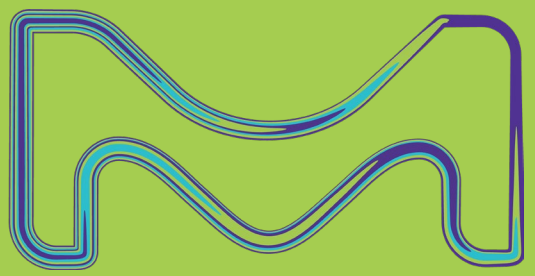
PQRI Workshop:
TiO2 Use in Pharmaceuticals
Global Regulatory and Technical Challenges
June 13-14, 2023

What could be next?: e.g. E172

PQRI Workshop:
TiO2 Use in Pharmaceuticals – Global Regulatory and Technical Challenges

14 June 2023

Thomas Broschard, EMD Serono



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E172
Agenda

1. Nanomaterials in Food: The ANSES list
2. E172 What and Where?
3. EFSA Opinion/Requests and Consortium
4. Studies and Results
5. Summary & Conclusion

E172
Ban of E171: End or Beginning?

"Substances that occur in human food and for which the presence of manufactured nanomaterials has been proven (characterization using EM in data and literature)"

EU No.	Chemical name
E 170	Calcium carbonate
E 171	Titanium dioxide
E 172 i, ii, iii	Iron oxide
E 341	Calcium monohydrogen phosphate
E 551	Silicon dioxide
E 552	Calcium silicate
	organic compounds and composites (nanoemulsions, liposomes, micelles, nanocapsules, nanoparticles of lipids)

E172
Ban of E171: End or Beginning?

"substances present in human food and for which the presence of manufactured nanomaterials is suspected and not confirmed after review of the literature and data"

EU No.	Chemical name
E 173	Aluminum
E 341	Calcium phosphate
E 343	Magnesium phosphate
E 421	Mannitol
E 504	Magnesium carbonate
E 554	Sodium aluminosilicate
	….and many others (30 compounds) https://www.anses.fr/fr/system/files/ERCA2016SA0226Ra.pdf

E172
What?…

Red Iron Oxide

Fe_2O_3

Yellow Iron Oxide

$FeO(OH) \cdot H_2O$

Black Iron Oxide

$FeO \cdot Fe_2O_3$

and Where?

E172
Timetable

EFSA Opinion on E172

Studies performed

EBRC Consulting GmbH

EFSA Nano Guidance

Tel Conf. with EFSA 02/2022

Further studies performed

VENATOR MERCK

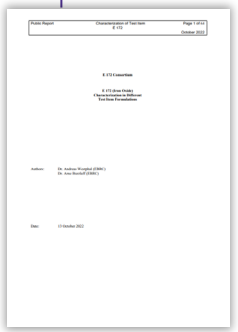
2015 2016 2017 2018 2019 2020 2021 2022 2023

JONES DAY®

E172 Consortium Signed by members

Data Submission to EU COM 03/2020

EFSA Request for further data 11/2022



Data Submission to EFSA 12/2022

MERCK

E172
EFSA Opinion 2015

efsa
European Food Safety Authority

EFSA Journal 2015;13(12):4317

SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of iron oxides and hydroxides (E 172) as food additives[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of iron oxides and hydroxides used as food additives (E 172): yellow iron oxide ($FeO(OH)\cdot H_2O$), red iron oxide (Fe_2O_3) and black iron oxide ($FeO\cdot Fe_2O_3$). Brown Iron Oxide has been included in this assessment for completeness, due to its importance as a commercial blend. The Panel considered that the particle size and particle size distribution should be included in the specifications. In 1980, an ADI of 0-0.5 mg/kg bw/day was established by JECFA. Absorption of iron from iron oxides is low. The acute oral toxicity of iron oxides is greater than 10 g iron oxide/kg bw. From a subacute and a subchronic toxicity study, the Panel identified a NOAEL for red iron oxide of 1 000 mg/kg bw/day, the highest dose tested. Red (Fe_2O_3) and black ($FeO\cdot Fe_2O_3$) iron oxide, both in nano- and micro-form, were positive in *in vitro* genotoxicity assays in mammalian cells. Due to the limitations of the database, and considering the impossibility to read-across between iron oxides with different redox state, the Panel considered that the genotoxicity of iron oxides cannot be evaluated based on the available data. Concerning carcinogenicity and reproductive and developmental toxicity, no signs of toxicity were observed in unpublished studies which were not available and could not be evaluated by the Panel. The Panel concluded that an adequate assessment of the safety of E 172 could not be carried out because a sufficient biological and toxicological database was not available. Refined exposure estimates show that exposure to E 172 ranged from 0.03 mg/kg bw/day for infants to 3.7 mg/kg bw/day for toddlers at the mean and from 0.1 mg/kg bw/day for infants to 9.5 mg/kg bw/day for toddlers at the 95th percentile for the non-brand-loyal scenario.

KEY WORDS

red iron oxide, black iron oxide, yellow iron oxide-hydroxide, E 172, CI 77492, CI 77491, CI 77499

[1] On request from the European Commission, Question No EFSA-Q-2011-00347, adopted on 17 November 2015.

[2] Panel members: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Younes Maged. Correspondence: fip@efsa.europa.eu

[3] Acknowledgements: The Panel wishes to thank the members of the former Working Group 'A' Food Additives and Nutrient Sources (2011–2014) and the members of the Standing Working Group on the re-evaluation of food colours: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Maria Jose Frutos, Pierre Galtier, David Gott, Claude Lambré, Jean-Charles Leblanc, Agneta Oskarsson, Jeanne Stadler, Paul Tobback, Ine Waalkens-Berendsen and Rudolf Antonius Woutersen for the preparatory work on this scientific opinion and EFSA staff members: Ana Maria Rincon, Alexandra Tard and Stavroula Tasiopoulou for the support provided to this scientific opinion. The ANS Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2015. Scientific Opinion on the re-evaluation of iron oxides and hydroxides (E 172) as food additives. EFSA Journal 2015;13(12):4317, 57 pp. doi:10.2903/j.efsa.2015.4317

Available online: www.efsa.europa.eu/efsajournal

The Panel recommended that the minimum, Tier 1 testing according to the EFSA guidance (2012), should be conducted for the material as marketed as the food additive (E 172):

- In vivo genotoxicity at the site of contact (gastrointestinal tract) and subchronic toxicity

- complete set of genotoxicity studies and subchronic toxicity

- absorption, distribution, metabolism and excretion (ADME), in vivo genotoxicity and subchronic toxicity

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E172
Timetable

EFSA Opinion on E172

Studies performed

EBRC Consulting GmbH

EFSA Nano Guidance

Tel Conf. with EFSA 02/2022

Further studies performed

VENATOR MERCK

2015 2016 2017 2018 2019 2020 2021 2022 2023

JONES DAY

E172 Consortium signed by members

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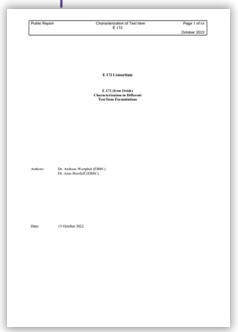
EFSA Request for further data 11/2022



Data Submission to EFSA12/2022

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E172
Consortium Members

MERCK

E172
Timetable

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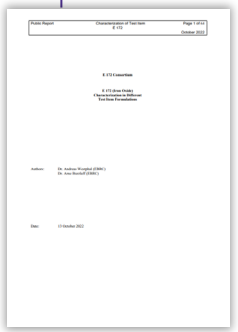

Data Submission to EFSA12/2022

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E172
Toxicological Studies Performed

Red
In vivo Comet Assay (OECD 489) Rat, oral

Black
In vivo Comet Assay (OECD 489) Rat, oral
90-day oral toxicity, rat (OECD 408)

Yellow
Ames test (OECD 471)
HPRT (OECD 476), L5178Y cells
In vitro MNT (OECD 487), CHO cells
Cellular Uptake (Leeds)
90-day oral toxicity, rat (OECD 408)

In vivo

In vitro

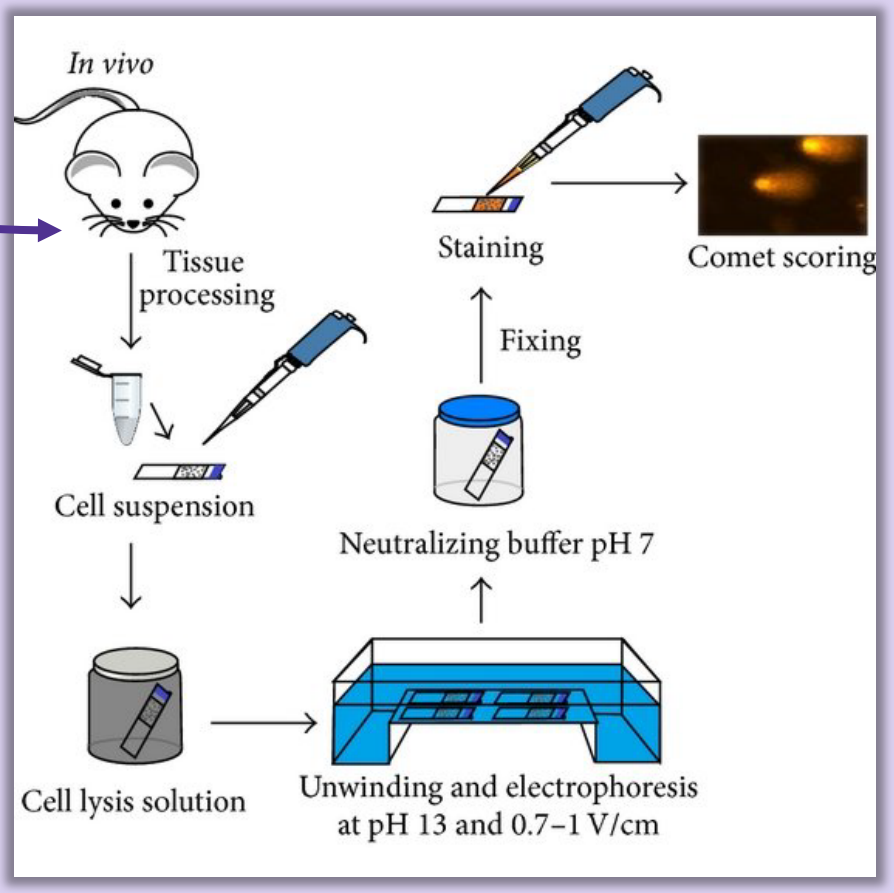
- All studies performed under GLP and according to OECD Guidelines (exception: Cellular uptake)
- Submission in 03/2020

E172
In vivo Comet Assay (OECD 489)


or


- Male rats, 6/group
- Dose levels: 500, 1,000 and 2,000 mg/kg bw/day
- 2 oral admin. (day 1,2)
- Vehicle: 0.5% HPMC
- Stomach and Duodenum

- Red E172: Clearly negative in both organs
- Black E172: Clearly negative in duodenum, Highest dose resulted in increase of tail intensity in stomach of 2/6 animals. However, considered to be an artefact and biologically not relevant



Tan & Bajo (2014) Arch Toxicol

E172
90-day Oral Toxicity Study (OECD 408)


or

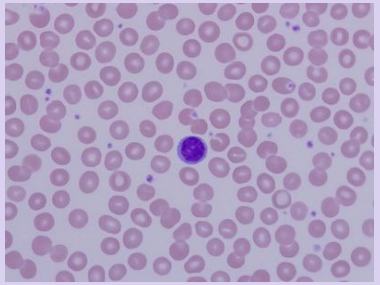

Treatment: 13 weeks → Recovery: 4 weeks

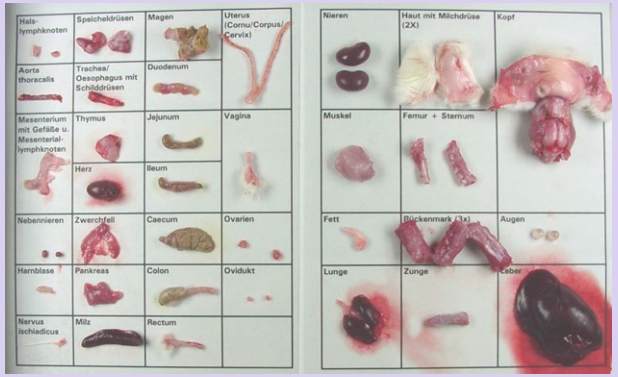
- Rats (m/f)
- Dose levels:
  0, 100, 300 and 1,000 mg/kg bw/day
- 10 animals/sex/group
- Vehicle: Diet

- Results:
- No adverse effects up to the highest dose
- No aberrant crypt foci
- NOAEL = 1,000 mg/kg bw/day

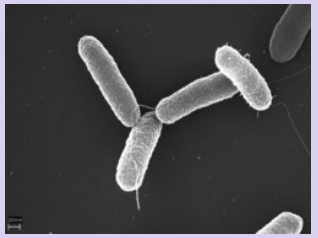
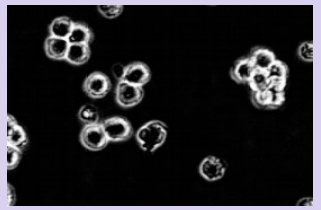
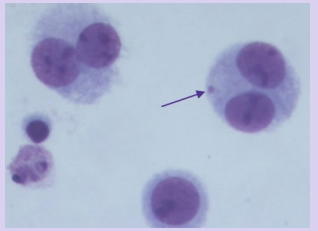
Investigations:

- Clinical symptoms
- Functional Observation Battery
- Body weight and body weight gain
- Food/water consumption
- Hematology, clinical chemistry
- Urinalysis
- Gross necropsy
- Organ weights
- Histopathology
- Aberrant Crypt Foci
- Toxicokinetics





E172
In Vitro Genotoxicity with 

Test		Result
• **OECD 471** (Ames test) up to 5,000 µg/plate +/- S9		• Negative (not relevant for insoluble particles)
• **OECD 476** (HPRT test) gene mutation L5178Y mouse lymphoma cells up to 100 µg/mL, +/-S9		• Negative
• **OECD 487** (in vitro MNT test) clastogenicity/aneugenicity CHO cells up to 300 µg/mL, +/-S9		• Negative

E172
In vitro Cellular Uptake

Swansea University & University of Leeds

L5178Y cells (HPRT):

No cellular uptake,

test material attached to cell surface

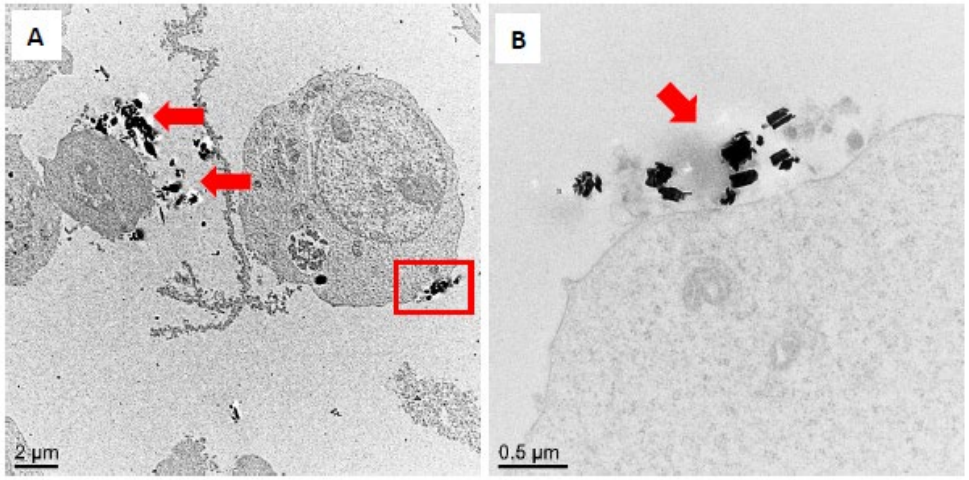


Figure 5 – Example electron micrographs of L5178Y cells treated with 100.1 µg/ml IRON OXIDE SICOVIT® YELLOW 10 E172. (A) - TEM image of L5178Y cells showing the presence of the test material at the cell surface (highlighted by the red arrows and the red box). No evidence of cellular uptake of test material is visible. The region highlight in the red box is displayed in (B) at higher magnification, the red arrow showing the location and morphology of the test material.

CHO cells (in vitro MNT):

Clear cellular uptake

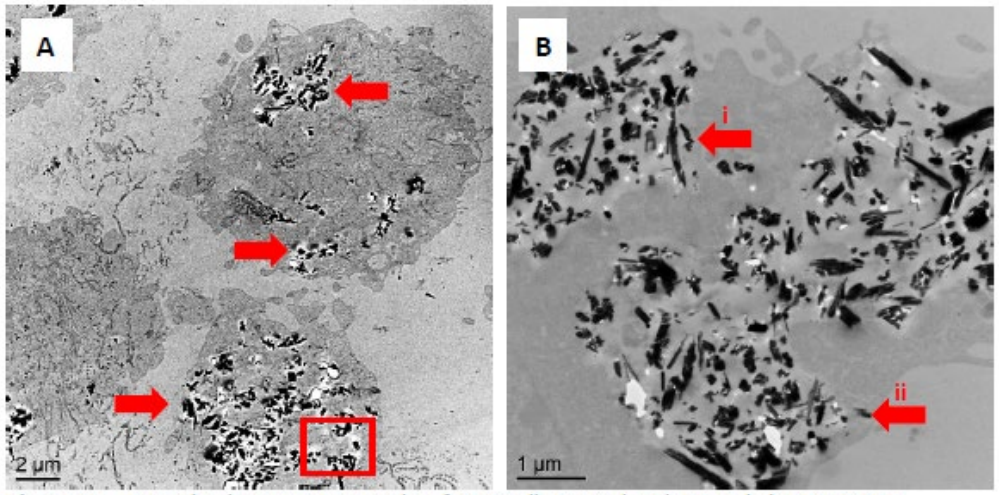


Figure 11 – Example electron micrographs of CHO cells treated with 75 µg/ml IRON OXIDE SICOVIT® YELLOW 10 E172. (A) - TEM image showing localisation of the test material in CHO cells highlighted by the red arrows and the red box). The region highlight in the red box is displayed in (B) at higher magnification, the red arrows showing membrane bound test material (i) and test material free in the cytoplasm (ii).

E172
Timetable

EFSA Opinion on E172

Studies performed

EBRC Consulting GmbH

EFSA Nano Guidance

efsa

Tel Conf. with EFSA 02/2022

Further Studies performed

VENATOR
MERCK

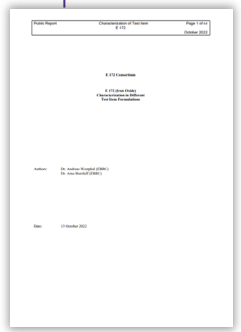
2015 2016 2017 2018 2019 2020 2021 2022 2023

JONES DAY

E172 Consortium signed by members

Data Submission to EU COM 03/2020

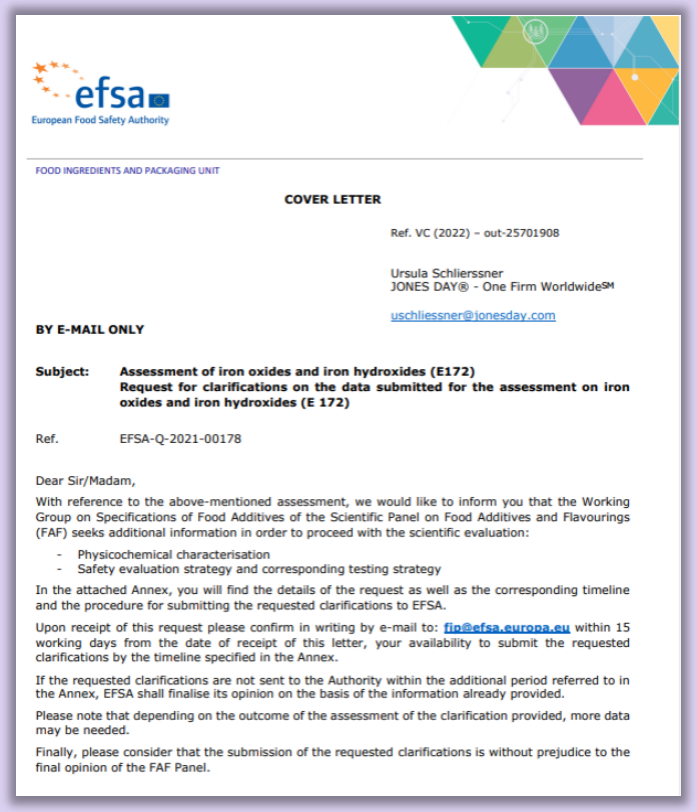
EFSA Request for further data 11/2021



Data Submission to EFSA12/2022

MERCK

E172
EFSA Request for Clarification (11/2022)



efsa
European Food Safety Authority

FOOD INGREDIENTS AND PACKAGING UNIT

COVER LETTER

Ref. VC (2022) – out-25701908

Ursula Schlierssner
JONES DAY® - One Firm Worldwide℠

uschliessner@jonesday.com

BY E-MAIL ONLY

Subject: **Assessment of iron oxides and iron hydroxides (E172)**
Request for clarifications on the data submitted for the assessment on iron oxides and iron hydroxides (E 172)

Ref. EFSA-Q-2021-00178

Dear Sir/Madam,

With reference to the above-mentioned assessment, we would like to inform you that the Working Group on Specifications of Food Additives of the Scientific Panel on Food Additives and Flavourings (FAF) seeks additional information in order to proceed with the scientific evaluation:

- Physicochemical characterisation
- Safety evaluation strategy and corresponding testing strategy

In the attached Annex, you will find the details of the request as well as the corresponding timeline and the procedure for submitting the requested clarifications to EFSA.

Upon receipt of this request please confirm in writing by e-mail to: fip@efsa.europa.eu within 15 working days from the date of receipt of this letter, your availability to submit the requested clarifications by the timeline specified in the Annex.

If the requested clarifications are not sent to the Authority within the additional period referred to in the Annex, EFSA shall finalise its opinion on the basis of the information already provided.

Please note that depending on the outcome of the assessment of the clarification provided, more data may be needed.

Finally, please consider that the submission of the requested clarifications is without prejudice to the final opinion of the FAF Panel.

Request for clarifications on the data submitted for the assessment on iron oxides and iron hydroxides (E 172):

- Physicochemical Characterisation
  - Composition, Coating, Particle Size Analysis
- Safety evaluation strategy and corresponding testing strategy
  - Reference to EFSA SC Guidance on particle-TR
  - *…you are invited to provide scientific evidence, supported by data, confirming that the safety studies provided for the assessment of E 172 are adequate for addressing the safety of the fraction of small particles, including nanoparticles, according to the principles indicated in Section 4 of the EFSA SC Guidance on particle-TR. P*
  - *…demonstrate that the test material(s) used in the safety studies included the fraction of small particles*
  - *demonstrate the adequacy of the study design of the existing toxicity and genotoxicity studies for covering the hazard assessment of the fraction of small particles,*

E172
Vehicles and Preparations (in vivo)

Study	Test Item	Vehicle	Dose/Conc.	Comment
90-day, oral rat (OECD 408)		Diet (ssniff®-R/M-H V1530, ssniff® Spezialdiäten GmbH, 59494 Soest, Germany)	100, 300, 1,000 mg/kg bw/day (1, 5 and 20 g/kg diet)	Premix, mixer (Röhnradmischer)
In vivo Comet Assay (OECD 489)		Aqueous Hydroxypropyl methylcellulose 0.5%	500, 1,000, 2,000 mg/kg bw/day (50, 100, 200 mg/mL)	*The test article was weighed into a formulation bottle. Vehicle was added to achieve the final volume. Formulations were then vortex mixed to stir*

E172
Vehicles and Preparations (in vitro)

Study	Test Item	Vehicle	Dose/Conc.	Comment
HPRT (OECD 476) L5178Y Mouse lymphoma cells		RPMI 1640 with additives and 5% horse serum	0.2-100 µg/mL	Vortex, ultrasonication, warming
In vitro MNT (OECD 487)		McCoy's 5A Medium +10% FCS	<300 µg/mL	Mixing

E172
Timetable

EFSA Opinion on E172

Studies performed

EBRC Consulting GmbH

EFSA Nano Guidance

Tel Conf. with EFSA 02/2022

Further Studies performed

VENATOR MERCK

2015 2016 2017 2018 2019 2020 2021 2022 2023

JONES DAY

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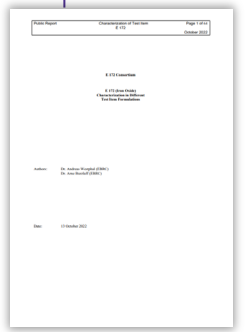


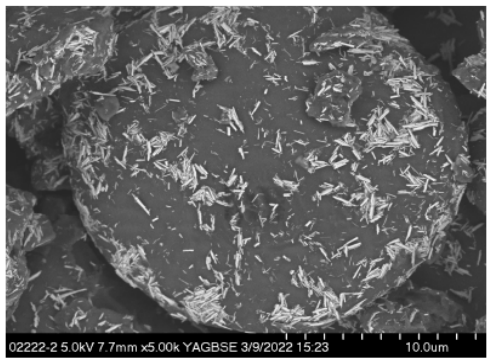
Data Submission to EFSA12/2022

MERCK

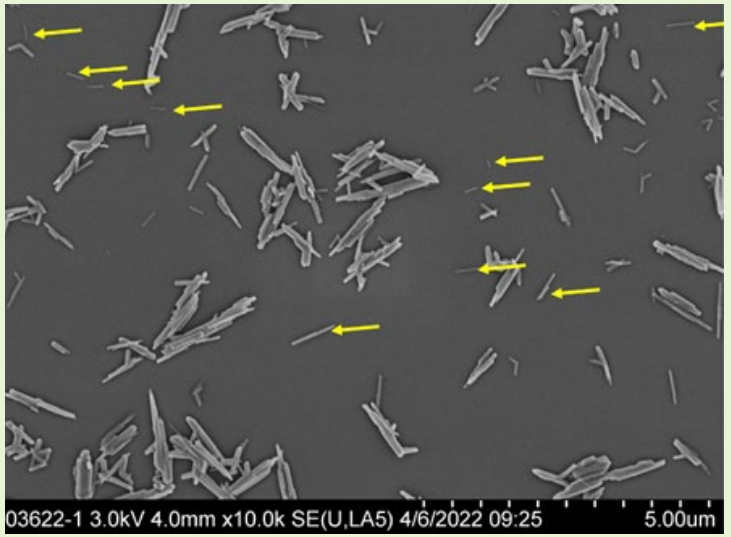
E172
Particle Characterization

- Preparation of test item/diet mixtures comparable to in vivo/in vitro studies
- Analysis by SEM
- Nanofraction in tox studies comparable to or exceeding real samples

E172 Yellow in rat diet (0.5%)



E172 Yellow in water (1 mg/mL)



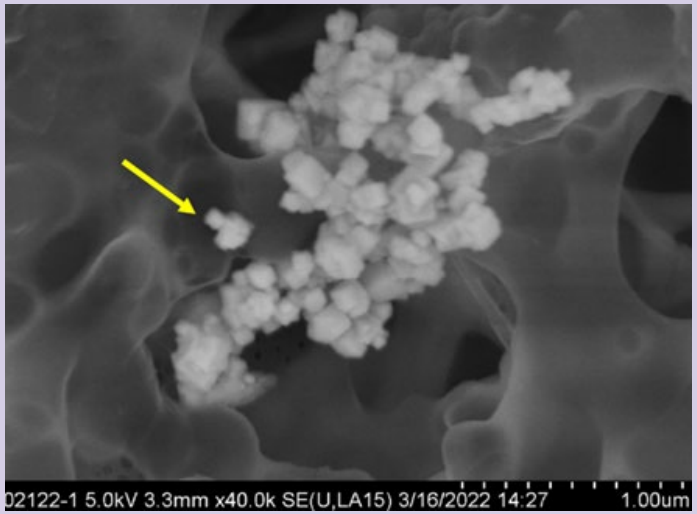
% Nano fraction

Rat diet	Gentox in vitro	Capsule	Hard candy shell
51-66.7%	30.2%	1.4 – 3.7%	42.5%

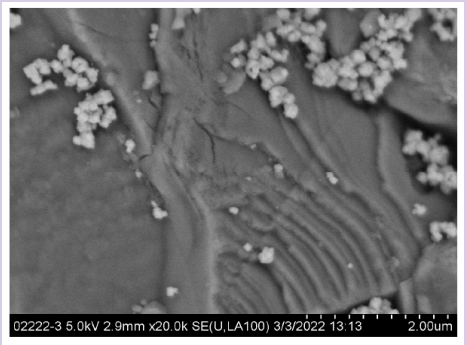
E172
Particle Characterization

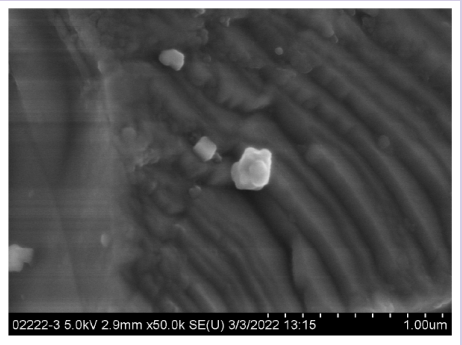
- Preparation of test item/diet mixtures comparable to in vivo studies
- Analysis by SEM
- % Nano fraction exceeds real samples

E172 Black in 0.5% HPMC (5 mg/mL)



E172 Black in rat diet (2.3 %)



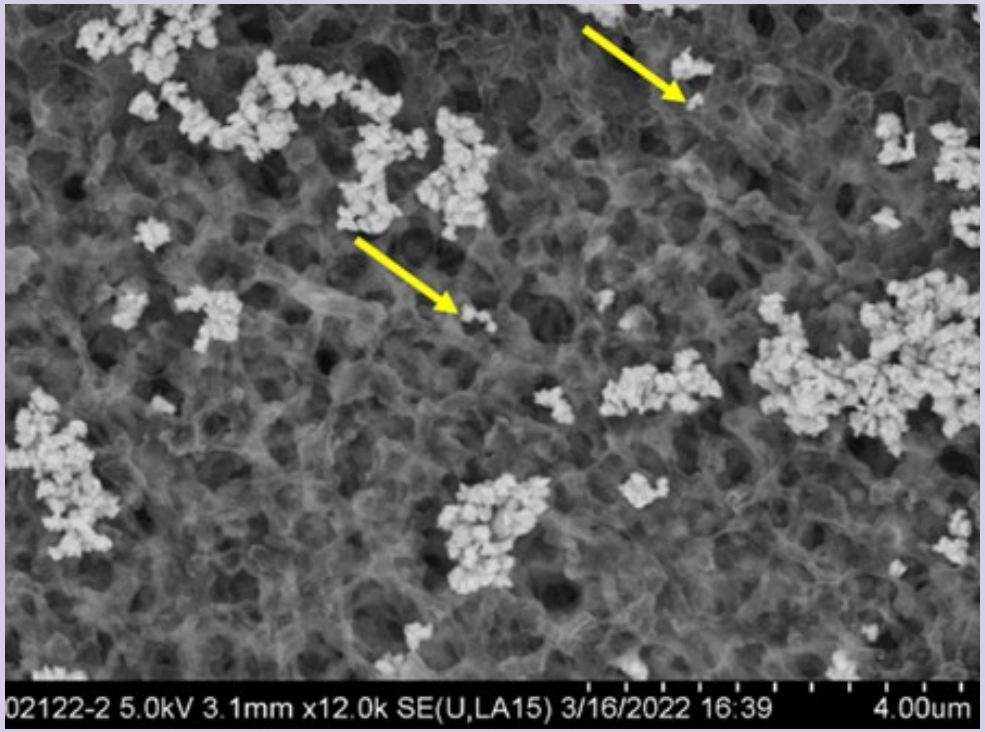


% Nano fraction

Rat diet	0.5% HPMC	Capsule	Hard candy shell
8-11%	28%	0-2.1%	0%

E172
Particle Characterization

E172 Red in 0.5% HPMC
(0.5 %)



- Preparation of test item in Methocel comparable to in vivo studies
- Analysis by SEM
- % nanofraction comparable to real sample

% Nano fraction

0.5% HPMC	Capsule
6.3%	4.1-6.5%

E172
Timetable

EFSA Opinion on E172

Studies performed

EBRC Consulting GmbH

EFSA Nano Guidance

Tel Conf. with EFSA 02/2022

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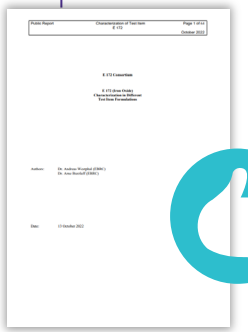

Data Submission to EFSA12/2022

MERCK

E172
Summary & Conclusion

- In 2015, Efsa has identified data gaps for three different E172 qualities; red, yellow and black
- An Industry Consortium has been formed which performed all studies requested by EFSA
- Based on the studies performed (and literature data) no adverse effect/no hazard has been identified for red, black or yellow E172
- Additional analytical investigations showed that the % nano fraction in the different test item formulation are comparable to real samples
- Based on the available data, red, yellow and black E172 are considered to be safe for food and pharmaceuticals
- EFSA opinion is expected to be published in 2024 

E172
Acknowledgments

- Martin Zilse
- Mark Booth
- Markus Rohe
- David Lockley
- Andreas Westphal
- Arne Burzlaff
- Roger Battersby
- Ursula Schliessner
- E172 Consortium
- Kevin Hughes
- Bram Baert

…and you for your attention