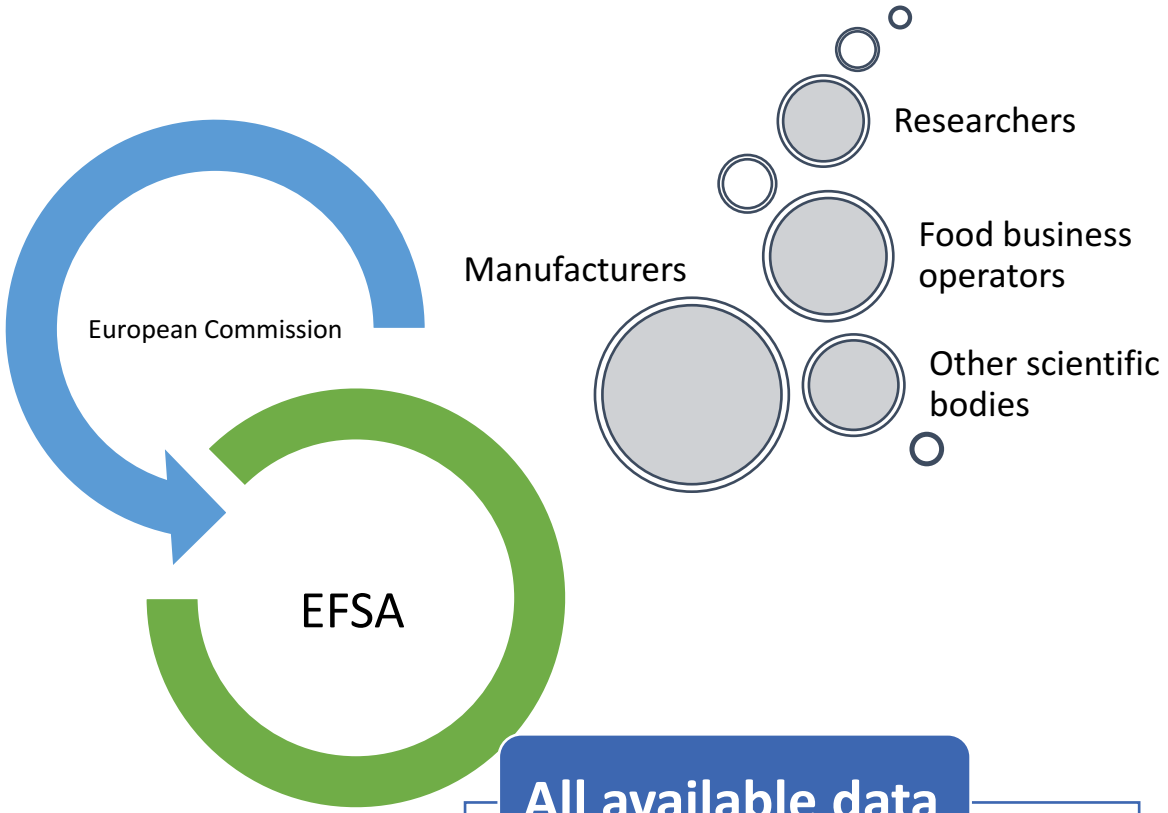
This guidance document refers to the applications for authorisation of a new food additive or to a modification of an already authorised food additive, combining in a single document the description of the data requirements and their context, and also a description of the risk assessment paradigm applied. The document is arranged in four main sections: chemistry and specifications, existing authorisations and evaluations, proposed uses and exp… assessment, and toxicological studies. Assessment of the exposure to food additives is based on info… on known or anticipated human exposure to the proposed additive or toxicologically relevant com… the additive from food, and any other potential dietary sources. For the toxicological studies, this guid… s a tiered approach which balances data requirements against the risk, taking into con… welfare by adopting animal testing strategies in line with the 3-Rs (replacement, …his tiered approach for toxicological studies consists of 3 tiers, for which the testing …d triggers are described. According to this tiered approach, a minimal dataset …been developed under Tier 1, while Tier 2 testing, generating more extensive …s which are absorbed and/or demonstrate (geno)toxicity in Tier 1 tests. Tier …ase basis taking into consideration all the available data, to elucidate …ation of findings in Tier 2 tests. This guidance document replaces the …Committee for Food published in 2001.

…cation, Tiered approach, Risk assessment, Toxicological studies

New applications

20 Jan 2009

RE-EVALUATION VS NEW APPLICATIONS



All available data

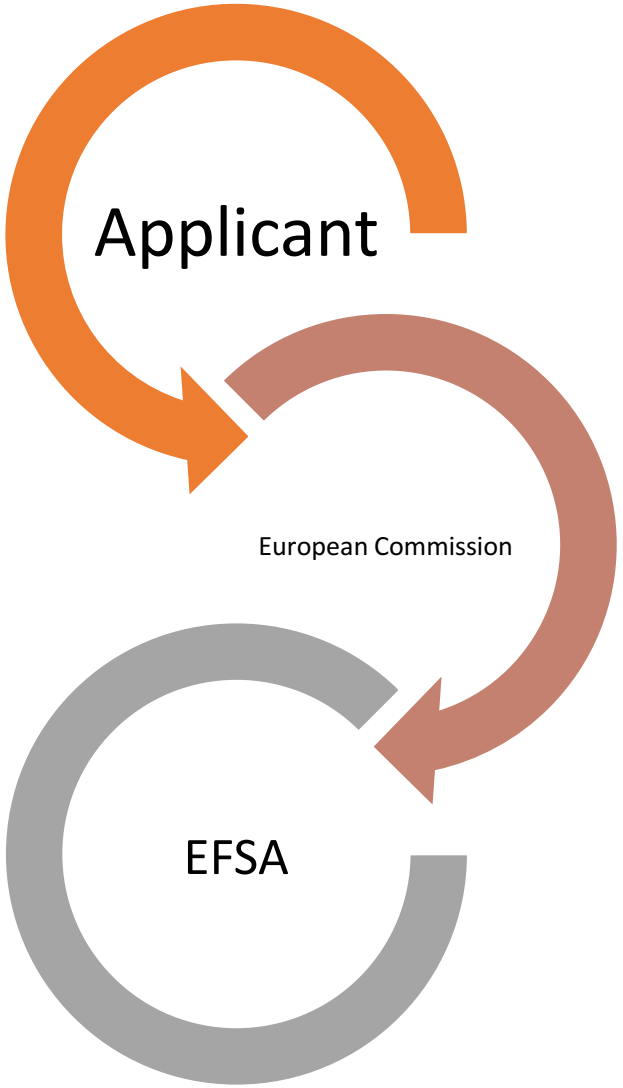
- Published literature
- Previous evaluations
- Unpublished reports

20 Jan 2009

Dossier

- Scientific information

9 months
(+ stop-the-clock) from receipt of a valid application



SCIENTIFIC ASSESSMENT

In the re-evaluation of food additives the available pieces of the jigsaw are put together

There may be missing pieces, but can we still understand the picture?

How do we piece together the jigsaw?

- Consider all the data available
- Evaluate the data and the data gaps
- Assess the significance of the data gaps
- Use read across and extrapolation as appropriate
- Use a weight of evidence evaluation
- In considering need for additional studies against uncertainty take account of other societal values e.g. animal welfare

SCIENTIFIC ASSESSMENT

Technical part

- Identity of the substance
- Specifications
- Analytical results
- Manufacturing process
- Methods of analysis in food
- Stability and fate in food

Questions

- What is the food additive?
- Are we talking about the same substance that it was assessed at the time of the initial authorisation?
- What are residuals/by products resulting from manufacturing process/storage/interaction with food?

HAZARD IDENTIFICATION

SCIENTIFIC ASSESSMENT

Biological and toxicological data

- ADME (absorption, metabolism, distribution, excretion)
- Genotoxicity (in vitro, in vivo)
- General toxicity (short-term, sub-chronic, chronic, carcinogenicity)
- Reproductive toxicity
- Immunotoxicity
- Other studies

Questions

- What happen to the additive once it is ingested with the diet? Is it absorbed? To what is it metabolised?
- Are adverse effects identified from the available studies?
- If yes: can a dose response be identified?
- If no: true lack of effect or lack of data?
- Are the data available still reliable compared to today's standards?

HAZARD CHARACTERISATION

SCIENTIFIC ASSESSMENT

Dietary exposure

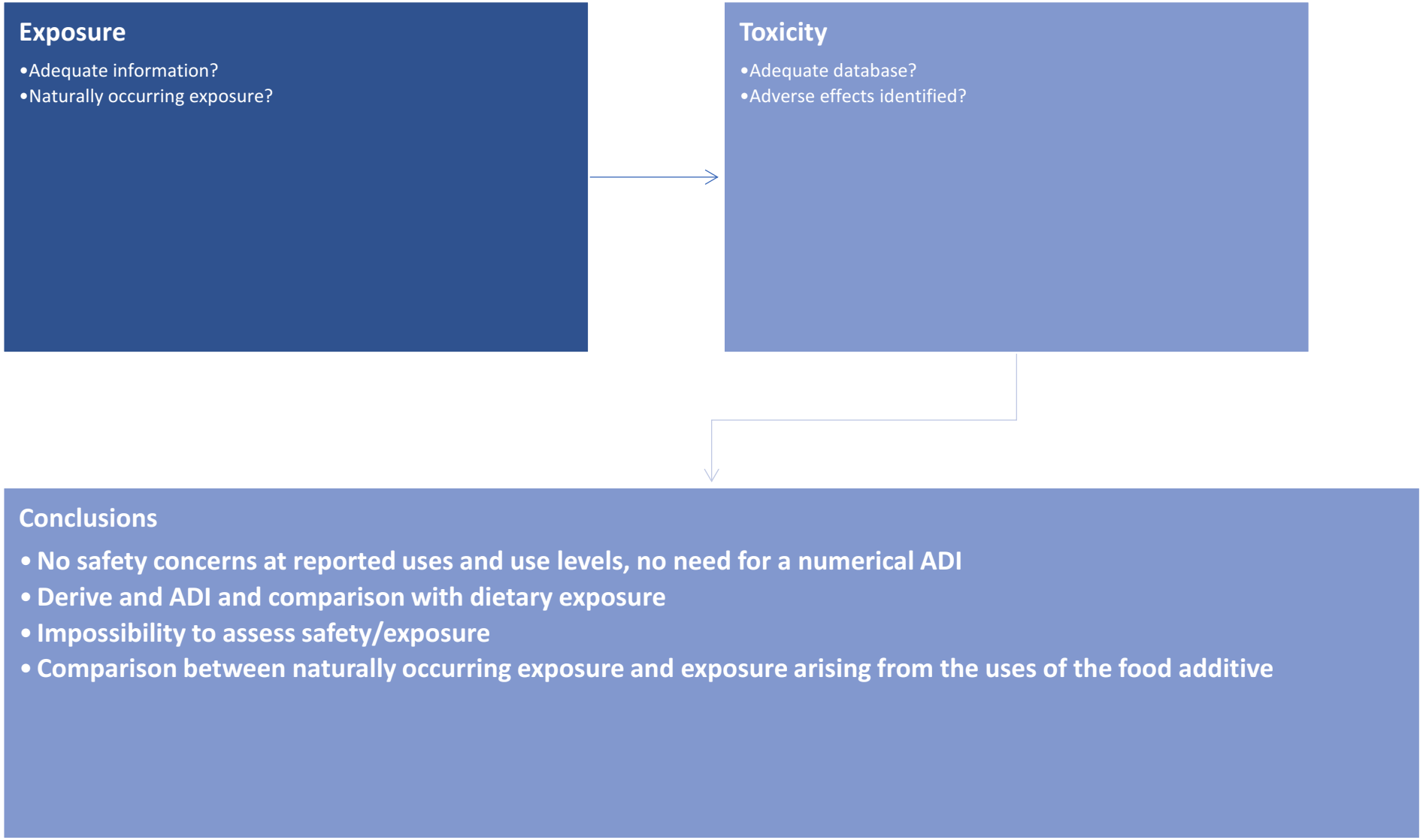
- Maximum permitted levels given in the legislation
- Typical uses and use levels
- Analytical data
- Other sources of exposure

Questions

- How much is the daily intake of the food additive in the EU population?
- Are there groups of the populaton that are exposed to it more than others?
- Is the additive really used in all the food categories in which it is authorised?
- Which food categories contribute most to the exposure?

EXPOSURE ASSESSMENT

CONCEPTUAL FRAMEWORK FOR RE-EVALUATION



Re-evaluation of titanium dioxide E171

- The last food colours to be re-evaluated
  - Number of calls for data
- Previous evaluations
  - JECFA 1969
    - an acceptable daily intake (ADI) ‘not limited except for good manufacturing practice’
  - SCF 1977
    - colours for which an ADI was not established but which could be used in food
- Also used as a feed additive: ongoing evaluation by the EFSA FEEDAP Panel

The nanomaterial issue

- All particulate food additives contain a distribution of particle sizes some of which are likely to be in the nano range
- Information provided on E171
  - would not be considered as a nanomaterial according to the EU Recommendation on the definition of a nanomaterial
- Currently no set limits for the particle size of TiO_2 in the EU specifications
- Panel recommended specifications for E 171 should include a characterisation of particle size distribution using appropriate statistical descriptors (e.g. range, median, quartiles) as well as the percentage (in number and by mass) of particles in the nanoscale

During re-evaluation Panel considered

- manufacturing process for powdered or particulate food additives resulted in material with a range of sizes.
- median size of the particles is generally significantly greater than 100 nm, a small fraction will always be, and has been, with at least one dimension below 100 nm
- material used for toxicological testing would have contained this nano fraction.
- in principle, for a specific food additive containing a fraction of particles with at least one dimension below 100 nm, adequately conducted toxicity tests should be able to detect hazards associated with this food additive, including its nanoparticulate fraction
- for the re-evaluation of food additives, this procedure would be sufficient for evaluating constituent nanoform fraction in accordance with the recommendation of the EFSA Nano Network in 2014

How big a problem is it?

- analytical data provided by interested parties confirmed the small percentage in the nanoscale
- BUT actual values depended on the method used
- Based on this information a percentage value of up to 3.2% of nanoparticles by mass was considered a reasonably conservative estimate

ADME conclusions

- the absorption of orally administered TiO_2 is extremely low
- the bioavailability of TiO_2 (measured either as particles or as titanium) is low
- the bioavailability measured as titanium appeared to be independent of particle size
- the vast majority of an oral dose of TiO_2 is eliminated unchanged in faeces
- a small amount (maximum of 0.1%) of orally ingested TiO_2 was absorbed by the GALT and subsequently distributed to various organs and elimination rates from these organs were variable
- there were significant and highly variable background (basal) levels of titanium in animals and humans, which presented challenges in the analysis at the low levels of titanium uptake reported and could complicate interpretation of the reported findings in some studies

short-term and subchronic toxicity

- rather limited information available

- 28-day gavage study in rats, NOAEL for the study was 24,000 mg/kg bw per day but material was not E171 but useful as supporting evidence
- 90-day study, doses up to 16,900 mg /kg bw per day for male mice and up to 8,100 mg /kg bw per day for male rats
- No differences in body weight or in relevant gross or microscopic pathology BUT no haematological parameters and no biochemical parameters in urine and blood were measured

Genotoxicity

- available mixed results provided some evidence of *in vitro* genotoxicity for TiO_2 micro- and nanoparticles
  - most positive results have been reported under experimental conditions associated with the induction of oxidative stress
  - genotoxic effects observed mainly in indicator assays, which may not be associated with permanent chromosome damage.
- *In vivo*
  - overall negative results were obtained in genotoxicity studies with microsized
  - Limited, if any, evidence in studies with orally administered nanoparticle
  - Limited or no indication of genotoxicity for nanoparticles using the intravenous route of administration

Carcinogenicity and chronic toxicity

- No evidence of carcinogenicity in rats and mice
  - Studies were old (1979)
  - Limited information on titanium dioxide tested
  - Carried out by US National Cancer Institute
  - Used oral administration

  - Evidence of carcinogenicity in inhalation studies but not relevant for this evaluation

- NOAEL was highest dose tested - 50000 mg/kg diet
  - Equivalent to
    - 6,500 and 8,350 mg/kg bw per day, for male and female mice, respectively
    - 2,250 and 2,900 mg/kg bw per day, for male and female rats, respectively

Promotion initiation studies

- Urrutia-Ortega et al. (2016),
- Intragastric administration of TiO_2 (E 171) in a chemically colitis-associated colorectal cancer (CAC) model in 24 mice in 4 groups
    - (a) control;
    - (b) 5 mg/kg bw foodgrade TiO2 (E 171; 99% pure) by gavage, 5 days/week for 10 weeks;
    - (c) the chemically colitis associated cancer (CAC) group received a single i.p. dose of 12.5 mg/kg bw azoxymethane (AOM) and 2% dextran sulfate sodium (DSS) in the third, sixth and ninth week in water ad libitum;
    - (d) the CAC + TiO2 (E 171) group: AOM, DSS and TiO2 (E 171).
  - TiO_2 (E 171) in combination with the initiator increased the expression of markers of tumour progression including COX2, Ki67 and b-catenin.
  - TiO_2 (E 171) alone did not show any enhancing effect on tumour markers.
- Further research needed but study cannot be used for risk assessment

Reproductive and developmental toxicity

- No reproductive (one- or two-generation toxicity) studies with TiO_2 (as the food additive, micro- or nanosized) performed according to the OECD guidelines were available for evaluation
- prenatal developmental studies with three pigment-grade (pg-1, pg-2 and pg-3) and three ultrafine (uf-1, uf-2 and uf-3)/nanoscale (anatase and/or rutile) TiO_2 particulates performed according to the OECD guidelines (TG 414) did not give concern for maternal or developmental toxicity up to the highest dose tested (1,000 mg/kg bw per day).
- Results from other reproductive and developmental studies with titanium nanoparticles (Jia et al., 2014 and Tassinari et al., 2014) showed contradictory results in the change in hormone levels
- Due to deficiencies in the study designs and inadequate data reporting, relevance of these findings is currently uncertain for the risk assessment

Immunotoxicity

- available studies on the effects of TiO2 (nano)particles on the immune systems pointed to different outcomes
- reported effects were dependent on
    - the core composition,
    - size and concentration of the particles,
    - and on the duration and route of exposure
- given the absence of clear characterisation of the material used, the difference in effects observed following various routes of administration and the diversity in the effects reported, a conclusion on the possible immunotoxic effects of the food additive TiO_2 cannot be reached
- However, the Panel noted that the larger the TiO_2 particles, the lower their potential to induce effects, and that from animal data it appeared that the route of injection influences the response, TiO_2 particles being less reactive after oral administration

Conclusions on the toxicity database

- based on information reported in the examined literature and information supplied following calls for data
- Considering
    - the food additive E 171 mainly consists of microsized TiO_2 particles, with a nanosized (< 100 nm) fraction less than 3.2% by mass;
    - the absorption of orally administered TiO_2 particles (micro- and nanosized) in the gastrointestinal tract is negligible, estimated at most as 0.02–0.1% of the administered dose;
    - no difference is observed in the absorption, distribution, and excretion of orally administered microsized and nanosized TiO_2 particles;
    - no adverse effect resulting from the eventual accumulation of the absorbed particles is expected based on the results of long-term studies which did not highlight any toxicity up to the highest administered dose;
    - the uncertainties in the toxicological database arising from limitations in the available reproductive toxicity studies
- an ADI should not be established, and that a margin of safety (MoS) approach would be appropriate

Exposure assessment

- Calculated by two approaches
  - maximum levels provided to EFSA and
  - reported use levels as provided by industry and Member States
- Two scenarios
  - Brand loyal
    - assumed that the population is exposed over a long period of time to the food additive present at the maximum reported use/analytical levels for one food category and to a mean reported use/analytical level for the remaining food categories
  - Non-brand loyal
    - assumed that the population is exposed over a long period of time to the food additive present at the mean reported use/analytical levels in all relevant food categories

The Panel considered

- the refined exposure assessment approach was a more realistic scenario,
  - because it was based on the range of usage and analytical data, assumed that the processed foods and beverages contain the additive at the mean concentration level for all products (non-brand-loyal consumer scenario) and considers one product containing TiO2 at the maximum concentration level (brand-loyal consumer scenario)
- However
  - due to the low amount of data provided to EFSA (reported use levels or analytical data) only 14 food categories were taken into account, representing between 60% and 80% of food (by weight) authorised to contain TiO_2

Estimated exposures

- Ranges from lowest to highest covering different population groups
- Maximum
  - Mean
    - 0.4 mg/kg bw per day for infants and the elderly to 10.4 mg/kg bw per day for children
  - 95%
    - 1.2 mg/kg bw per day for the elderly to 32.4 mg/kg bw per day for children
- Brand loyal
  - Mean
    - 0.4 mg/kg bw per day for infants and the elderly to 8.8 mg/kg bw per day for children
  - 95%
    - 1.1 mg/kg bw per day for the elderly to 30.2 mg/kg bw per day for children.
- Non-brand loyal
  - Mean
    - 0.2 mg/kg bw per day for infants and the elderly to 5.5 mg/kg bw per day for children.
  - 95%
    - 0.5 mg/kg bw per day for the elderly to 14.8 mg/kg bw per day for children

For risk characterisation

- Considered non-brand-loyal scenario covering the general population was the more appropriate and realistic scenario

- Used these exposures and NOAEL of 2,250 mg TiO2/kg bw per day from the NCI study to estimate margins of safety

- Guidance for submission of food additives (EFSA ANS Panel, 2012), the Panel considered that, for non-genotoxic and non-carcinogenic compounds “a MoS of 100 or more between a NOAEL or BMDL and the anticipated exposure would be sufficient to account for uncertainty factors for extrapolating between individuals and species”.

Margins of safety

(min–max across the 6 dietary surveys)

Population groups	Mean	95%
Infants	2,800–11,000	600–3,200
Toddlers	500–3,800	350–1,200
Children	400–2,500	150–950
Adolescents	550–5,700	200–1,800
Adults	550–7,500	250–2,100
The elderly	800–11,000	300–4,500

Nanoparticle exposure

- Ranges from lowest to highest covering different population groups using highest reported weight percentage value of 3.2% of nanoparticles by mass
- Maximum
  - Mean
    - 0.01 mg/kg bw per day for infants and the elderly to 0.33 mg/kg bw per day for children
  - 95%
    - 0.04 mg/kg bw per day for infant and theelderly to 1.04 mg/kg bw per day for children
- Brand loyal
  - Mean
    - 0.01 mg/kg bw per day for infants and the elderly to 0.28 mg/kg bw per day for children
  - 95%
    - 0.03 mg/kg bw per day for the elderly to 0.97 mg/kg bw per day for children.
- Non-brand loyal
  - Mean
    - 0.01 mg/kg bw per day for infants, adolescent, adults and the elderly to 0.18 mg/kg bw per day for children.
  - 95%
    - 0.02 mg/kg bw per day for infant and the elderly to 0.47 mg/kg bw per day for children

Overall conclusions

- possible adverse effects in the reproductive system were identified in some studies conducted with material which was either non-food-grade or inadequately characterised nanomaterial (i.e. not E 171).
- There were no such indications in the available, albeit limited, database on reproductive endpoints for the food additive (E 171).
- The Panel was unable to reach a definitive conclusion on this endpoint due to the lack of an extended 90-day study as in the Guidance for submission of food additives (EFSA ANS Panel, 2012) or a multigeneration or extended-one generation reproduction toxicity study with the food additive (E 171).

Therefore

- on the database currently available and the considerations on the absorption of TiO_2 the margins of safety calculated from the NOAEL of 2,250 mg TiO_2/kg bw per day identified in the toxicological data available and exposure data obtained from the reported use/analytical levels of TiO_2 (E 171) considered in this opinion would not be of concern.

Data required

- In order to enable the Panel to establish a health-based guidance value (ADI) for the food additive TiO_2 (E 171), additional testing could be performed.
- An extended 90-day study or a multigeneration or extended-one generation reproduction toxicity study according to the current OECD guidelines could be considered.
- Such studies should be performed with TiO_2 (E 171) complying with the EU specifications and additionally including a characterisation of the particle size distribution of the test material.
- However, in deciding on actual testing, considerations of animal welfare need to be balanced against the improvement in the toxicological database within a tiered testing approach.

Some food for thought

- Tier 1 requirements for a new additive
  - Absorption data
  - Extended 90 day toxicity test
  - *In vitro* genotoxicity

- Triggers for tier 2 testing
  - Absorption data – absorption is not negligible
  - Extended 90 day toxicity test – adverse effects in systemic or local tissues or additional screening endpoints
  - *In vitro* genotoxicity – positive or equivocal requires *in vivo* genotoxicity

So if titanium dioxide were a new additive

- Absorption of TiO_2 is negligible
- *In vitro* genotoxicity equivocal triggers tier 2 genotoxicity but negative *in vivo* so not considered genotoxic
- No extended 90 day study but based on long term toxicity data unlikely to have adverse effects at limit dose, limited data on extended parameters but from available data effects unlikely
- Testing would probably stop at tier 1

The new EFSA evaluation

- Asked to comment on 4 specific papers by end of June
  - In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority (EFSA) to provide a scientific opinion in relation to four new studies on the potential toxicity of titanium dioxide used as a food additive (E 171). In particular, EFSA is requested to carry out a scientific evaluation of those studies and to indicate whether they would merit re-opening the existing opinion of EFSA related to the safety of titanium dioxide (E 171) as a food additive.
- Key question is do they alter the weight of evidence

Which 4 papers?

- Bettini S., Boutet-Robinet E., Cartier C., Coméra C., Gaultier E., Dupuy J., Naud N., Taché S., Grysan P., Reguer S., Thieriet N., Réfrégiers M., Thiaudière D., Cravedi J.-P., Carrière M., Audinot J.-N., Pierre F.H., Guzylack-Piriou L., Houdeau E. (2017). Food-grade TiO2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon. Sci Rep. 2017, 7:40373.
- Guo Z., Martucci N., Moreno-Olivas F., Tako E., Mahler G., Titanium dioxide nanoparticle ingestion alters nutrient absorption in an in vitro model of the small intestine. NanoImpact, 5 : 70-82, janvier 2017
- Heringa MB, Geraets L, van Eijkeren JCH, Vandebriel RJ, de Jong W and Oomen AG, 2016. Risk assessment of titanium dioxide nanoparticles via oral exposure, including toxicokinetic considerations. Nanotoxicology Vol. 10, Iss. 10, 2016
- Proquin H., Rodríguez-Ibarra C., Moonen C., Urrutia Ortega I., Briedé J., de Kok T., van Loveren H., Chirino Y. , Titanium dioxide food additive (E171) induces ROS formation and genotoxicity: contribution of micro and nano-sized fractions. Mutagenesis, Volume 32, Issue 1, 1 January 2017, Pages 139–149 doi: 10.1093/mutage/gew051. Epub 2016 Oct 27.

considered in the context of the conclusions of
the EFSA opinion of 2016.

Conclusions on the Bettini et al.(2017)

- Based on the Bettini et al. study, and the negative results of the NCI (1979) carcinogenicity studies in mice and rats, new findings not sufficient to raise a concern on the potential initiation or promotion properties of TiO_2 (E 171) on colon carcinogenesis;

- Determination of biomarkers for putative preneoplastic lesions in the colon, as an additional parameter to be examined in the ongoing extended one generation reproductive toxicity study.

- Not enough justification for a new carcinogenicity study (large number of animals), but that if additional mechanistic studies provided useful information on the relevance of the reported results, this could be reconsidered in future;

Conclusions on the Proquin et al.(2017)

- Useful for hazard evaluation of TiO_2 nanoparticles (under the specific conditions of the study protocol). However, the relevance of the results for risk assessment of the food additive E 171 has not been established;

- Do not change the conclusion on the genotoxicity of TiO_2 as stated in the previous EFSA Opinion (2016) on the safety of TiO_2 (E171) when used as a food additive;

- One of the authors mentioned that consistent results appeared to be observed in mouse colon in ongoing in vivo transcriptomics studies. Therefore, the overall database might require reassessment when these studies are completed, if necessary, but that any such assessment should consider the entire literature available at that time.

Conclusions on the Guo et al.(2017)

- The results from the Guo et al. (2017) study cannot be extrapolated to the human situation and cannot be used for the risk assessment of the food additive TiO_2 (E171).

Conclusions on the Heringa et al.(2016)

- Significant uncertainty in the assessment carried;
- Not a weight of evidence analysis of the whole database on E171;
- This assessment consistent with a hazard from TiO_2 NP when dosed as in the selected studies, but the relevance to nanoparticles in a food matrix could not be assessed;
- The additional studies called for in its 2016 opinion should provide a more robust basis for addressing the reported effects in reproductive organs in the studies used by Heringa et al.(2016).

What did the EFSA ANS Panel conclude?

- The results of the Bettini et al. (2017) study did not provide enough justification for a new carcinogenicity study, but, should additional useful mechanistic information become available, this could be reconsidered in future;
- the new in vitro findings in the study by Proquin et al. (2017) did not modify the conclusion on the genotoxicity of TiO_2 as stated in the previous EFSA opinion (EFSA ANS Panel, 2016) on the safety of TiO_2 (E171) when used as a food additive;
- the effects of engineered TiO_2 nanoparticles reported by the Guo et al. (2017) study were of uncertain biological significance and therefore of limited relevance for the risk assessment of the food additive TiO_2 (E171);
- there was significant uncertainty in the risk assessment performed by Heringa et al. (2016), which did not include a weight of evidence analysis of the whole database;
- the four studies evaluated, highlighted some concerns but with uncertainties, therefore their relevance for the risk assessment was considered limited and further research would be needed to decrease the level of uncertainties.

What did the EFSA ANS Panel conclude?

- Overall, three of the studies assessed in this opinion reported that TiO_2 was able to induce various effects in in vitro and in vivo models. These studies may be useful for hazard identification of TiO_2. The Panel considered that the limited relevance of the protocols of these studies to the use of E 171 under realistic conditions in food, hampered the use of the data in the risk assessment of the food additive E 171.
- In the fourth study by Heringa et al. (2016), numerous assumptions were made, which resulted in large uncertainty in their conclusion.
- More research exploring the possible effects observed in three of the four studies could address their applicability to the risk assessment of the food additive E 171 under realistic conditions of use.
- Altogether, the Panel concluded that the outcome of the four studies *did not merit re-opening the existing opinion of EFSA related to the safety of titanium dioxide (E 171) as a food additive*.

What did the EFSA ANS Panel recommend?

- in order to substantiate the observations in the Bettini et al. (2017), biomarkers for putative preneoplastic lesions in the colon as additional parameters should be examined in the extended one generation reproductive toxicity study recommended by EFSA (EFSA ANS Panel, 2016);

- further studies on TiO_2 NP should include administration in a food matrix.

Titanium dioxide (E 171): update on EFSA's activities

Federica Lodi
Food Additives Team
Food Ingredients and Packaging Unit

Workshop on Possible adverse effects of food additive E171 (titanium dioxide)

5-6 July 2018, Amsterdam

efsa
European Food Safety Authority

RE-EVALUATION MANDATE

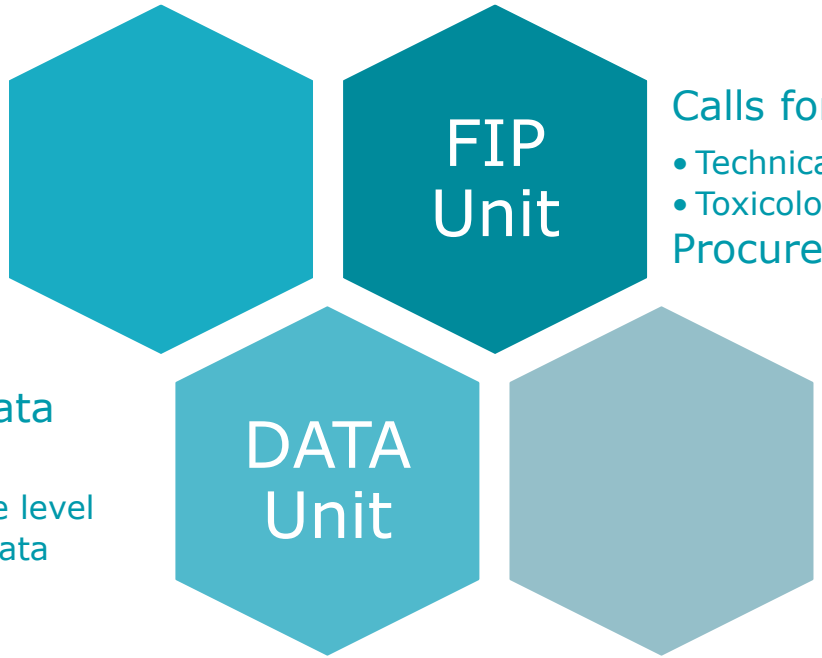
European Commission

Single mandate covering all food additives to be re-evaluated

Interested parties

Calls for data

RE-EVALUATION: DATA COLLECTION



Calls for data

- Technical
- Toxicological

Procurement

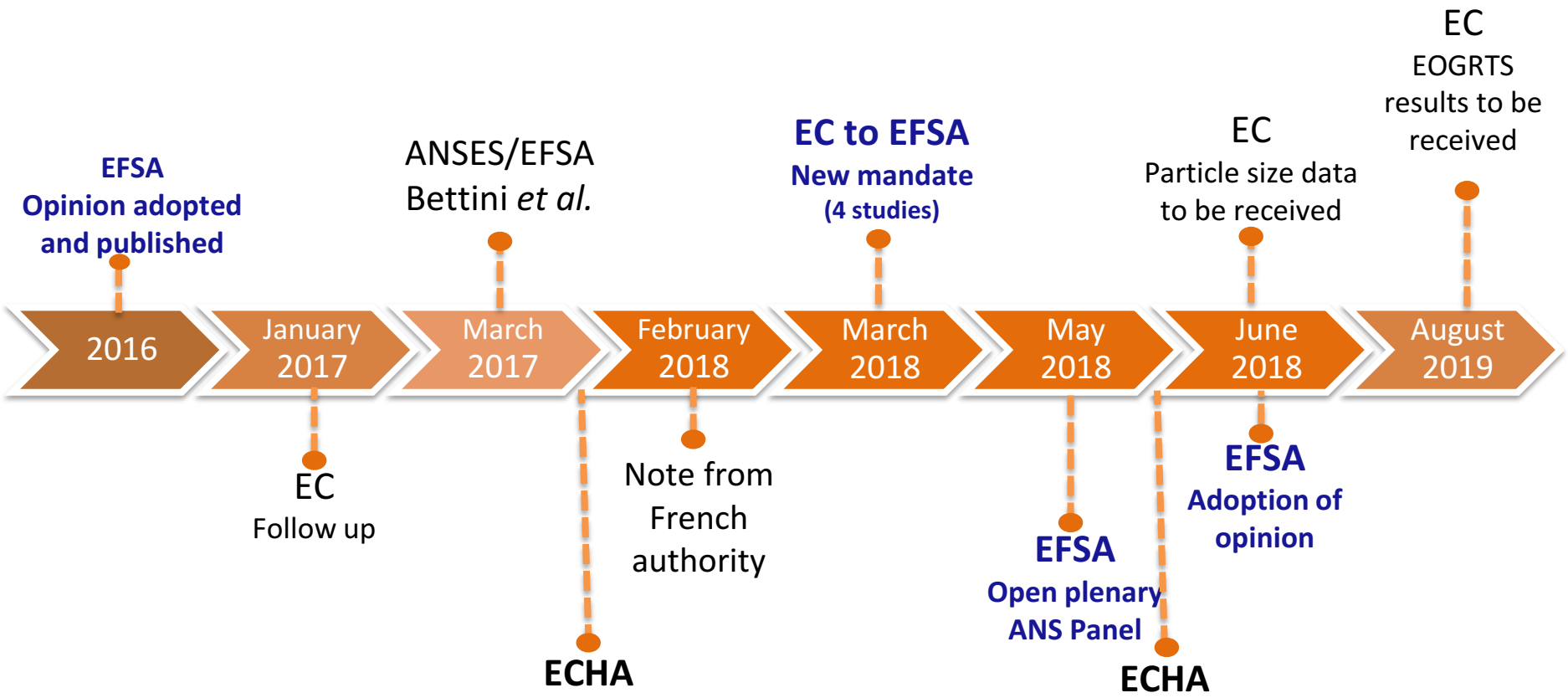
Calls for data exposure

- Use and use level
- Analytical data

All available data

- Published literature
- Previous evaluations
- Unpublished reports

TITANIUM DIOXIDE (E 171) TIMELINE



FOLLOW UP OF EFSA OPINION FROM EC

- Call for scientific and technical data:

https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en

- **Published:** 30/01/2017.
- **Registration of the contact details of business operators interested in submitting data (step 1) Deadline:** 02/03/2017
- **Confirmation of data submission, deadlines and milestones (step2) Deadline:** 30/07/2017)

FOLLOW UP OF EFSA OPINION FROM EC

- **Data on particle size and particle size distribution:** The Titanium Dioxide Manufacturers Association (TDMA) has committed to provide update specifications, including information on particle size and particle size distribution, for the food additive E 171. The data expected to be submitted by 30 June 2018.

- **Toxicological data:** dietary Extended One-Generation Reproductive Toxicity Study with E171 food-grade titanium dioxide in rats (by The Titanium Dioxide Manufacturers Association, TDMA), including cohort 1 (extension by mating of F1 animals to the F2 generation), cohort 2 (for developmental neurotoxicity) and cohort 3 (for developmental immunotoxicity). Deadline: August 2019.

FOLLOW UP OF EFSA OPINION FROM EC

- Data on the lowest achievable limits for the impurities of toxic elements (arsenic, lead, mercury and cadmium) in titanium dioxide (E 171).

- Data on the actual use of alumina (aluminium oxide) in E171 formulations/lowest achievable limit for the use of alumina in those formulations.

COLLABORATION WITH ANSES (1Q 2017)

- **March 2017** → ANSES assessed the impact of the Bettini et al. 2017 study

- **Collaboration with ANSES**

- **ANSES technical hearing** with the authors of the study

- **Joint meetings** between ANSES and EFSA experts

- **ANSES conclusion: no need to reopen the EFSA Opinion**

RECENT UPDATE FROM ANSES

- Working group dealing with nano in food but not specifically on titanium dioxide (E171).

- ANSES is the Member State to carry out the assessment of titanium dioxide in the Community Rolling Action Plan (**CoRAP**) under the REACH Regulation, and the deadline to complete this task is **March 2019**.

ECHA ACTIVITIES

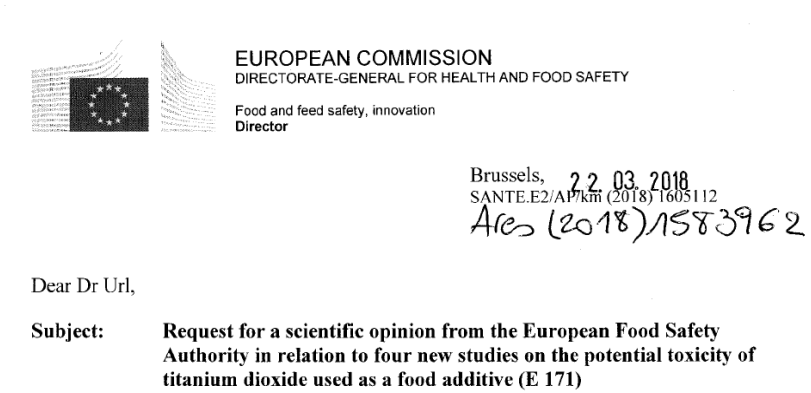
- **2Q 2017**: **TC meeting between ECHA and EFSA, updating on current activities.**

- EFSA followed the discussion at the RAC.
- **ECHA conclusions (June 2017)**:
  - Classification of titanium dioxide as a "substance suspected of causing cancer" (**category 2**) (**via inhalation**) → Relevance to FEEDAP for occupational exposure

- **No concern** about carcinogenicity of titanium dioxide **via oral or dermal administration**.

RECENT MANDATE FROM THE EC (MARCH 2018)

- The European Commission sent a **mandate** to EFSA requesting the assessment of four studies published after the publication of the 2016 ANS Panel opinion, in accordance with Article 29 (1)(a) of the Regulation (EC) No 178/2002, and indicating whether these studies would merit to reopen the existing opinion.



EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Food and feed safety, innovation
Director

Brussels, 22 03 2018
SANTE.E2/AP/km (2018) 1605112
Ares (2018)1583962

Dear Dr Url,

Subject: **Request for a scientific opinion from the European Food Safety Authority in relation to four new studies on the potential toxicity of titanium dioxide used as a food additive (E 171)**

- Bettini et al., 2017
- Heringa et al., 2016
- Proquin et al., 2017
- Guo et al., 2017

PAFF COMMITTEE (APRIL 2018)

- Note from the French authorities sent to the EC on 15 February 2018 requesting interim protective measures (in accordance with Article 53 of the General Food Law) to address the uncertainties in respect of the impact on human health of titanium dioxide in food.

- Implementation of measures for suspension of the placing on the market or use of the food additive titanium dioxide (E171) in all food of European origin and measures for suspension of imports of all food containing that food additive from third countries.

PAFF COMMITTEE (APRIL 2018)

- France based this request on the fact that:
  - in the re-evaluation of the safety of titanium dioxide (E 171) as a food additive (Scientific Opinion published on 14 September 2016) EFSA was unable to reach a definitive conclusion on the reproductive and developmental toxicity endpoint and therefore EFSA did not establish an acceptable daily intake (ADI).
  - four studies on the potential toxicity of titanium dioxide used as a food additive (E 171), which were published after the adoption of EFSA's Opinion, pose some concern.

https://ec.europa.eu/food/sites/food/files/safety/docs/reg-com_toxic_20180417_sum.pdf

RECENT MANDATE FROM THE EC (MARCH 2018)

- The WG on the Re-evaluation of Food Colours of the ANS Panel was preparing this scientific opinion for further discussion and adoption by the ANS Panel:

  - **15-17 May 2018:** first discussion at Plenary open to observers.
    - **Authors** of the studies attended as **hearing experts**: overview on their findings and answer to questions

  - **Adoption by the ANS Panel: 26 June 2018**

  - **Publication**: **4th July 2018**

TITANIUM DIOXIDE AS A FEED ADDITIVE

- Assessment of titanium dioxide as feed additive **ongoing:** by EFSA FEEDAP Panel;

- Same EOGRTS study has been asked to their applicant: waiting for additional data.

NEXT STEPS

- The outcome of the **recently adopted opinion** (EFSA, June 2018) will be considered by **risk managers**.

- **EC to receive the following data**:
  - particle size and particle size distribution **by 30 June 2018**
  - EOGRTS reproductive toxicity study **by August 2019**.

- Other **ongoing evaluations**: EFSA FEEDAP Panel, ANSES/ECHA, …..

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