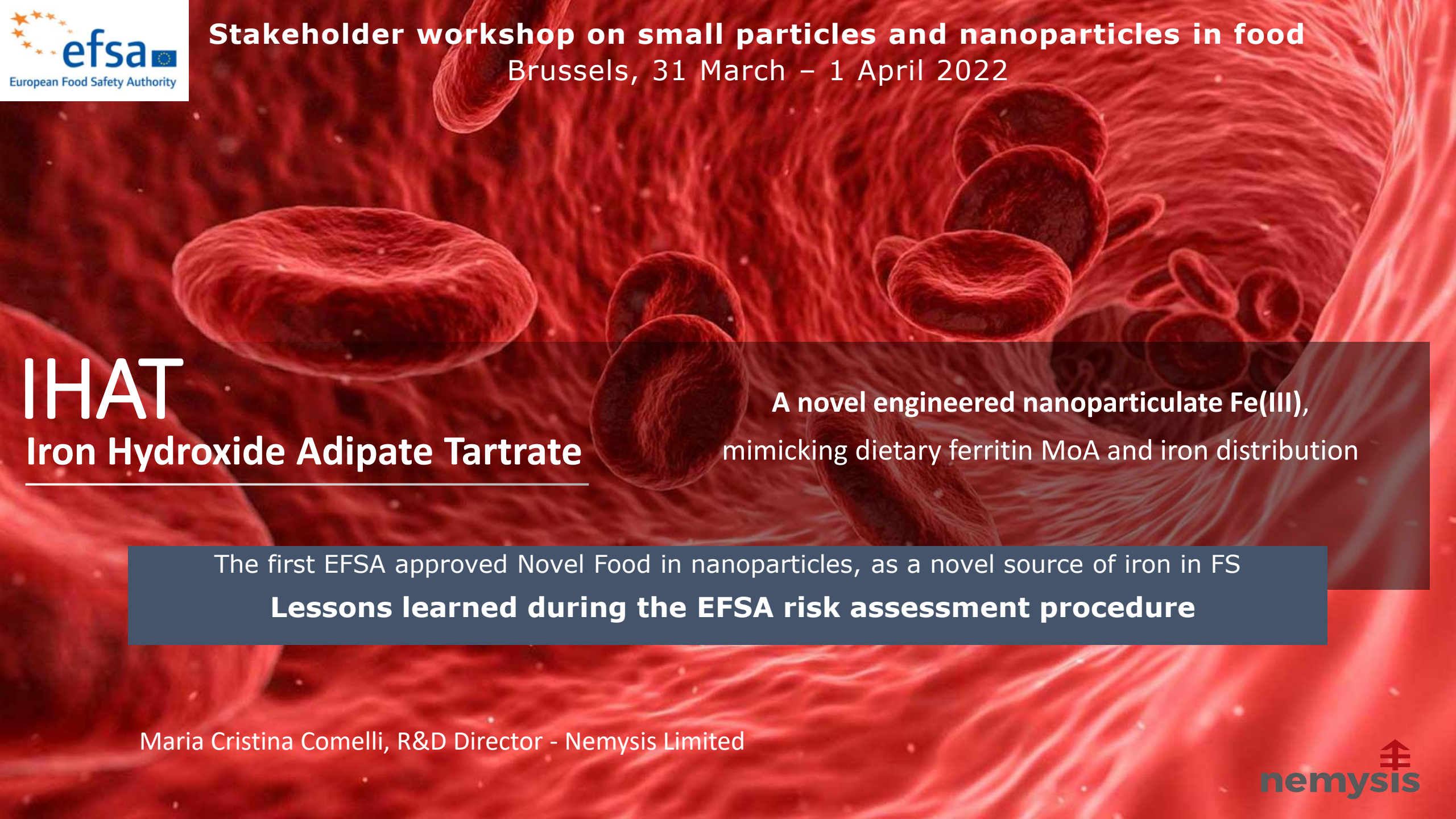

efsa
European Food Safety Authority

Stakeholder workshop on small particles and nanoparticles in food
Brussels, 31 March – 1 April 2022

IHAT
Iron Hydroxide Adipate Tartrate

A novel engineered nanoparticulate Fe(III),
mimicking dietary ferritin MoA and iron distribution

The first EFSA approved Novel Food in nanoparticles, as a novel source of iron in FS

Lessons learned during the EFSA risk assessment procedure

Maria Cristina Comelli, R&D Director - Nemysis Limited

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IHAT : Regulatory/legal framework of the application NF 2019/1417

IHAT is a novel food: for placement on the EU market, it was submitted for assessment of **SAFETY** as per Article 10 of Regulation (EU) No 2015/228

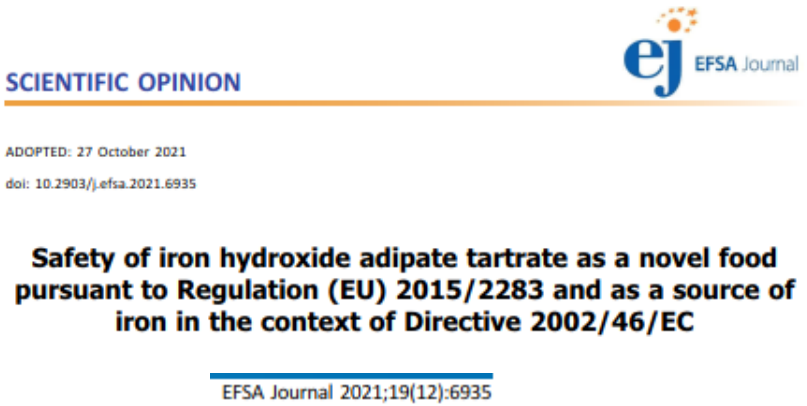
IHAT is a new source of iron: **BIOAVAILABILITY** was addressed in the context of Directive 2002/46/EC on food supplements

IHAT is an engineered nanomaterial: as defined in Article 3.2(f) of the Novel Food Regulation (EU) 2015/2283 and Commission Recommendation 2011/696/EU "*intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale".*

Within the application, IHAT was characterized and presented in compliance with:

- EFSA guidance on NF applications (EFSA NDA Panel, 2016)
- Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources (EFSA ANS Panel, 2018)
- **Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health'**:
  - EFSA Scientific Committee, 2018 - **as per 2019 submitted dossier**
  - **due to additional request of information by EFSA during the risk assessment, <u>also</u> compliant with the implemented 2021 guideline**

WHERE WE CURRENTLY STAND WITH IHAT-NF?


SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 October 2021

doi: 10.2903/j.efsa.2021.6935

Safety of iron hydroxide adipate tartrate as a novel food pursuant to Regulation (EU) 2015/2283 and as a source of iron in the context of Directive 2002/46/EC

EFSA Journal 2021;19(12):6935

Proposed uses and use levels

The applicant intends to market the NF for use in food supplements, up to a maximum dose of 100 mg per day, corresponding to a maximum daily intake of iron of 36 mg; the highest dose is to be defined in accordance with the equivalent maximum amount of iron supplementation permitted at the national level → **EU Commission for regulated authorization to market**

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LESSON 1: BUILDING A NETWORK OF EXCELLENCE

- highly experienced scientists from the (mineral) nano-world
- with specific knowledge/experience on iron metabolism
- highly familiar with NF/nano regulation, guidelines, and EU Commission-EFSA application procedures

Prof. Jonathan Powell

Head of Bio-Mineral Research at Cambridge University

- **Prof. Powell and his team at Cambridge University work with trace elements and mineral structures, especially nanoparticles, trying to identify their benefit to health as part of normal physiology**
- Former Head of Bio-mineral Research at the MRC's Human Nutrition Research (''HNR'') Elsie Widdowson Laboratory in Cambridge, **Prof. Powell and his team were the inventors of the patented technology behind IHAT which Nemysis Ltd acquired the license from the MRC in late 2017**

Dr. Nuno Faria

Chief Scientific Officer at NoBACZ Healthcare Ltd

- Formerly worked with Prof. Powell at the Bio-Mineral Research at the MRC's Human Nutrition Research (''HNR'') Elsie Widdowson Laboratory in Cambridge
- **Chemist by training, he developed the production process and analytical tools for IHAT at the lab scale, contributing to the tech transfer at industrial scale**
- Contributed to invention of the patented technology behind IHAT

Dr. Dora Pereira

Medical advisor at Vifor, UK

- Former Research Group Leader at the Department of Pathology, University of Cambridge
- Research focuses on **understanding the risk-benefit balance of oral iron in iron deficiency, the gut microbiome and enteric infections**
- **Principal Investigator with the MRC Unit The Gambia on a Phase II clinical trial investigating the safety and efficacy of oral iron supplements in young children**
- Contributed to invention of the patented technology behind IHAT

Dr. Katharina Kessler

R&D, Nemysis Ltd

- Postdoctoral Research at the University of Cambridge; PhD in Medical Sciences and MSc in Nutritional Sciences
- **Research focus: effect of macronutrients, micronutrients (iron and silicon) and certain plant extracts on human health**
- Katharina joined Nemysis Ltd in 2020

Dr. Federico Benetti

Executive Director

ecsin
european center for the sustainable impact of nanotechnology

- Expertise and state-of-the-art equipment and methodologies, for the measurement of different physico-chemical properties in the nano-specific matrix.
- **Testing IHAT nano-identity, its dissolution/ dispersion properties in culture media,and cellular uptake within the toxicological assessment of IHAT with validated industrial batches.**

Patrick Coppens

Managing Director EU&MEA

EAS) Strategies

Expert in food law through his work over almost 30 years with the European Commission, EFSA and other decision-making bodies.

Specialisation particularly in novel foods, health claims, additives and contaminants

Provides trainings and strategic insights to companies, trade associations and government authorities.

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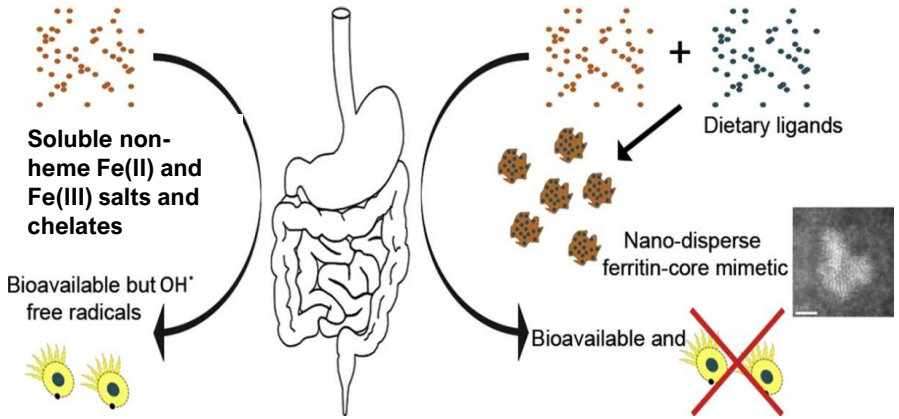
IHAT CONCEPT

A UNIQUE FERRITIN CORE MIMETIC MIMICKING THE STRUCTURE AND GI SPECIATION OF DIETARY FERRITIN

A PARADIGM SHIFT IN IRON SUPPLEMENTATION



IHAT = nano Fe(III)

Chemically organic ligands «doped» ferrihydrite to form:

- a ferritin core mimetic with increased Fe(III) lability and bioavailability
- strictly adhering to homeostatic pathway of iron efflux via ferroportin
- Not releasing free iron along the GI tract beyond the duodenum (absorption site) → no selection of potential harmful components of the microbiome
- repleting iron stores on increased body iron demand

2.8 ADME

Pereira D et al. **Caco-2 Cell Acquisition of Dietary Iron(III) Invokes a Nanoparticulate Endocytic Pathway.** PLoS ONE. 2013; 8(11): e81250

Powell JJ et al. **A nano-disperse ferritin-core mimetic that efficiently corrects anemia without luminal iron redox activity**. Nanomedicine. 2014;10(7):1529-38

Aslam MF et al. **Ferroportin mediates the intestinal absorption of iron from a nanoparticulate ferritin core mimetic in mice.** FASEB J. 2017; 28(8):3671-3678

Pereira D et al. **Nanoparticulate iron(III) oxo-hydroxide delivers safe iron that is well absorbed and utilised in humans.** Nanomedicine. 2014;10(7):1877-1886

Latunde-Dada GO et al. **A Nanoparticulate Ferritin-Core Mimetic Is Well Taken Up by HuTu 80 Duodenal Cells and Its Absorption in Mice Is Regulated by Body Iron.** J. Nutr. 2014; 144:1896-1902

RATIONALE TO IHAT DEVELOPMENT

- **Lack of bio-similarity of currently available oral iron preparations with dietary ferritin results in an array of unwanted side-effects** in the gut lumen with increased oxidative stress to cells and tissues, negative impact on commensal microbiota, and direct relationship to disease states e.g. activation of oncogenic pathways.
- **IHAT** may have beneficial application in one key global health priority, namely the prevention and treatment of iron deficiency (ID) and iron deficiency with anemia (IDA) without side effect-inducing redox cycling in the GI tract and no impact on the gut microbiome, thus opposing to the facilitated growth of potentially pathogenic bacteria.

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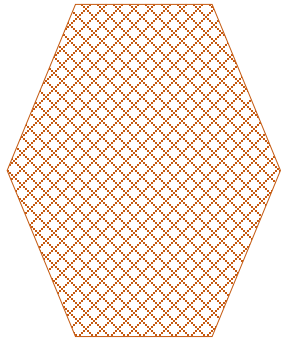
How nature inspired the formulation of IHAT

Synthetic/geological ferrihydrite

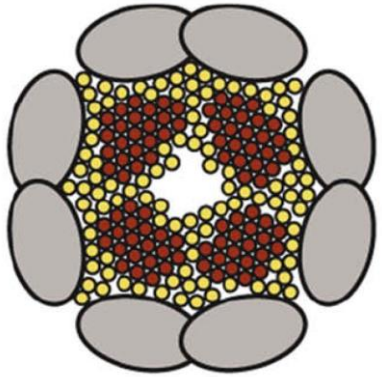
Hydrous ferric oxohydroxide nanoparticle
$(5(Fe3+)2O3{\cdot}9H2O)$



More organised,
"crystalline" atomic structure

Low bioavailability

Ferritin



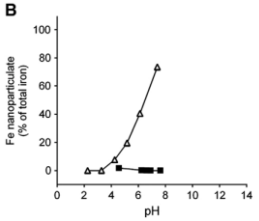
Destabilized structure

protein-encapsulated ferrihydrite nanostructures (∅ 5 nm)

present in meat and plant-based foods of the human diet and physiological iron storage form within cells

High bioavailability

US 8,058,462 LIGAND MODIFIED POLY OXO-HYDROXY METAL ION MATERIALS, THEIR USES AND PROCESSES FOR THEIR PREPARATION

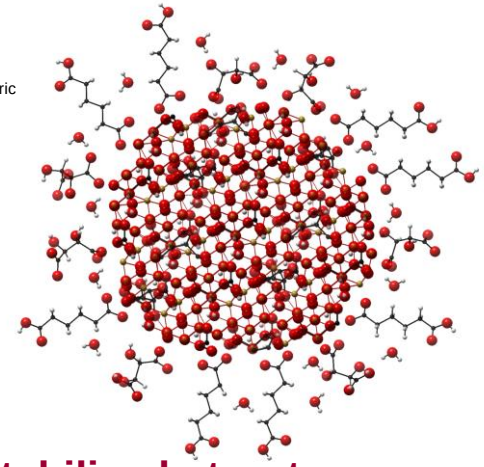


IHAT is manufactured by a chemical synthesis.

An acidic aqueous solution comprising iron (III) chloride, L-(+)-tartaric acid and adipic acid is neutralised through the addition of sodium hydroxide, resulting in the formation of Iron Hydroxide Adipate Tartrate (IHAT).

The product is then precipitated, recovered through a physical separation process (e.g. filtration or centrifugation), and dried.

IHAT



Destabilized structure

A ferrihydrite nanocore "doped" with tartrate and adipate as an aiding manufacturing buffer; (∅ 2-6 nm) amorphous with respect to ferrihydrite

High bioavailability

POTENTIAL CLINICAL SIGNIFICANCE

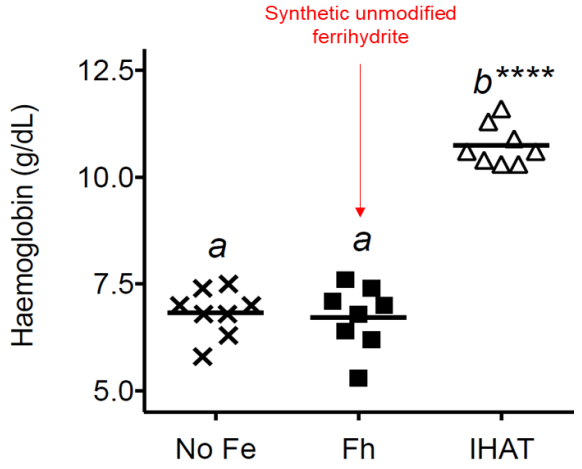
Nanomedicine: Nanotechnology, Biology, and Medicine
10 (2014) 1529–1538

A nano-disperse ferritin-core mimetic that efficiently corrects anemia without luminal iron redox activity

Jonathan J. Powell, PhD[a,*,1], Sylvaine F.A. Bruggraber, PhD[a,1], Nuno Faria, PhD[a], Lynsey K. Poots, BSc[a], Nicole Hondow, PhD[b], Timothy J. Pennycook, PhD[c,d], Gladys O. Latunde-Dada, PhD[e], Robert J. Simpson, PhD[e], Andy P. Brown, PhD[b,1], Dora I.A. Pereira, PhD[a,1]

PROOF OF CONCEPT

Hemoglobin levels of **anemic** Sprague–Dawley male rats following **2-week treatment** with diets fortified with different iron compounds at similar iron equivalent concentration



No Fe = control diet with no supplemental iron (3.1 ± 0.6 mg Fe/kgdiet)
Fh = synthetic ferrihydrite (35.7 ± 0.1 mg Fe/kgdiet)
LM-Fh (IHAT) = tartrate-modified ferrihydrite (31.4 ±0.5 mg Fe/kgdiet)

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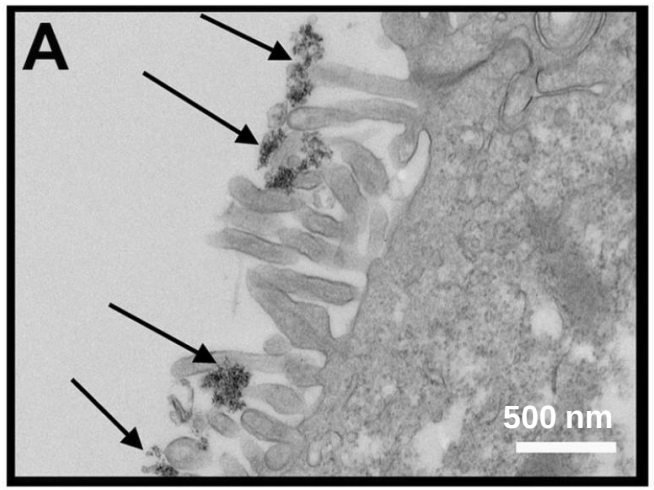
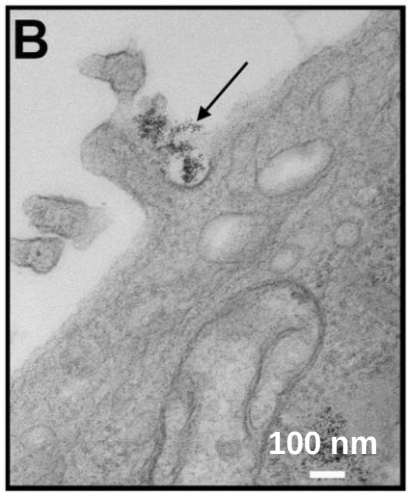
IHAT is taken up via endocytosis by the duodenal enterocytes (as proposed for ferritin)

TEM images showing differentiated Caco-2 cells incubated with IHAT

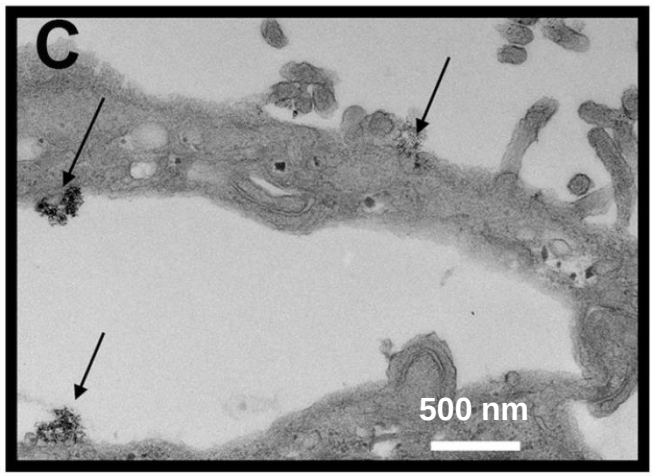
IHAT adhesion to cell membrane microvilli

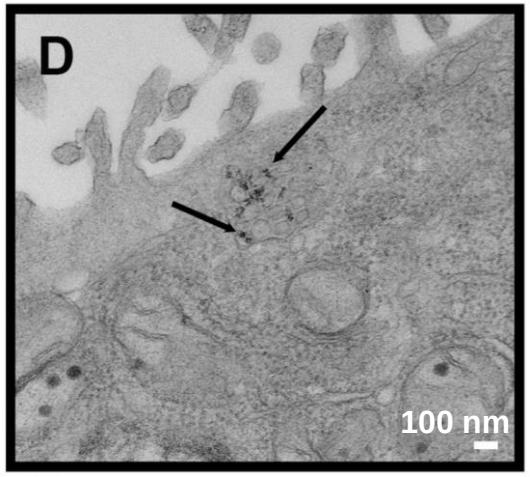


Invagination on cell membrane

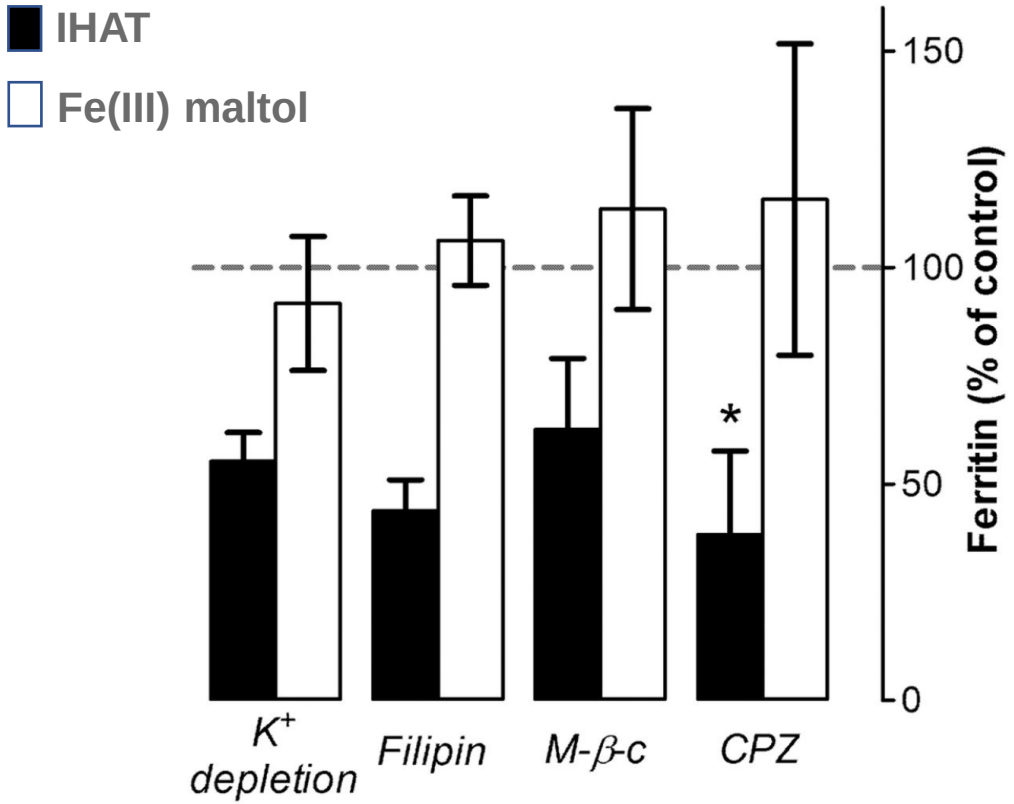


Iron accumulation inside cell





Inhibition of the endocytotic pathway by inhibitors decreases uptake of IHAT and formation of ferritin by differentiated Caco-2 cells



Data are shown as a percentage of the controls (without inhibitor) after a 1 h exposure of differentiated Caco-2 cells to IHAT (black bars) or Fe(III) maltol (open bars), co-incubated with either chlorpromazine (CPZ), potassium-free BSS (K+ depletion), filipin or methyl-β-cyclodextrin (M-β-c).

Pereira et al., PLoS ONE 8(11): e81250. doi:10.1371/journal.pone.0081250

DATA PROVIDED IN THE APPLICATION FOR IHAT CHARACTERIZATION

Is the material legally defined as engineered nanomaterial/nanoform? (Section 4.1) or does the material have properties characteristic of the nanoscale? (Section 4.3)

Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health

EFSA Journal 2021;19(8):6768

Physicochemical characterisation (section 5), including solubility and dissolution/degradation rate

↓

Are the nanospecific properties maintained?

↓ YES

Section 2.8 ADME:
- Uptake of IHAT and iron utilization in HuTu-80 cells (Latunde Data, 2014)

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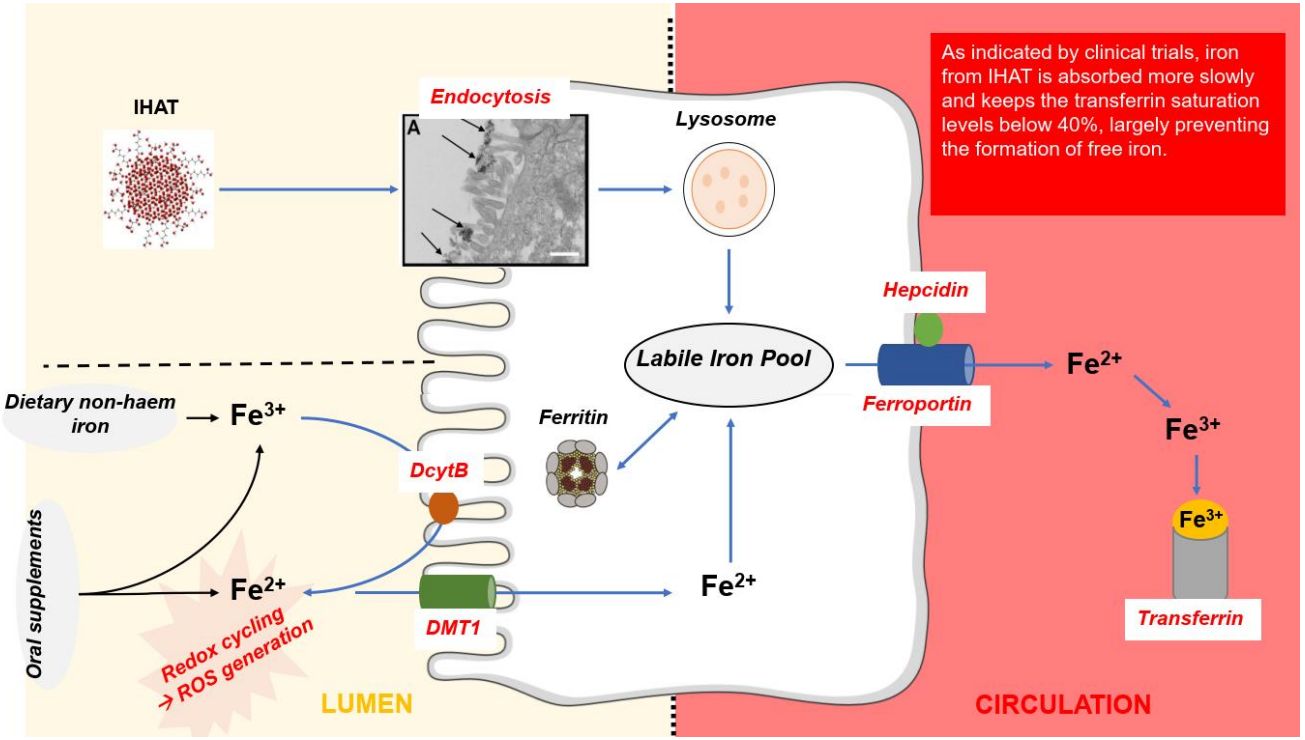
Table 2: Specifications of the NF

Description: Iron hydroxide adipate tartrate (IHAT) is a red-brown micro powder, odourless, insoluble in water

Parameter	Specification
Physical/chemical	
Iron (% dry matter)	24–36
Adipate (% dry matter)	1.5–4.5
Tartrate (% dry matter)	28–40
Water (%)	10–21
Sodium (% dry matter)	9–11
Chloride (% dry matter)	2.6–4.2
Phase distribution (in water)	
Soluble	2–4%
Nano	92–98%
Micro	0–3%
Primary particle size	
Median diameter(1)	1.5–2.3 nm
Mean diameter(1)	1.8–2.8 nm
Dv(10)(2)	1.5–2.5 nm
Dv(50)(2)	2.5–3.5 nm
Dv(90)(2)	5.0–6.0 nm
Heavy metals	
Arsenic	< 0.80 mg/kg
Nickel	< 50 mg/kg
Residual solvents	
Ethanol	< 5,000 mg/kg
Microbiological	
TAMC	< 10 CFU/g
TYMC	< 10 CFU/g

CFU: colony forming units; Dv: percentile of the volume-based particle size distribution; TAMC: total aerobic microbial count; TYMC: total yeast and mould count.
(1): Number-based (by TEM).
(2): Volume-based (hydrodynamic diameter by DLS).



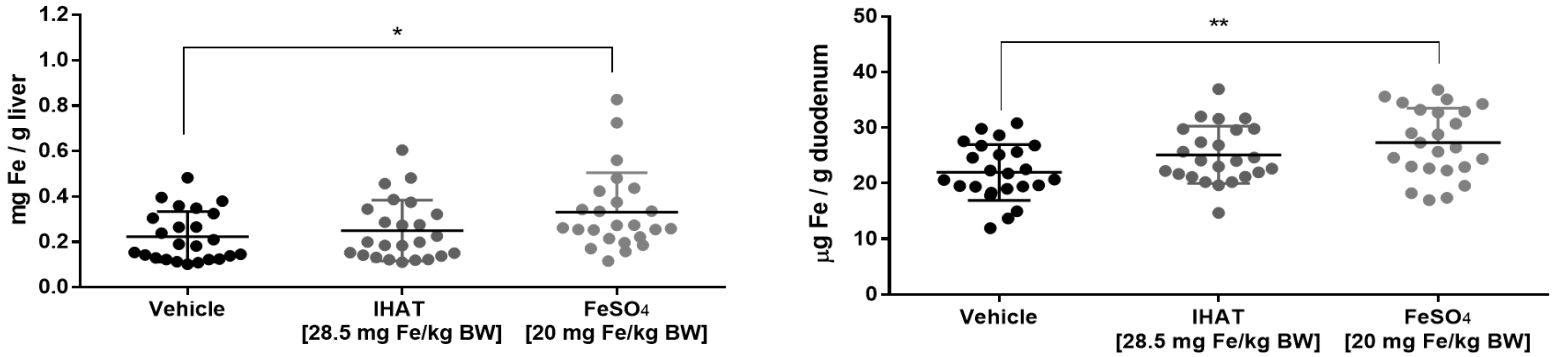
a. IHAT [NanoFe(III)]: endocytotic uptake, SIMILARLY to dietary ferritin and DIFFERENTLY from soluble Fe(II) or Fe(III) salts and chelates in FS as source of iron

b. IHAT DISSOLVES INTO THE LYSOSOME and the released IRON enters the LABILE IRON POOL, ready for EXPORT to the systemic circulation via EXCLUSIVELY FERROPORTIN upon INCREASED BODY IRON DEMAND

IHAT and iron-IHAT do not bioaccumulate in the intestinal mucosa, Payer's patches, Mesentheric Lymph Nodes, liver and spleen after 90 days of dose feeding (ICP-OES and Perls' Prussian Blue HC)

72 rats in total were gavaged with IHAT, $FeSO_4$ or placebo for either 28 days or 90 days

Study	Test item	Concentration test item	Necropsy 28 days	Necropsy 90 days
N25-001	IHAT	51.1 mM iron (28.5 mg Fe/kg body weight)	6 female & 6 male	6 female & 6 male
N25-001	Vehicle	0 mM iron	6 female & 6 male	6 female & 6 male
N25-002	$FeSO_4$	35.81 mM iron (20 mg Fe/kg body weight)	6 female & 6 male	6 female & 6 male



Total hepatic and duodenal iron content (ICP-OES) in vehicle controls (n = 24), IHAT-gavaged animals (n = 24) and $FeSO_4$-gavaged animals (n = 24).

- **NONE OF THE SELECTED TISSUES SHOWED A SIGNIFICANT DIFFERENCE IN TOTAL IRON CONCENTRATIONS BETWEEN THE IHAT AND THE CONTROL GROUP**
- Iron from FeSO4, but **not from IHAT**, accumulated in liver and duodenum samples.

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The «EFSA CLOCK»
EFSA claims 9 mo for the closing of the risk assessment and the NDA Panel issuing the final scientific opinion

Dec 9, 2019: Nemysis submitted the request to EC Commission in accordance with Article 10 of Regulation (EU) No 2015/2283
→ Feb 19, 2020: NF complete dossier submitted → mandate to EFSA

Jan 27, 2021
20210127_EFSA_Q-2020-00200_IHAT_CSL - REQUEST OF INFORMATION
How the NF as intended to be placed on the market, relates to the test material used in ADME with regards to its **physicochemical and nanospecific properties, including dispersion**

«CLOCK STOP»
+2 months

Additional data submitted

…even a stationary clock marks the right time twice a day…
Herman Hesse

April 24, 2021
20210420_EFSA-Q-2020-00200_IHAT_CSL - REQUEST FOR CLARIFICATION
Updating table and sections in compliance with provided data

«CLOCK STOP»
+15 working days

May 7, 2021
Clarification submitted

June 18, 2021
20210618_EFSA-Q-2020-00200_IHAT_CSL - REQUEST FOR INFORMATION
Genotoxicity: to perform a new MN study testing higher doses to achieve the expected cytotoxicity according to OECD TG 487 at the top concentration scored. **Size distribution and dispersion state of the test material in the culture medium should be in line with the previous tests provided**.

«CLOCK STOP»
+6 months

Sept 24th, 2021
Additional data submitted

Oct 13, 2021
20211013_EFSA-Q-2020-00200_IHAT_CSL - REQUEST FOR CLARIFICATION
Updating table and sections in compliance with provided data

«CLOCK STOP»
+15 working days

EFSA Journal 2021;19(12):6935

SCIENTIFIC OPINION

ADOPTED: 27 October 2021

- ❑ **From the first request of additional information by EFSA, 9 months were needed for the completion of the risk assessment**
- ❑ Overall, 18 months were needed for the whole procedure

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LESSON 2: ALL YOUR NANO-DATA NEED TO BE PERFECTLY COHERENT

Particular attention to properly bridge physico-chemical and nanospecific properties of lab (ADME), clinical and commercial batches (as intended to be placed on the market!)

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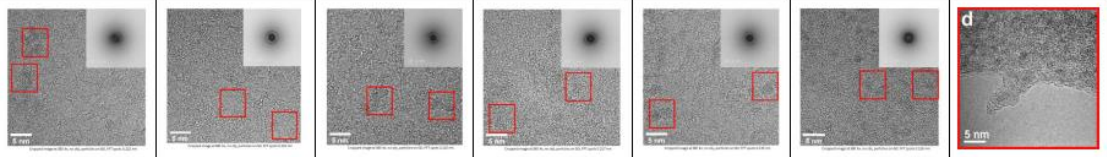
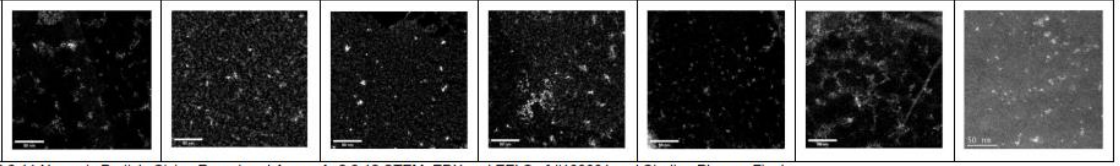
The applicant provided comparative data substantiating the equivalence of the novel food batches analysed and reported in the dossier, with a sample manufactured by Sterling Limited and used for the scale up process. Since no such comparison has been provided for the test material used in the studies by Powell at al. (2014) which is instrumental in documenting: i) the physicochemical characterization of the product and ii) the in vitro/in vivo studies carried out in the light of the physico-chemical and nanospecific properties, the applicant is requested to discuss and document how the NF as intended to be placed on the market, relates to the test material used in those studies with regards to its physico-chemical and nanospecific properties, including dispersion (Pereira et al, 2013; Latunde-Dada, 2014; Powell et al, 2014; Aslam et al, 2014; Pereira et al, 2014).

Table 1: particle size, lattice spacing, representative BF-TEM and HAADF-STEM images for all IHAT batches

	#180001[1]	#180002[1]	#180003[1]	#180004[1]	#180005[1]	Sterling Pharma[1]	Cambridge lab batch[2]
Particle size	2.18 ± 1.17 nm	2.12 ± 1.15 nm	2.61 ± 1.72 nm	2.38 ± 1.40 nm	2.32 ±1.48 nm	2.17 ± 1.27 nm	2-5 nm
Lattice spacing	2.22 Å	2.16 Å	2.10 Å	2.17 Å	2.15 Å	2.16 Å	~2.7 Å
Bright field TEM[3]							
HAADF STEM							

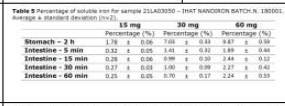
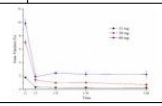
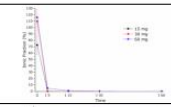
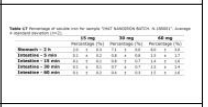
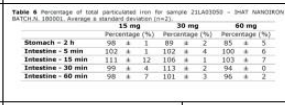
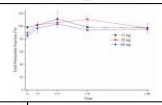
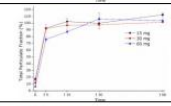
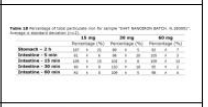
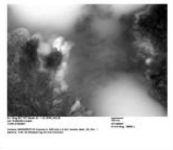
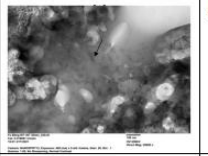
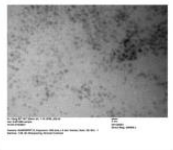
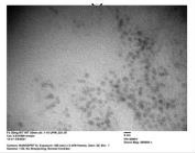
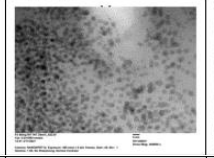
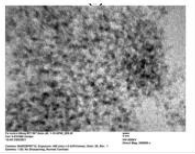
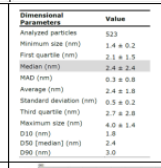
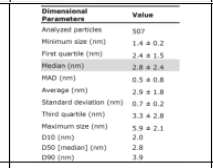
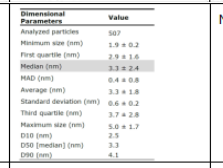
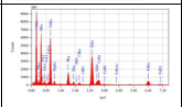
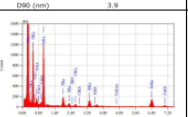
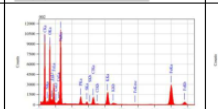
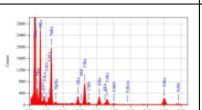
[1]refer to A_2.3.11 Nemysis Particle Sizing Report and Annex A_2.3.12 STEM, EDX and EELS of #180001 and Sterling Pharma Final
[2]refer to Powell et al, 2014 (Nanomedicine: Nanotechnology, Biology, and Medicine 10 (2014) 1529–1538), shown are Figure 2C (HAADF-STEM) and Figure 4D (BF-TEM).
[3]The red squares highlight particles with disordered lattice. The insert refers to the Fast Fourier Transform (FFT), which was used to determine the spacings between the lattice fringes by measuring the distance from the centre to one of the spots that can be seen.

efsa
European Food Safety Authority

The applicant provided volume-based particle size distribution of the nanoparticulate fraction for 5 batches of the NF performed by Dynamic Light Scattering. In line with the requirements set out in the EFSA Guidance for the risk assessment of nanomaterials[1] the applicant is requested to provide number-based particle size distribution to characterize the nanoparticulate fraction of the 5 batches, performed via electron microscopy (e.g. TEM).

According to the EFSA Guidance on nanomaterials[1] , at least three different concentrations - with a middle concentration that is calculated to be representative for human exposure - should be tested. On the contrary, 2 concentrations were tested, with the highest considered to be representative of the expected exposure based on use levels, quantified in 20 mg day as iron. Regarding this intake it is notedthat the NF is intended to be used in food supplements at a maximum recommended daily dose of 100 mg NF, corresponding a maximum daily intake of 30 mg iron (24-36 mg).

Table 5: IHAT dissolution in an in vitro gastrointestinal assay (as per Minekus et al, 2014; (A_10.12_Report_GIT dissolution IHAT 180001)

	Without addition of second food[1]				With addition of second food[2]
	15 mg iron as IHAT	30 mg iron as IHAT	60 mg iron as IHAT	$FeCl_2$ (15 mg, 30 mg, 60 mg Fe)	IHAT (15 mg, 30 mg, 60 mg Fe)
Kinetic soluble fraction					
Kinetic total particulate fraction[3]					
Representative TEM image[4] Intestinal phase 30 min Mag: 10000 x / 20000 x				Not determined	Not determined
Representative TEM image[4] Intestinal phase 30 min Mag: 200000 x					
Particle size distribution[4] Intestinal phase 30 min				Not determined	Not determined
EDX[4] Intestinal phase 30 min					Not determined

[1]The Novel Food IHAT is the only source of food.
[2]Mixture of powdered protein and lipid, corresponding to a protein-to-lipid ratio of 1.7, equivalent to 1 g of chicken meat, was co-digested with IHAT.
[3]The soluble and total particulate iron fraction were separated via 3 KDa filtration and the total particulate fraction was subjected to TEM analysis.
[4]For ease of reading, the Applicant has decided to show the results for degradation in the intestinal phase at 30 mins. As shown by the dissolution kinetics, similar results were obtained at 5, 15 and 60 min of the intestinal phase.

Table 1: Batch to batch analysis of the NF

Parameter	Batch number					Method of analysis
	1	2	3	4	5[a]	
Physical/chemical						As originally submitted
Iron % w/w (dry matter)	30.3	32.2	34.7	33.6	35.1	ICP-OES
Tartaric acid % w/w (dry matter)	32.8	33.9	35.0	32.8	28.9	HPLC-DAD
Adipic acid % w/w (dry matter)	2.1	2.5	1.9	2.1	2.0	HPLC-DAD
Sodium % w/w (dry matter)	10.5	10.5	10.5	11.0	11.0	ICP-OES
Chloride % w/w (dry matter)	3.3	3.9	2.6	4.1	4.0	ICP-OES
Dry mass balance % w/w	79.0	83.0	84.7	83.6	81.0	Calculated
Water (%)	17.1	16.0	14.3	20.7	11.2[a]	Karl Fisher
Iron (%) % w/w (wet basis)	25.2	27.0	29.8	26.7	31.2	Considering water content
Tartaric acid % w/w (wet basis)	27.8	28.3	31.0	26.7	27.1	Considering water content
Adipic acid % w/w (wet basis)	1.7	2.1	1.7	1.7	1.9	Considering water content
Sodium % w/w (wet basis)	8.7	8.8	9.0	8.8	9.7	Considering water content
Chloride % w/w (wet basis)	2.8	3.3	2.3	3.4	3.7	Considering water content
Phase distribution (in water)						
Soluble (%)	3.2	3.5	2.2	3.3	2.7	ICP-OES
Nano (%)	95.0	96.5	94.9	93.7	97.3	ICP-OES
Micro (%)	1.8	0.0	2.9	2.9	0.0	ICP-OES
Primary particle size						
Median diameter (nm)	1.88	1.82	2.15	2.00	1.88	HAADF-STEM[b]
Mean diameter (nm)	2.18	2.12	2.61	2.38	2.32	HAADF-STEM[b]
Dv(10) (nm)	1.68	2.32	2.00	1.81	1.86	DLS
Dv(50) (nm)	3.25	3.47	2.99	2.89	2.90	DLS
Dv(90) (nm)	5.49	5.91	5.17	5.17	5.18	DLS
Particle size distribution (volume-based) of secondary microparticles in the dry powder						
Dv(10) (µm)	18.2	14.26	2.81	10.28	84.26	Laser diffraction
Dv(50) (µm)	569.14	598.70	498.21	457.86	614.65	Laser diffraction
Dv(90) (µm)	1,282.53	1,329.95	1,320.22	1,198.04	1,302.45	Laser diffraction
Density (cm^3)	2.09	2.16	2.17	2.13	2.16	Pycnometry
Microbiological						
TAMC (CFU/g)	–	< 10	< 10	< 10	–	Ph. Eur. 9.4
TYMC (CFU/g)	–	< 10	10	< 10	–	Ph. Eur. 9.4
Heavy metals						
Ni (mg/kg)	38.6	39.2	48.2	46.3	41.4	ICP-MS[c]
Cd (mg/kg)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	ICP-MS[d]
Pb (mg/kg)	0.17	0.16	0.19	0.20	0.18	ICP-MS[c]
Hg (mg/kg)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	ICP-MS[c]
As (mg/kg)	0.67	0.62	0.80	0.70	0.69	ICP-MS[c]
Cr (mg/kg)	52.9	53.4	68.0	64.6	56.8	ICP-MS[c]
Residual solvents						
Ethanol (mg/kg)	< 89	< 89	< 89	< 89	< 89	HS-GC
2-Propanol (mg/kg)	< 7.5	< 7.5	< 7.5	< 7.5	< 7.5	HS-GC

CFU: colony forming units; DLS: dynamic light scattering; Dv: percentile of the volume-based particle size distribution; HAADF-STEM: high-angle annular dark-field aberration-corrected scanning transmission electron microscopy; HPLC-DAD: high-performance liquid chromatography with a diode-array detector; HS-GC: headspace gas chromatography; ICP-OES: inductively coupled plasma optical emission spectrometry; LOQ: limit of quantification; Ph. Eur.: European Pharmacopeia; TAMC: total aerobic microbial count; TYMC: total yeast and mould count; UV–VIS: ultraviolet–visible spectroscopy.
(a): Batch number 5 was subject to a drying process lasting twice the time used for the other batches.
(b): Data obtained from number-based distributions.
(c): LOQ 0.2 mg/kg.
(d): LOQ 0.04 mg/kg.

- TEM; EDX for particle nanoenvironment for number-based distribution in media used in GIT dissolution assays (fed and fast conditions), and
- in culture media of MN and MLA genotoxicity assay, to exclude agglomeration and maintenance of pristine characteristics
- to demonstrate cellular uptake

nemysis

OVERVIEW OF SAFETY DATA PROVIDED ON IHAT

Table 5: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
CHELAB (2021)	*In vitro* mammalian cell micronucleus test (GLP, OECD 487:2016)	Chinese Hamster Ovary (CHO) cells	Up to 90.4 μg/mL without S9 mix. Up to 9.8 μg/mL with S9 mix.
CHELAB (2019)	L5178Y Tk+/− Mouse Lymphoma Mutation Assay (GLP, OECD 490:2016)	L5178 Tk+/− mouse lymphoma cells	Up to 2.5 mg/mL (with and without S9 mix)
Vivo Science (2019)	90-day repeated dose oral toxicity study (GLP, OECD TG 408 extended to some endocrine endpoints of OECD 407)	Wistar rats	Control and 3 doses up to 462.13 mg/kg bw/day (114.1 mg Fe/kg bw per day)

Table 6: Summary of studies in humans related to the NF

Reference	Study design	Study population	Duration of study	Doses; route of administration if relevant	Parameters investigated related to bioavailability and safety
MRC (2013)	Single-blind, single-dose, cross-over study comparing 15 ferric iron oxide organic acid preparations (Fe-OA) (including IHAT) against ferrous sulfate.	4 pre-menopausal women (aged 18 to 45 years, from the UK) with mild iron deficiency (serum ferritin < 12 μg/L) or mild-moderate iron deficiency anaemia (haemoglobin 10-11.9 g/dL plus either serum ferritin < 20 μg/L or transferrin saturation < 10%) for each tested Fe-OA preparation. In total, 67 finished the study.	14 days	1 × Fe-OA (60 mg Fe equivalent) [IHAT tested at a dose of 66.8 mg Fe-equivalent/person]. 1 × $FeSO_4$ (60 mg Fe equivalent). Oral administration via methylcellulose capsule, on empty stomach or with light breakfast.	Relative bioavailability of iron from Fe-OA compared to $FeSO_4$ No safety-related parameters were tested except for reporting of adverse events.
MRC (2019)	Randomised, double-blind, placebo-controlled, parallel study with 3 arms (IHAT, $FeSO_4$, placebo)	Per protocol population was 582 healthy young children. 189 subjects (aged 6-35 months, both sexes, from Gambia) with iron deficiency and anaemia were included in the IHAT study arm.	12 weeks	IHAT: IHAT powder providing 20 mg Fe, 21 mg tartaric acid and 4.7 mg adipic acid (1 capsule orally)/child per day for 12 weeks (assumed to be bioequivalent to 12.5 mg Fe of $FeSO_4$, assuming a 60% bioavailability of IHAT relative to $FeSO_4$). $FeSO_4$: 62.5 mg ferrous sulfate heptahydrate powder providing 12.5 mg Fe (1 capsule orally)/child per day for 12 weeks.	Inflammation marker in the gut (faecal calprotectin) and blood (serum C-reactive protein (CRP); alpha 1-acid glycoprotein (AGP)). Diarrhoea-related parameters Faecal microbiome Reporting of adverse events (including serious ones).
MRC (2020)	Double-blind, single-dose, randomised cross-over study comparing the IHAT against ferrous sulfate.	32 pre-menopausal healthy women (aged 18–52 years, from Gambia), non-pregnant, non-lactating, with normal C-reactive protein (CRP) at screening (CRP < 5 mg/L). 32 women completed the study – 10 non-anaemic – 22 anaemic, whereas iron deficiency anaemia (IDA) was defined as haemoglobin 9-11 g/dL and serum ferritin < 15 ng/mL.	14 days	Single oral dose of either IHAT(i) or IHAT(ii) as capsule (equivalent to 60 mg Fe) as well as a single oral dose of $FeSO_4$ as capsule (equivalent to 60 mg Fe), 14 days apart. Administered as single oral dose of IHAT(i)(tray-dried) or IHAT(ii) (ethanol precipitated and then tray-dried) (capsule) and single oral dose of $FeSO_4$ (capsule) 14 days apart.	Relative bioavailability of iron from IHAT compared to $FeSO_4$. Serum iron, transferrin saturation, plasma iron, hepcidin concentration in blood. Pathogen growth in blood samples. Reporting of serious adverse events and adverse events.
JM-USDA (2019)	Randomised, double-blind, placebo-controlled, parallel study with 6 arms (IHAT, placebo, 3 $FeSO_4$ groups differing in dose and one plus micronutrients and another Fe-product under investigation)	Per protocol population was 160 subjects. 27 iron-replete non-anaemic post-menopausal women and age-comparable men (aged 50–77 years, 15 female, 12 male, mainly white or Caucasian, from US), were enrolled to the IHAT arm.	28 days	IHAT: 60 mg Fe/day as capsule. Three FeSO4 groups: (1) 60 mg Fe/day, (2) 420 mg Fe/week, (3) 60 mg Fe/day plus micronutrients. Another Fe-product under investigation, 60 mg Fe/day. IHAT providing 60 mg Fe (1 capsule orally)/fasted (12 h) person per day for 28 days.	*Ex vivo* malarial infectivity; *Ex vivo* bacterial proliferation potential (*E. coli*, *A. baumannii*, *K. pneumonia*, *S. aureus*, *Salmonella* Typhimurium). Gut inflammation markers (faecal calprotectin, myeloperoxidase, α-1 antitrypsin, tumour necrosis factor-α with LPS) and gut irritation questionnaire. Reporting of adverse events.

IHAT as a novel iron source was compared to placebo and to an active comparator (FeSO4 - golden standard of iron supplementation)

IHAT-Gut

A novel nano-iron supplement (IHAT) to safely combat iron deficiency and anaemia (IDA) in young children: a double-blind randomised controlled trial

PRIMARY ENDPOINTS

Summary of the four primary comparisons the trial.

Groups compared	Outcome	Comparison	Population	Method	Decision
IHAT Vs FeSO4	IDA correction/ response probability	Non-inferiority	ITT, PP	Logistic regression	Declare non-inferiority if lower limit of 90% one-sided CI for OR >0.583.
IHAT Vs FeSO4	Incidence density of diarrhoea	Superiority	ITT	Poisson regression	Declare superiority if the one-sided p-value for the Wald test of the effect of IHAT is less than 0.1.
IHAT Vs FeSO4	Prevalence of diarrhoea	Superiority	ITT	Logistic regression	Declare superiority if the one-sided p-value for the Wald test of the effect of IHAT is less than 0.1.
Placebo Vs IHAT	Prevalence of diarrhoea	Non-inferiority	ITT, PP	Logistic regression	Declare non-inferiority if lower limit of 90% one-sided CI for OR >0.583.

Treatment effect

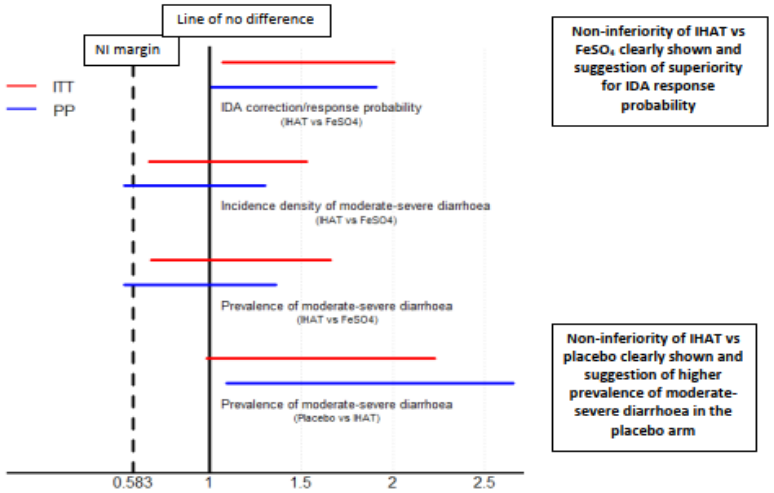


Figure 2. Primary endpoint summary conclusions based on the confidence interval approach to the analysis of a non-inferiority trial. ITT and PP analyses adjusted for Hb and age groups for the four primary objectives; horizontal bars are 90%CIs (Red: ITT, Blue:PP), the dashed vertical line is the non-inferiority margin on the OR scale, the solid vertical line is the line of no effect on the OR scale.

Secondary outcome

IHAT DOES NOT NEGATIVELY IMPACT ON THE GUT MICROBIOME

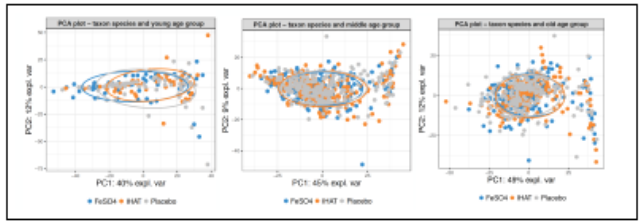


Figure 5. Principal component analysis (PCA) of the gut microbiome samples collected from children in the young (6-11 months), middle (12-23 months), and old (24-37 months) age groups separated by treatment group. The proportion of variance explained by the principle component 1 and 2 are mentioned after PC1 and PC2 on the x-axis and y-axis.

Table 18. Permutational Multivariate Analysis of Variance" (PERMANOVA) of the differences in microbial composition between variables: age group, season, geography, treatment, and timepoint.

taxon level	age group		season		geography		treatment		timepoint	
	R2	P value	R2	P value	R2	P value	R2	P value	R2	P value
Family	0.14121	0.0001	0.0347	0.0001	0.00434	0.0281	0.00209	0.0824	0.00641	0.0001
Genus	0.10611	0.0001	0.0279	0.0001	0.00464	0.0076	0.00184	0.109	0.0049	0.0001
Species	0.08516	0.0001	0.0224	0.0001	0.00488	0.0023	0.00196	0.0706	0.00436	0.0001

Pereira et al. Gates Open Res. 2018 Oct 11;2:48
Stelle I et al. Nutrients 2021, 13(4):1140

THANK YOU

nemysis

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BIOPERSISTENCE: IS THAT IHAT OR IRON per sè?

- LYSOSOMIAL ASSAY: 10 mmol/L citric acid, 0.9% NaCl, pH 5-

i. Even 'soluble inorganic iron' hitting the duodenum and proximal jejunum (where iron absorption occurs) is subjected to alkaline conditions → *as per IHAT nanoparticles precipitation during the production process*

ii. Consequently, **«soluble inorganic iron» reduces its solubility, and *in-situ* formation of nanoparticles of iron oxohydroxide (ferrihydrite) occurs in the lumen**. Growth and aggregation of these newly formed nanoparticles are restricted by an abundance of mucins → they remain just a few nm in size, readily available for enterocytic endocytosis (Bellmann et al., 2015, Theil EC, 2012).