Intestinal Uptake of Particles

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Oral uptake of Nanomaterials

- For a long period of time nanomaterial research was focussed on inhalation
- Based on some critical publications oral exposure gets more attention

In general for voluntary product stewardship programs by industry no animal experiments:

`in vitro´

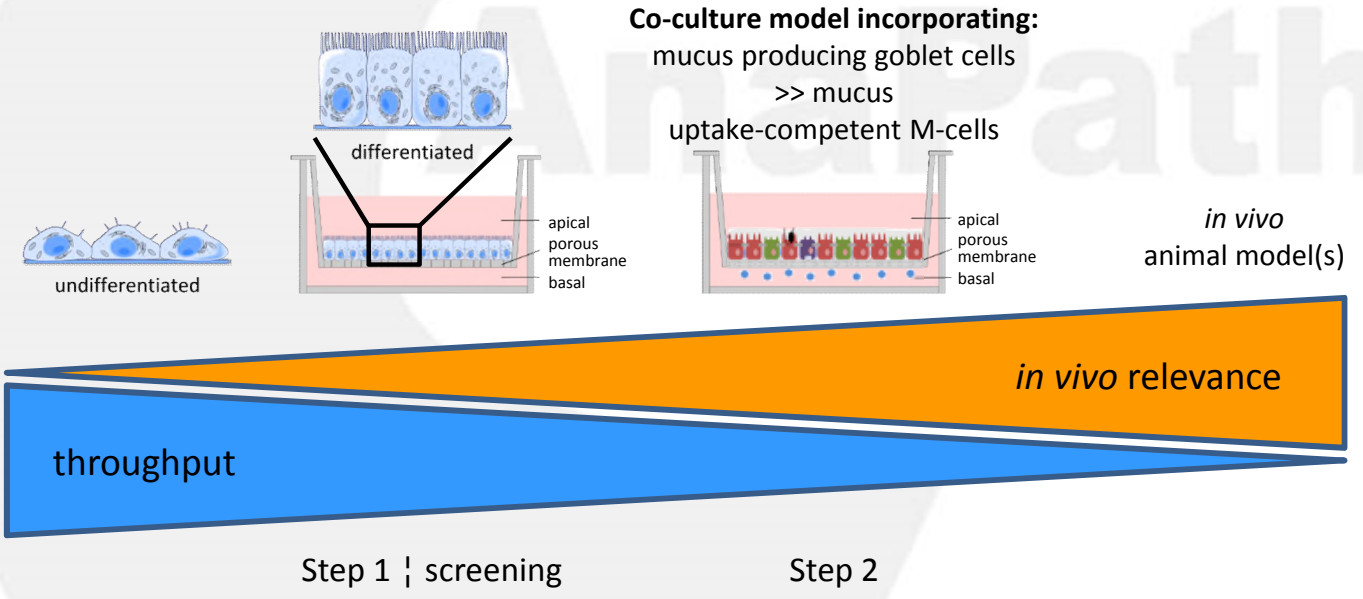
the only alternative?

in vitro: nanoscreen project to examine oral uptake of nanomaterials

nanoscreen.ch

Nanoscreen 2- step approach : screening and in vivo relevance

Implications of nanomaterials (e.g. SAS) on the human gut



Pathology: `in vivo´ alternative to avoid animal experiments

In general pathology is associated with animal experiments to identify target organs by morphological examination of H & E stained tissue sections

However, pathology offers much more possibilities:

Existing formalin fixed or parraffin embedded tissues available from old studies can be used to address new questions

- special stains
- Immunohistochemistry
- EDX
- etc…….can be applied in old material stored since decades to reduce new tests with animals.

Application of new methods with existing material from old studies is an alternative to new animal experiments and could be much more often used for regulatory purposes

Particle Uptake

Depends from:

- Particle size
- Particle surface (e.g. hydrophobicity/charge)
- Dose of particles administered
- Administration vehicle
- Use of targeted delivery to M* cells
- Fed state of the animal
- Age of the animal
- Species under investigation
- Method used to quantify uptake

*Microfold cells

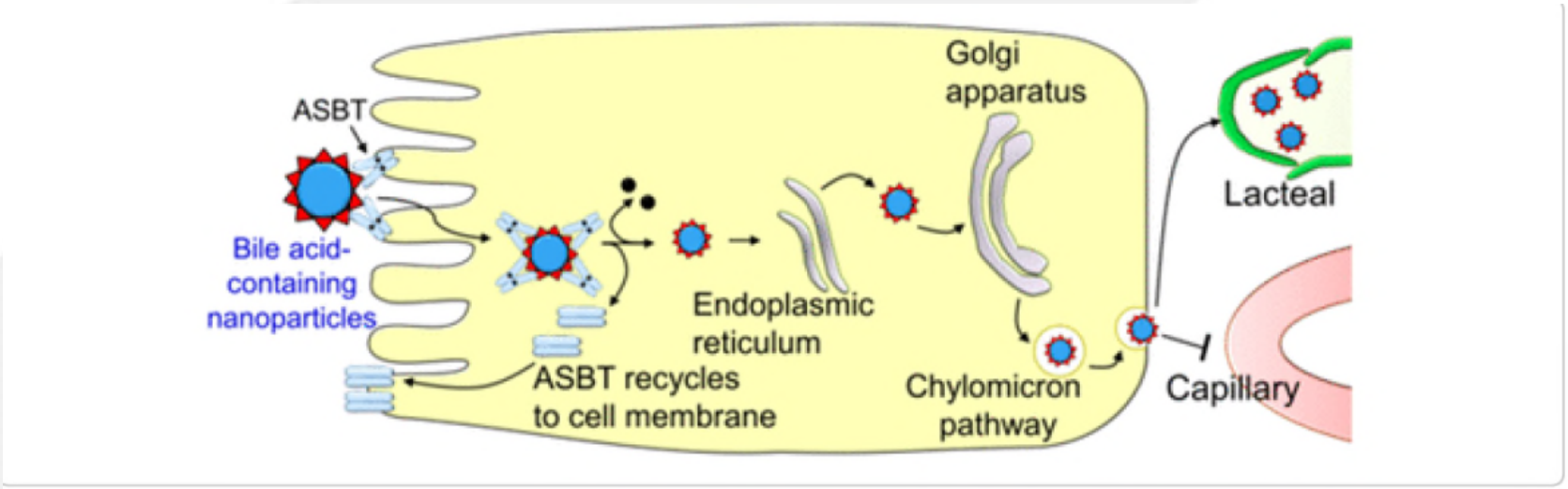
Particle Uptake

Site/Mechanism	Particle Size
Villus tips - resorption	5-150 nm
Intestinal macrophages - phagocytosis	1 µm
Enterocytes – endocytosis	<200 nm
Peyer's patches - transparacellular	<10 µm

(evaluated for Poly-styrene, -methyl methacrylate, -lactide, -lactide co-glycolide, and Ethyl cellulose)

O'Hagan DT (1996). The intestinal uptake of particles and the implications for drug and antigen delivery. J Anat. 189 (Pt 3):477-82.

Intestinal Resorption by GALT



- Nanoparticle transport: combination of apical sodium-dependent bile acid transporter-mediated cellular uptake and chylomicron transport pathways.
- Particle-size- and dose-dependent oral bioavailability was observed for oral nanoparticle dosing up to 20 mg/kg.
- Probe nanoparticles appeared to be transported to systemic circulation via the gut lymphatic system.

Kim KS, Suzuki K, Cho H, Youn YS, Bae YH (2018). Oral Nanoparticles Exhibit Specific High-Efficiency Intestinal Uptake and Lymphatic Transport. ACS Nano. 12(9):8893-8900.

Small Intestinal Transit Time

- In human, the median SITT: 219 min for females and 191 min for males.
- In rats: 1–2 h for transit of contents to reach the cecum, and 4–6 h to transit from the stomach to the colon.
- Longer retention period in human compared to rats

Fischer M, Fadda HM (2016). The Effect of Sex and Age on Small Intestinal Transit Times in Humans. J Pharm Sci. 105:682-686.

Horiuchi A, Tanaka N, Sakai R, Kawamata Y (2014). Effect of age and elemental diets on gastric emptying in rats. J Gastroenterol Hepatol Res. 3: 1340–3.

How quick work M-cells?

'…The formation of these "pockets" greatly reduces the intracellular distance that antigens have to travel and allows M cells to rapidly transport (within 10 to 15 min) antigenic materials to the basolateral membrane…'

Miller H, Zhang J, Kuolee R, Patel GB, Chen W (2007). Intestinal M cells: the fallible sentinels? World J Gastroenterol. 13(10):1477-86.

Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health: Page 35: Step 0 In vitro digestion

- consider the time for degradation
- consider the degraded amount
- consider the consitutents after degradation

Expected Pathology with High Uptake

1. Accumulation of reactive macrophages in Peyer’s patches
2. Accumulation of reactive macrophages in mesenteric lymph nodes and related inflammatory lesions (e.g., latex, carbon)
3. Presence of particles in different organs with not predictable pathological lesions.

Ikomi F, Kawai Y, Ohhashi T (2015). Recent advance in lymph dynamic analysis in lymphatics and lymph nodes. Ann Vasc Dis. 5:258-68.
LeFevre ME, Olivo R, Vanderhoff JW, Joel DD (1978). Accumulation of latex in Peyer's patches and its subsequent appearance in villi and mesenteric lymph nodes. Proc Soc Exp Biol Med. 159:298-302.

Guidance on risk assessment …1a/1b, 2a

‘…Review all existing physicochemical and toxicological information as well as information relevant to grouping/read-across…’

or

‘…Including dissolution under lysosomal conditions…’

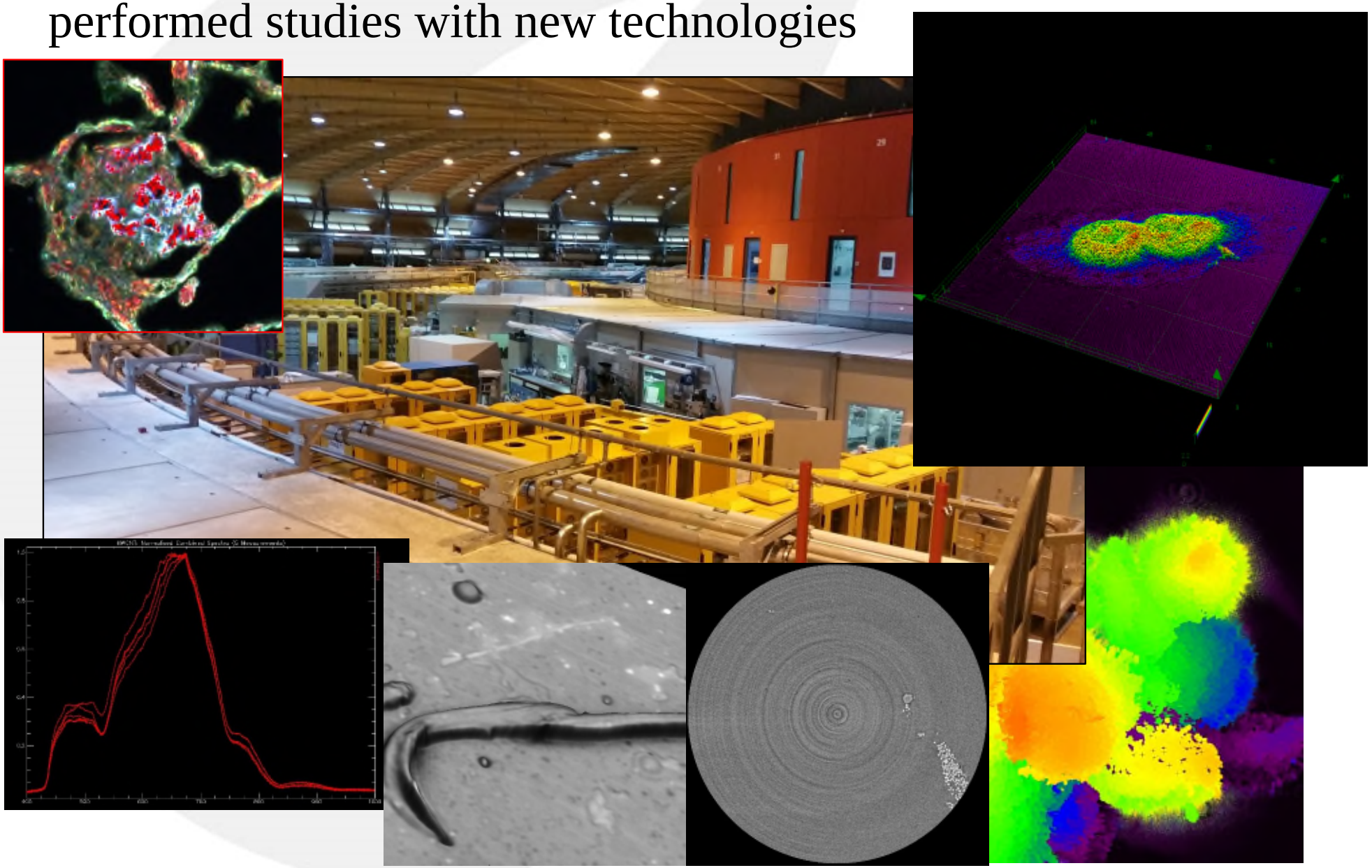
or

Is the nanomaterial non-persistent AND no indication of potential toxicity is observed

‘…2a) A pilot study for dose finding and assessment of absorption, tissue distribution and accumulation and elimination phases…’

Or:

Exploit, corrected and enhance the evaluation of previously performed studies with new technologies



Example for Evaluation of Published Data and Use of Material from Previously Performed Studies

- Data contradictory to present knowledge might be published in peer-reviewed journals
- Critical view on surprising data is necessary
- Previously performed studies might be ‘exploited’ for additional data in order to follow such new findings

‘…Step 1a Review existing information(b). See Sections 3, 4, 6.3: Review all existing physicochemical and toxicological information as well as information relevant to grouping/read-across…’

- See example

Evidence?

van der Zande M, Vandebriel R, Groot M, Kramer E, Herrera Rivera Z, Rasmussen K, Ossenkoppele J, Tromp P, Gremmer E, Peters R, Hendriksen P, Marvin H, Hoogenboom R, Peijnenburg A and Bouwmeester H. Sub-chronic toxicity study in rats orally exposed to nanostructured silica. Particle and Fibre Toxicology. 11: 8. 2014.

Morfeld P, Bosch A, Weber K, Heinemann M, Krueger N (2017). Synthetic amorphous silica in food: Findings about "liver fibrosis" and other study-related findings in van der Zande et al. (2014) are questionable. EC Pharmacology and Toxicology 3(2): 49-61

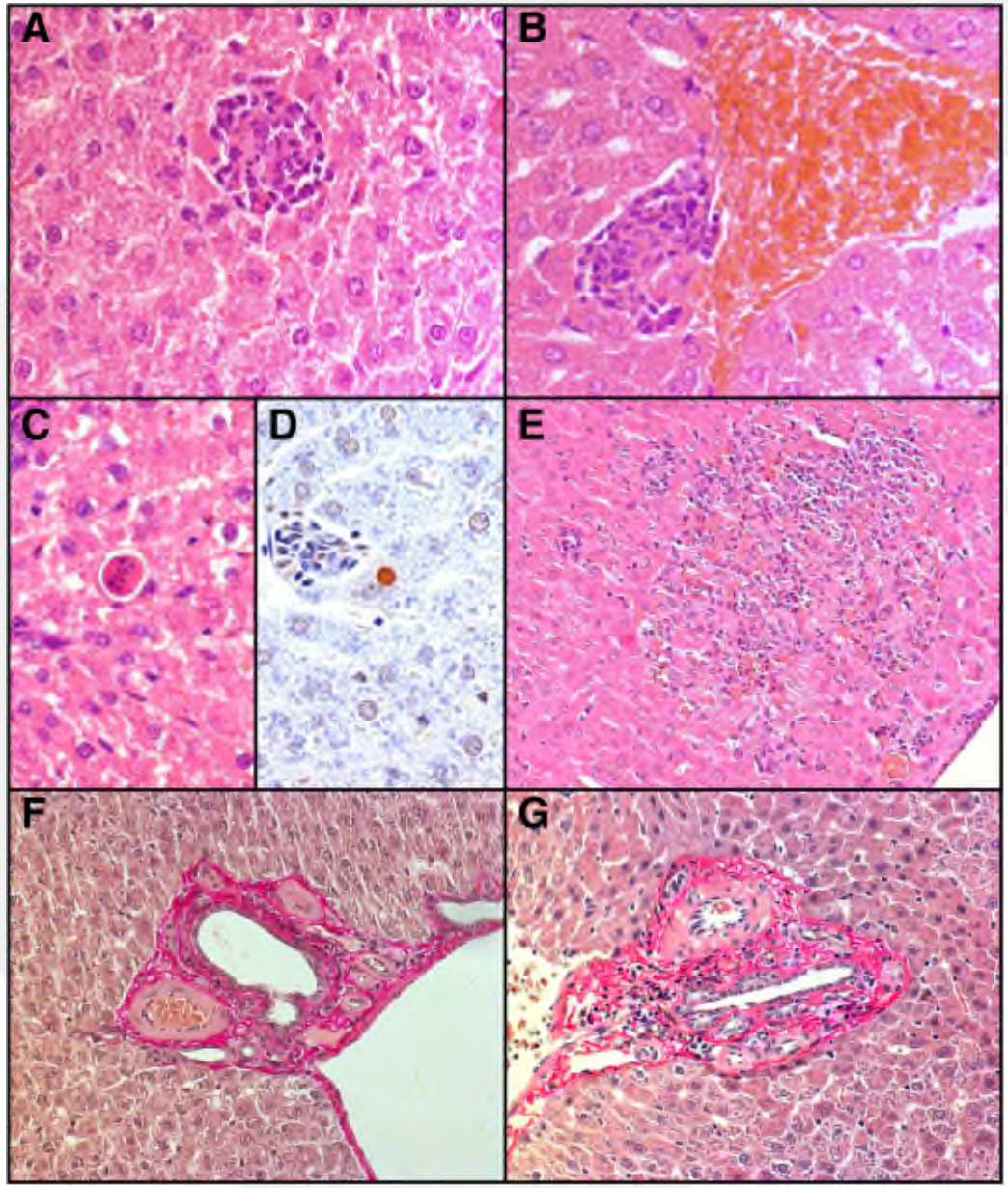
Study Outcome

- two SASs (identifiers: “SAS” and “NM-202”) were administered to male Sprague-Dawley rats via food for 29 days
- additional administration of the high dose groups up to 84 days
- Group size: 5 animals per sex

Conclusion:

- the study “…showed an increased incidence of liver fibrosis after 84-days of exposure…”
  and
  “…increased height of jejunal villi…”

Interpretation by an Experienced Pathologist



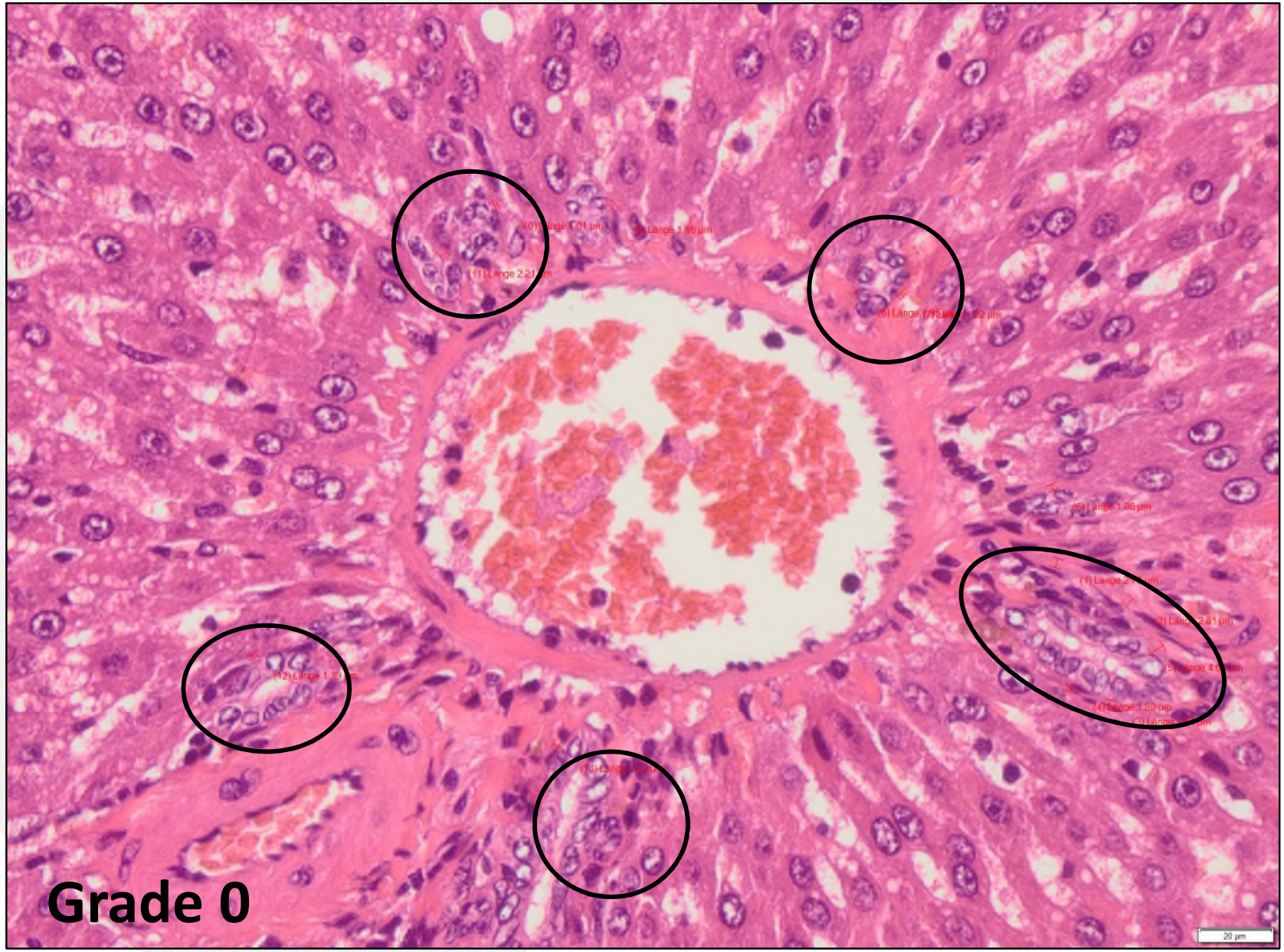
(A, B) ..inflammatory cell infiltrates as normal turnover of rat lives. Normal control lesion (up to 80-100%)
(C, D) Apoptosis yes, but is normal in rat livers, also in control animals.
(E) Necrosis yes. In control data e.g., RccHan™ rats 14-50%.
(F, G) minimal and expected peribiliar fibrosis after 84-days of exposure. Normal background finding in 13-week studies. Usually related to bile duct proliferation. Compare to pictures shown below.

The staining for F and G was not indicated. It is Sirius Red.

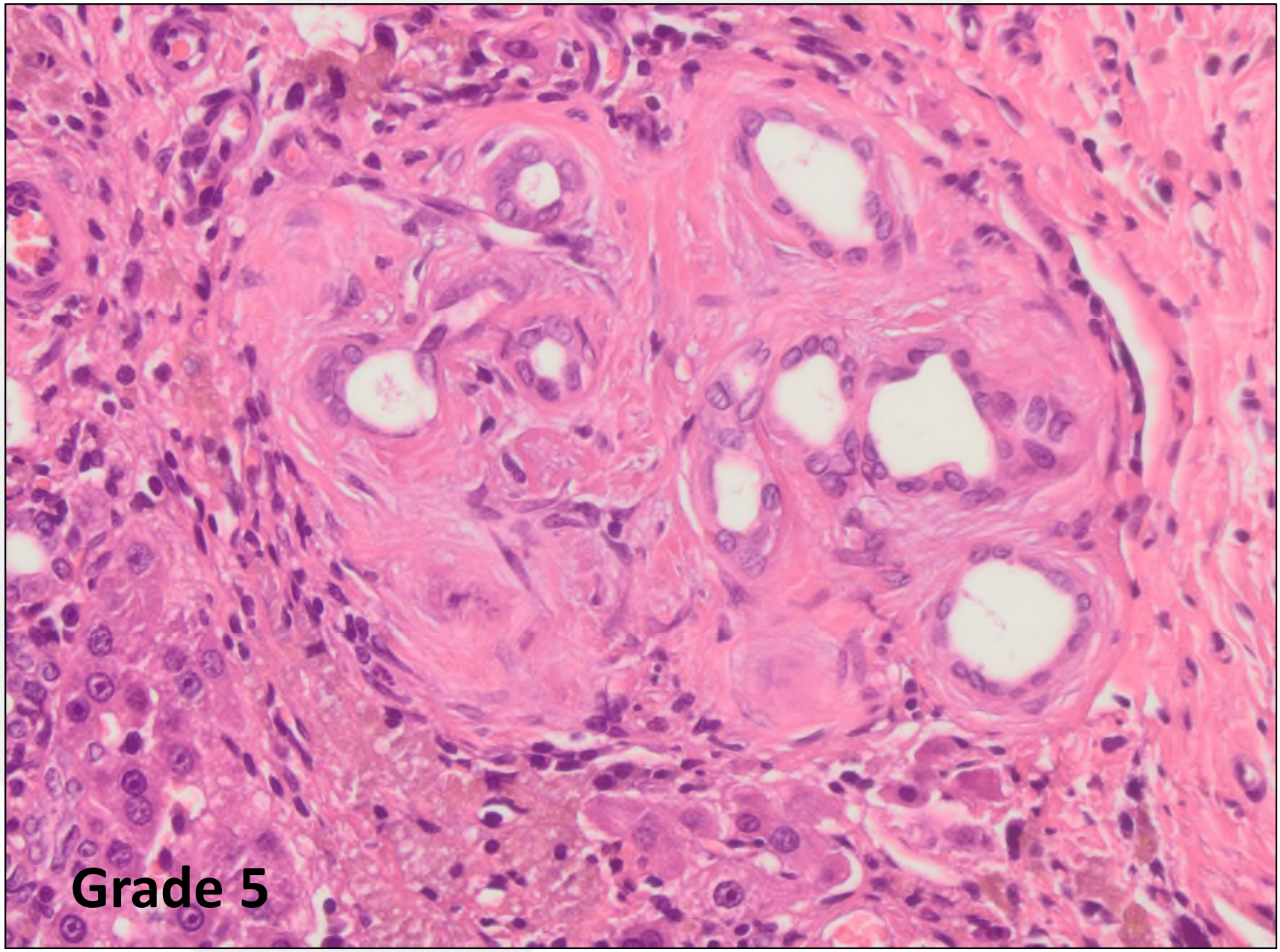
Endpoint liver fibrosis

- Liver fibrosis is defined by the presence of connective tissue in the liver (above the normal low rate seen in portal areas) as a reaction to acute or prolonged toxicity.
- The recent INHAND publication did not discuss gradings with the exception of cirrhotic changes representing a severe degree.
- The method section of the publication by van der Zande et al. does not provide a reference or standard for the definition of the 6 fibrosis severity categories that have been applied by the authors

Proposal: Gradings, Example by Measurement



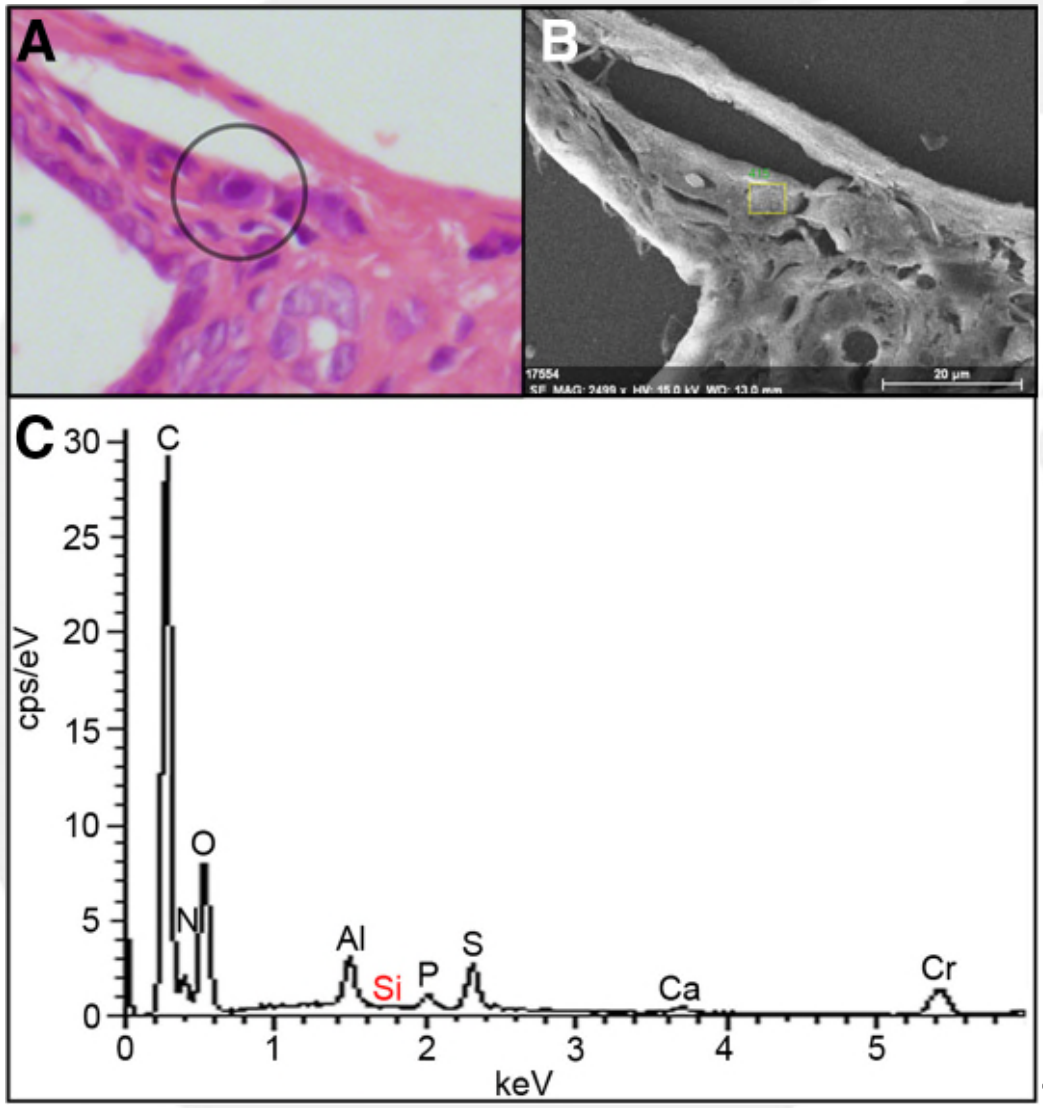
Proposal: Gradings, Example by Measurement



Silica Uptake and Organ Weights

- Analysis of silica uptake in the liver did not show significant differences except for the low dose.
- It may be expected that organ weight would be changed if silicon accumulates. However, absolute organ weights have been reported only in Table S4. When calculating relative organ weights, no difference can be established for liver, kidney and spleen. In contrast, this calculation reveals even lower organ/body weight ratios in several cases.

Silica Uptake and Organ Weights



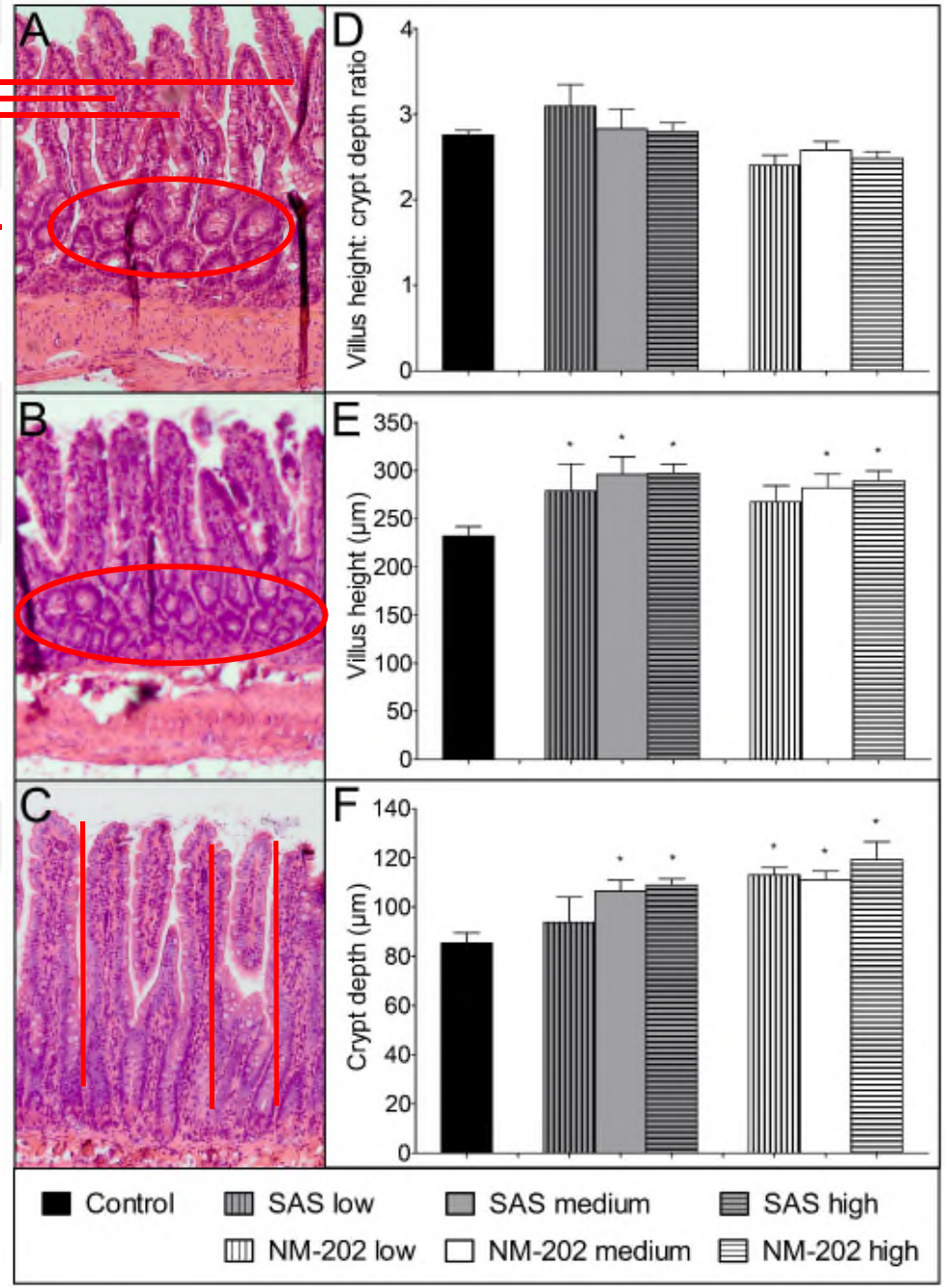
- In Figure 7A, a cell is shown that have been annotated as a macrophage. It is also possible that this cell represents an oval cell together with a few more cells shown in the same picture at the right, the underlying small bile duct and a few lymphocytes can be recognized.
- Figure 7C does not show any peak for silica.

Villi Height. No Comparison Possible!

Oblique section. Note: Several villi are cut in upper thirds only.
Crypts are visible by transversal section planes.

Again:
Crypts are visible by transversal section planes.

Note: Villi are cut longitudinally until the depths of crypts.



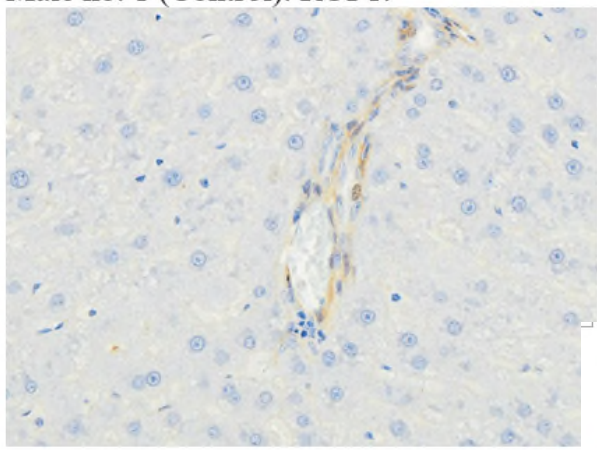
Proof

Previously performed reprotoxicity study with NM-200 underwent additional evaluation:

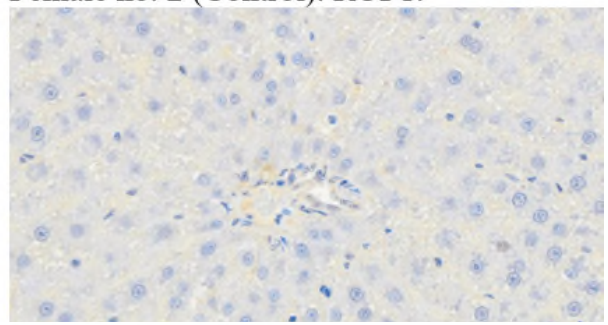
- Check on liver and intestine
- Liver: Sirius Red, Col I-III
- Intestine: Measurements of villus lenght
- Several organs: Si load (EDX)

Of course not: Liver

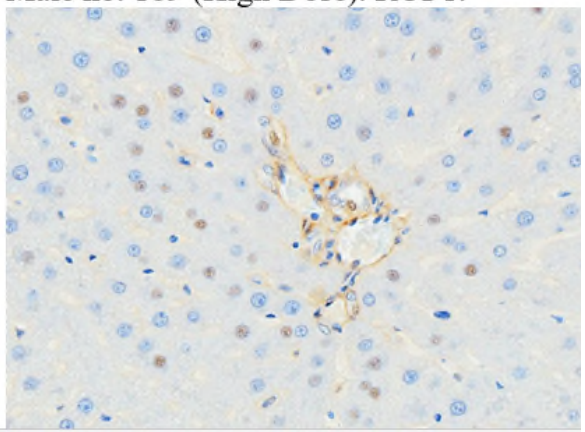
Male no. 1 (Control). ROI 1.



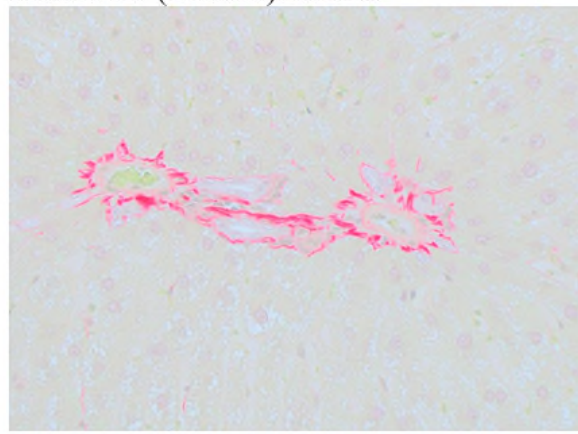
Female no. 2 (Control). ROI 1.



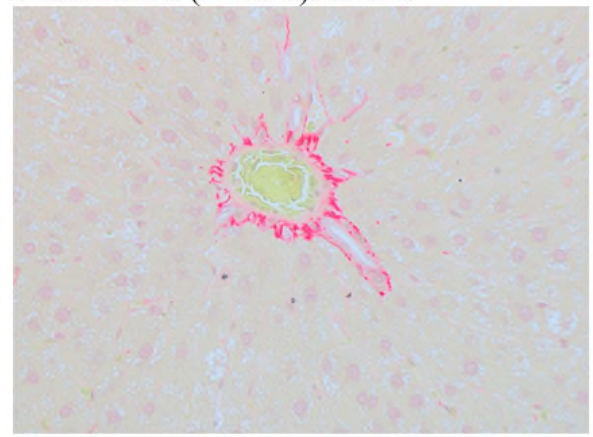
Male no. 169 (High Dose). ROI 1.



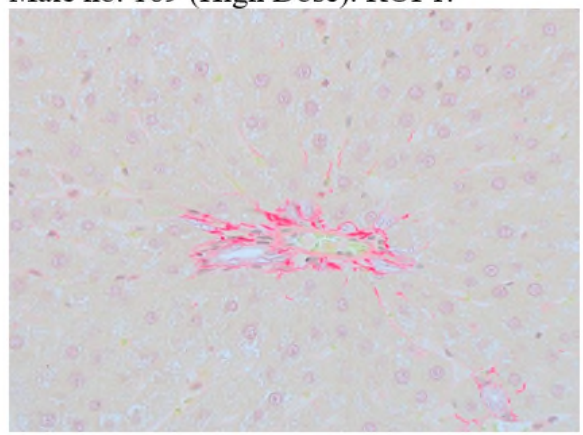
Male no. 1 (Control). ROI 1.



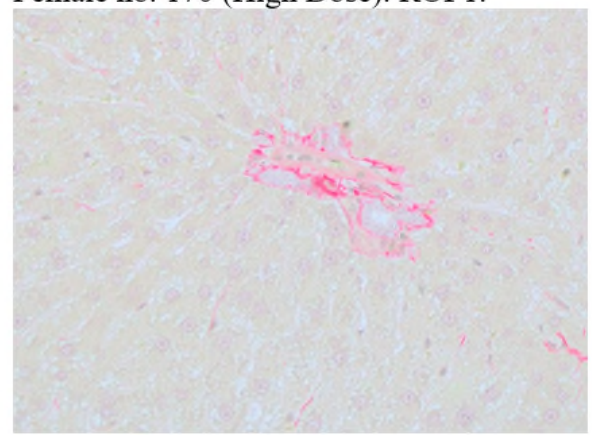
Female no. 2 (Control). ROI 1.



Male no. 169 (High Dose). ROI 1.

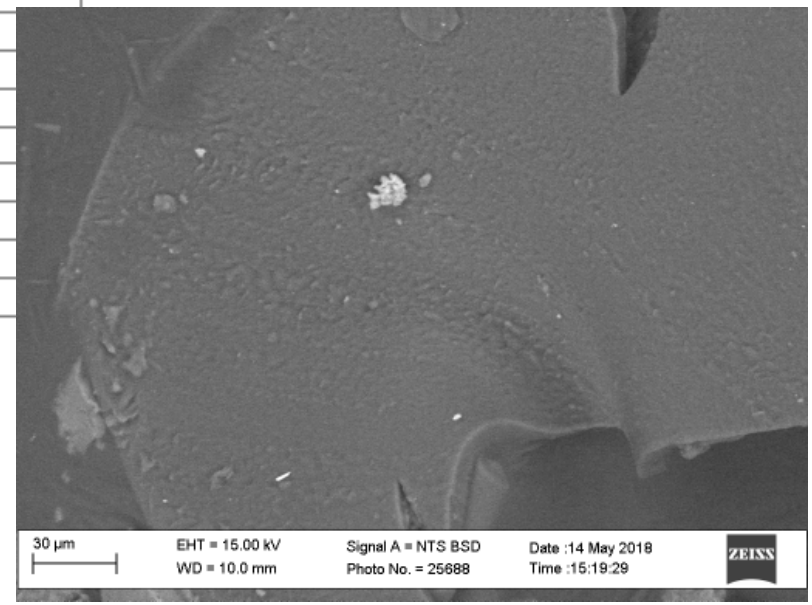


Female no. 170 (High Dose). ROI 1.

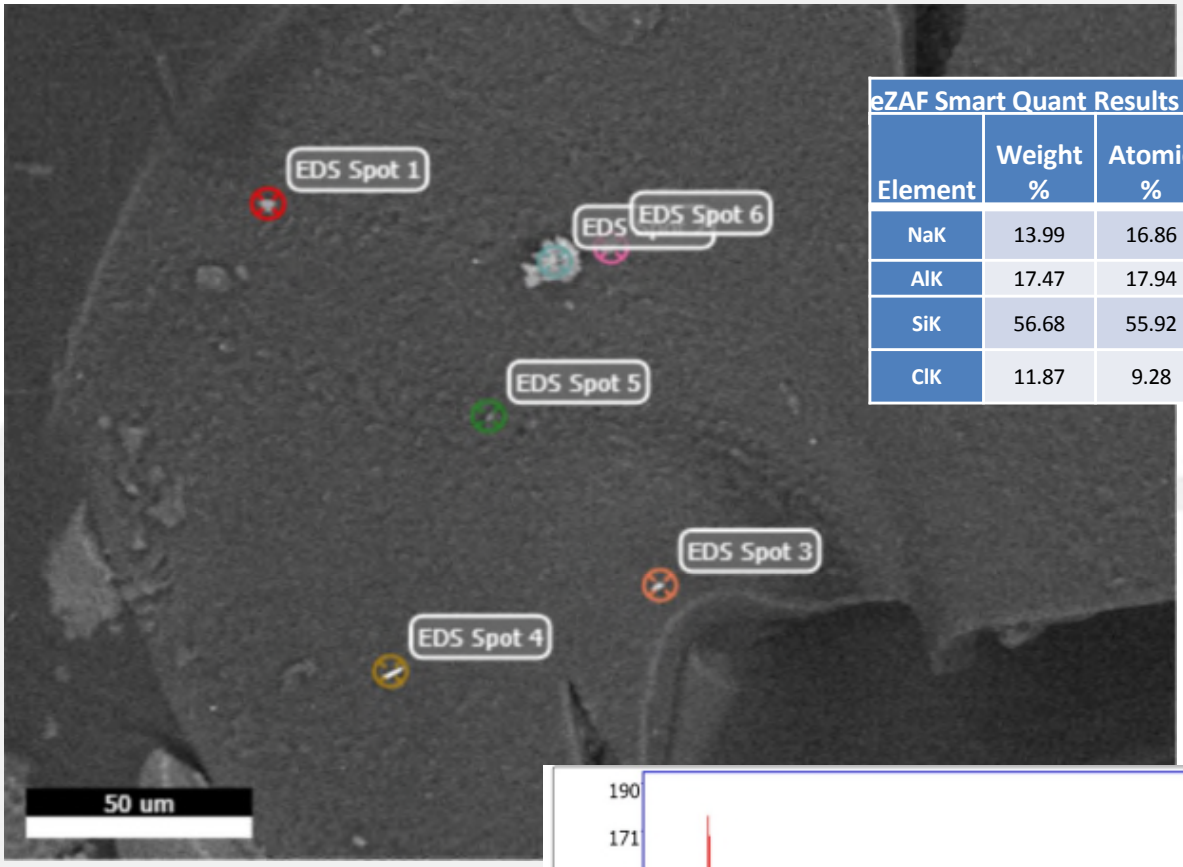


Of course not: Test Item load

Ileum	503_13_02	-		
	503_13_03	503_13_03	N	
	503_13_04	-		
	503_13_05	503_13_05	Y	Feldspar
Ceacum	503_14_01	-		
	503_14_02	-		
	503_14_03	503_14_03	N	
	503_14_04			
	503_14_05	503_14_05	N	
Colon	503_15_01	-		
	503_15_02	-		
	503_15_03	503_15_03	N	
	503_15_04	503_15_04	N	
Stomach, cardia	503_16_01	-		
	503_16_02	-		
	503_16_03	503_16_03	Y	Feldspar
Stomach, fundus	503_17_01	-		
	503_17_02	-		
	503_17_03	503_17_03	N	
	503_17_04	-		
	503_17_05	503_17_05	N	
Stomach, pylorus	503_18_01	-		
	503_18_02	-		
	503_18_03	503_18_03	N	
	503_18_04			



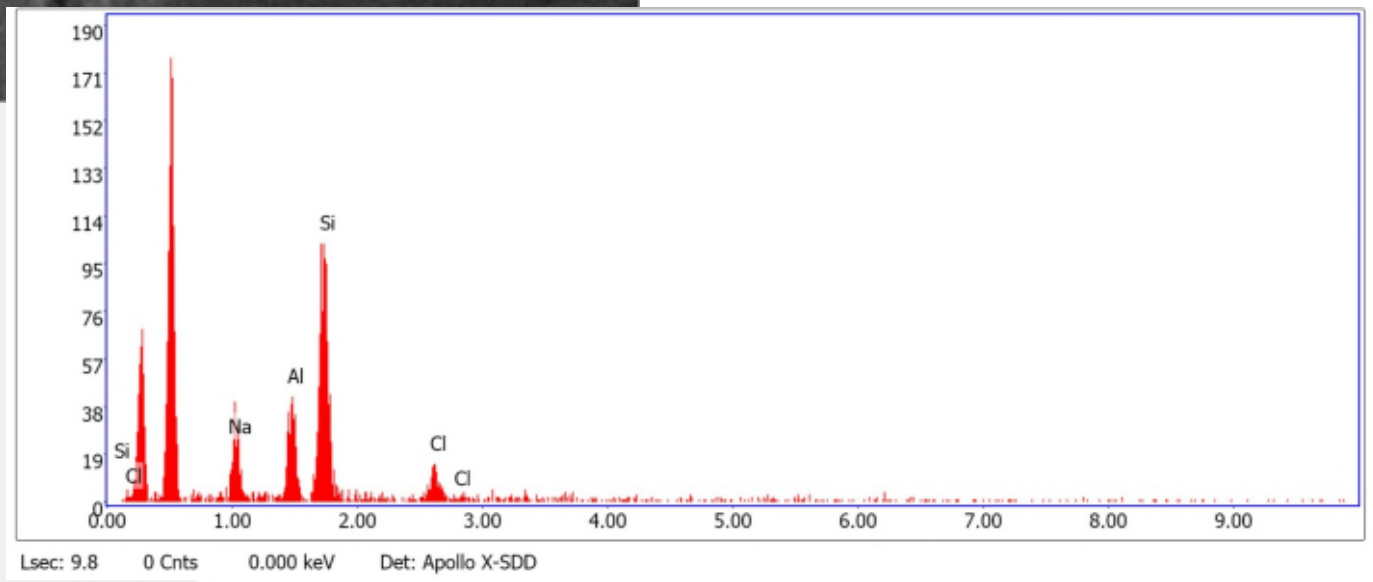
Of course not: Test Item load



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
NaK	13.99	16.86	19.58	16.60	0.1127	1.0140	0.7920	1.0036
AlK	17.47	17.94	30.42	11.71	0.1478	0.9910	0.8465	1.0086
SiK	56.68	55.92	87.81	8.41	0.4531	1.0117	0.7888	1.0018
ClK	11.87	9.28	11.58	25.53	0.0858	0.9398	0.7680	1.0019

$Na[AlSi_3O_8]$



Other Examples…

- Use of material from previously performed studies, e.g. material from reprotoxicity studies:
  F1-generation tissue material can be used in to order avoid new OECD 443 studies etc., e.g.,
  - application of measurements according to Garman et al. (2013) and special staining to avoid additional neurodevelopmental studies
  - enhanced histopathology of immune organs according to Elmore (2012)

Garman RH, Li AA, Kaufmann W, Auer RN, Bolon B (2016). Recommended Methods for Brain Processing and Quantitative Analysis in Rodent Developmental Neurotoxicity Studies. Toxicol Pathol. 44:14-42. Toxicol Pathol. 2012; 40(2): 148–156.

Elmore SA (2011). Enhanced Histopathology of the Immune System: A Review and Update. Toxicol Pathol, 40: 148-156.

Guidance on Nanotechnologies…

- If there is no quick degradation, follow from steps 2a onwards.

Questions:

- Is a 90-day study necessary? Or is a carfully designed 28-day study sufficient?
- Recovery groups are not recommaned but should be optional to the manufacturer
- Detection option for absorption might be an issue (e.g., carbonanotubes consist of approx. 98-99% Carbon only. Currently no well defined detection method is established.)

Guidance on Nanotechnologies…

- If there is no quick degradation, follow from steps 2a onwards.

Recommandations:

- Sampling and preservation of a full organ list
- Histological evaluation might be limited to selected organs, but local lymph nodes (e.g., mesenteric lymph nodes) and Peyer's patches should be included.
- For immune organs (including GALT and MALT), the STP described enhanced immune system evaluation might be considered as a tool on HE-stained sections
- Special stains (e.g., Masson's Trichrome) for intestinal mucosa and lympathic organs in order to detecd fibrosis
- Pathology evaluation is a key point. A Peer Review should be obligatory.

Guidance on Nanotechnologies…

- Step 3

Recommandations:

- An orderly performed 28 or 90-Day study might be a waiver for follow up studies (reproductive toxicology, cancerogenicity)
- Several in-vitro or ex-vivo models might elucidate further questions

Summary

- Particle uptake via intestine is possible mainly by M-cells into GALT possible for particles <10 µm (nanoparticles up to nanoparticle aggregtaes) (otherwise by endocytosis)
- Uptaken amount very limited:
  depending on concentration, diet, chemical surface, and prolonged transit times
- Solubility of particles causes resolution before inflammatory processes can start (no reports for Peyer' patches or mesenteric lymph nodes)
- Some publications very doubtful or wrong
- Own studies did not confirm previously reported results