Acute toxicity of Cobalt Ferrite and Gold nanoparticles: *in vitro* and *in vivo* study

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Origin of nanoparticles

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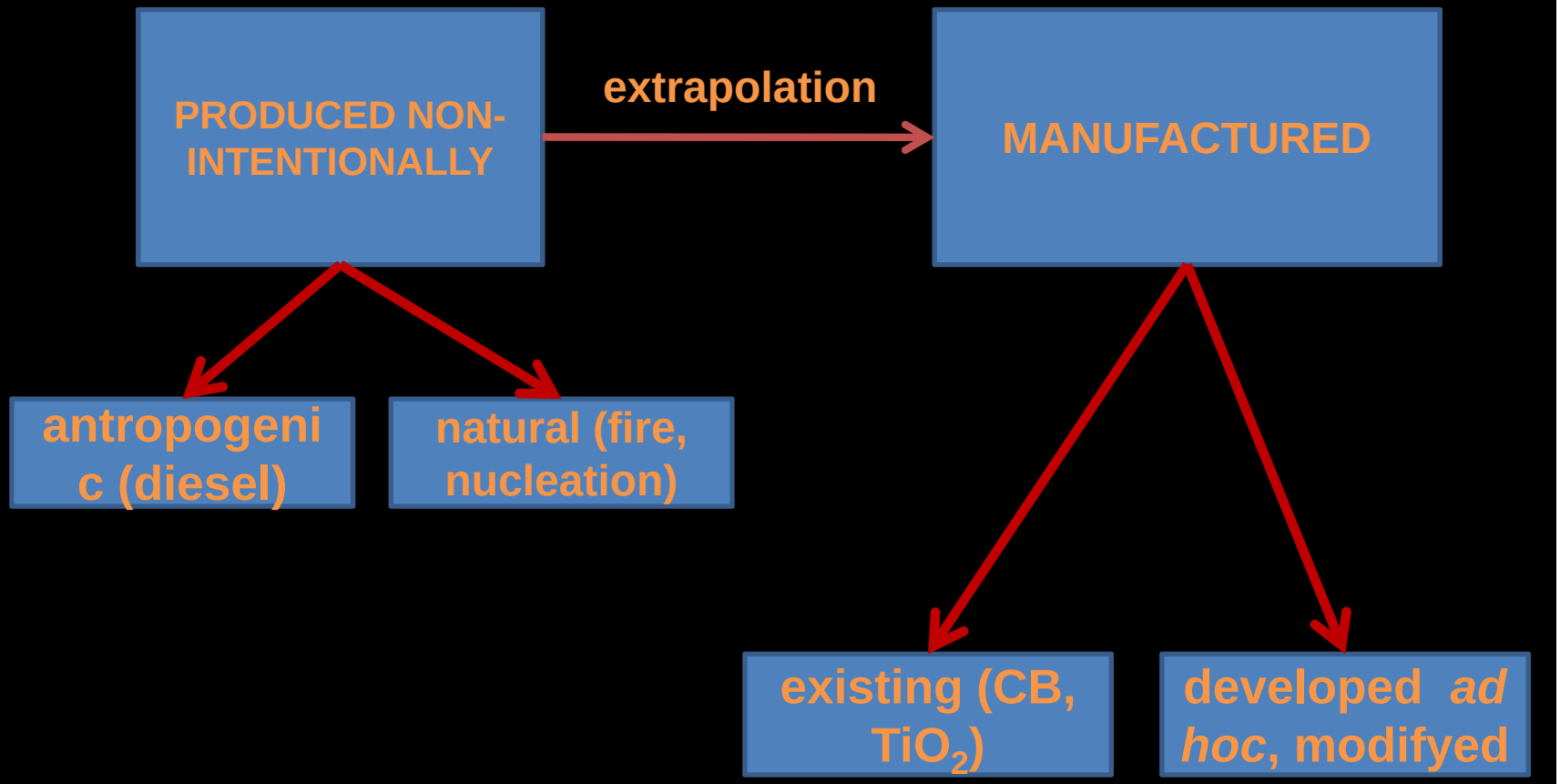

Some NP and their applications

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- Biomedical uses:
  - drug delivery
  - sensors or diagnostic systems working from **inside** the cell
  - structures for implants (scaffolds), may afford the possibility of imitating “porous” organs, or presenting capillary structures, as the liver
  - etc.

Risk evaluation

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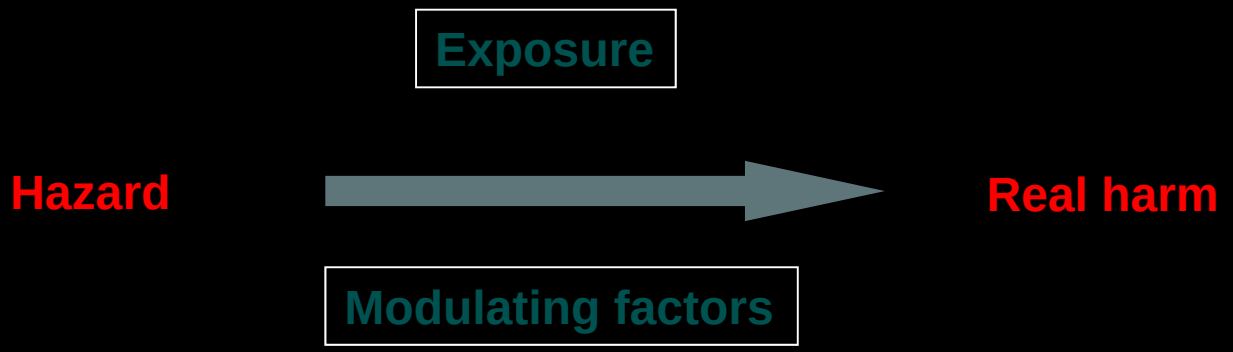
- In the scientific language:
  - Statistic concept (probability)



  - Assessment of exposure and effects: incertainity

Risk assesmment

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- Risk zero does not exist
- … and perhaps is even not desirable
- Risk / benefit balance

Biological risk of nanoparticles

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- Exposure
  - Non- intentional
    - Atmospheric (primary or secondary), traffic (60%), combustion processes, volcanoes, erosion, marine spray, etc..
  - Manufactured particles
    - Respiratory
    - Dermal
    - Oral
- 1. How does exposure occur?
- 2. What or who is exposed?
- 3. How much exposure occurs? When and where does it
- occur?
- 4. How does exposure vary?
- 5. How uncertain are exposure estimates?
- 6. What is the likelihood that exposure will occur?

Exposure

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- Present needs
  - Use of novel particle measurement techniques
  - Development of personal samplers to determine personal exposure to NP and their agglomerates
  - Development of a model describing the dispersion and transformation of NP and their agglomerates

Effects

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- Non-soluble Nanoparticles can stay for years in the lungs, GI-tract or brain; they are less well taken up by “professional” macrophages of the defense system but interact with cells of the epithelium, the interstitial tissue and vascular cells allowing pro-inflammatory reactions of these cells which usually do not see any particles.

- In addition, Nanoparticles can bind to proteins or translocate into the circulation and reach secondary target organs like liver, spleen, kidneys, heart and brain

Effects

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- Pulmonary Effects
  - Increased inflammatory effects (free radicals)
  - Very high load tumors (rat)
  - Effects on reticulum–endothelial system
- Cardiovascular effects and hemocompatibility
  - Hemolysis, coagulation
  - CV effects known but poorly understood
- Central Nervous System
  - Hypertension
  - Allergic Encephalomyelitis
  - Harm to BBB
  - Oxidative stress

As a resum: some toxicological specificities

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- Nanoparticles show special characteristics that may modulate its toxic potential:
  - increased absorption / through unusual ways
  - cross barriers
  - penetrate into otherwise scarcely accessible organs
  - interact with subcellular targets in special and poorly known conditions

- Drugs delivered at nano-scale:
  - reach new tissue, cell or molecular targets
  - and they do it in scarcely predictable conditions

- ***However, lower equipotent doses and a more accurate vectorization should contribute to a decrease of toxicity***

Toxicological methods

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- its assessment present special challenges.
  - Its behaviour toward biological systems is influenced by many factors including
    - shape,
    - Coatings
    - inherent heterogeneous size distribution.
  - In NPs the internalization process is a crucial event and should be characterized.
- In that complex context toxicological methods adapted to these new materials should be developed.

NANOSOST

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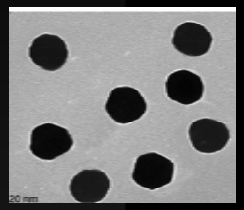
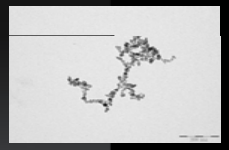
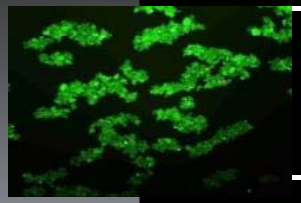
- Within the frame of NANOSOST, we have tested the toxicity of 4 kinds of nanoparticles (NPs):







  - gold (AuNP) diameter: 10,45nm
  - gold coated by hyaluronic acid (HA-AuNP) diameter: 30 nm
  - cobalt ferrite coated by silane (Si-$CoFe_2O_4$ NP) diameter: 17 nm
  - cobalt ferrite coated by silane and gold (Au-Si-$CoFe_2O_4$ NP) diameter: 45 nm.

In vivo assay

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- Acute toxicity in rats by intraperitoneal (IP) administration. The specific aim of the study was to determine a DL_{50} by the Up&Down protocol (OECD 425) and compare these results with the values obtained in parallel for gold (III) chloride ($HAuCl_4$) and $CoFe_2O_4$ in solution.
  - Clinical signs,
  - necropsy,
  - hematological parameters and
  - histopathological exam (kidney, liver, spleen and lung)
- Biodistribution by TEM (liver, spleen, kidney and lung)
- ICP-MS of different tissues (liver, spleen, pancreas, lymph node, kidney, lung and brain) at 14 days from the administration was assessed.

In vivo results

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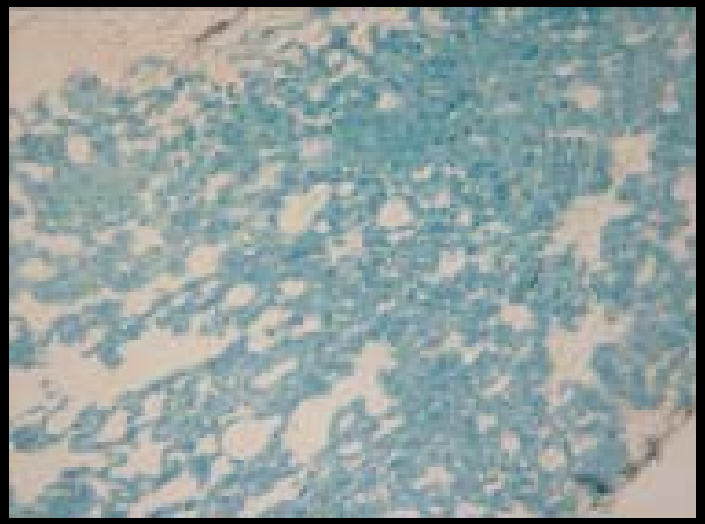
	LD $_{50}$	Low Lim LD $_{50}$	Up Lim LD $_{50}$
Au salts	106,56	42,63	266,41
Cobalt ferrite salts	121,9	48,76	304,74



Not differences in hematological values
Not differences in biochemical parameters
Some differences in histophatological evaluation but it's because pH

In vivo results

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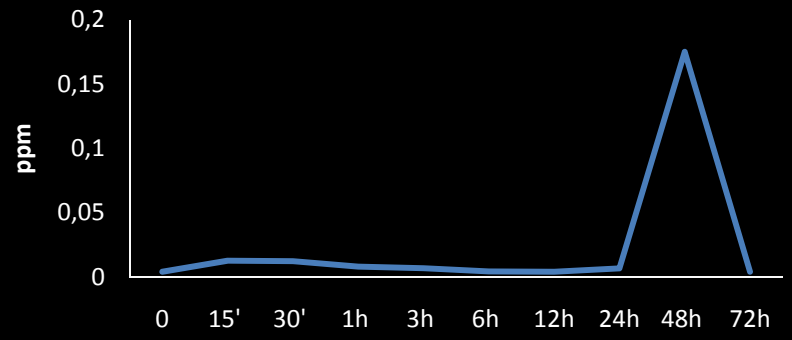
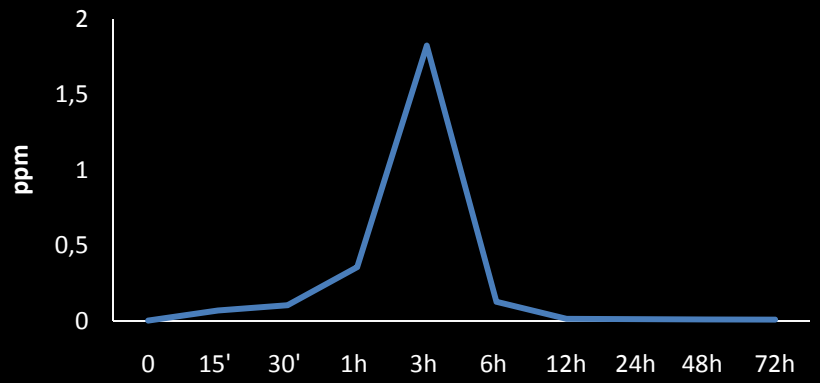
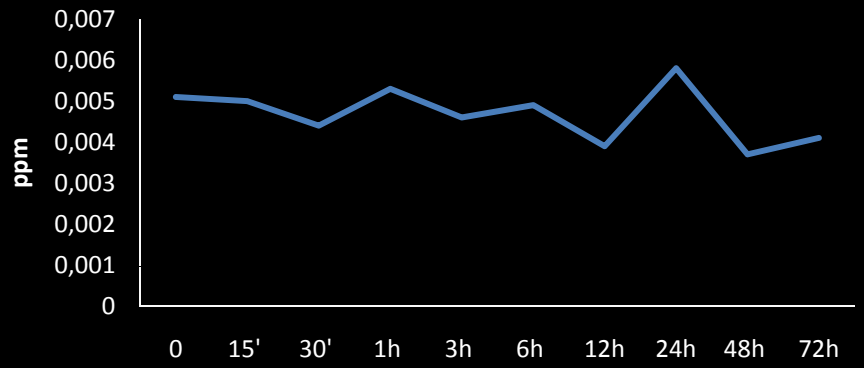
AuNP



AuNP con HA



NP ferrita cobalt



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In vitro assay

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- ***Cytotoxicity :*** LLC-PK1 and Hep2 (representing potential target organs following systemic administration) by means of WST-1 and LDH methods.

- ***Mutagenicity:*** Bacterial mutagenicity was assessed in 4 *Salmonella thyphimurium* and 1 *Escherichia coli* strain with and without metabolic activation according to Ames protocol.

- ***Uptake*** of the NPs by cells was studied by fluorescence microscopy and ICP-MS.

Mutagenicity results

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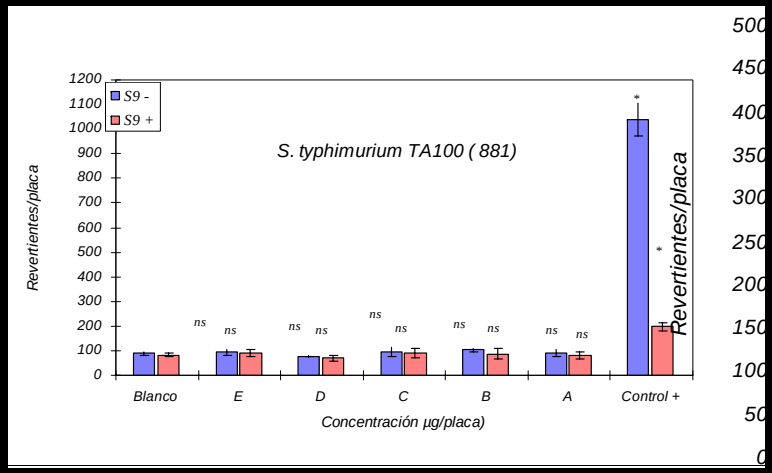
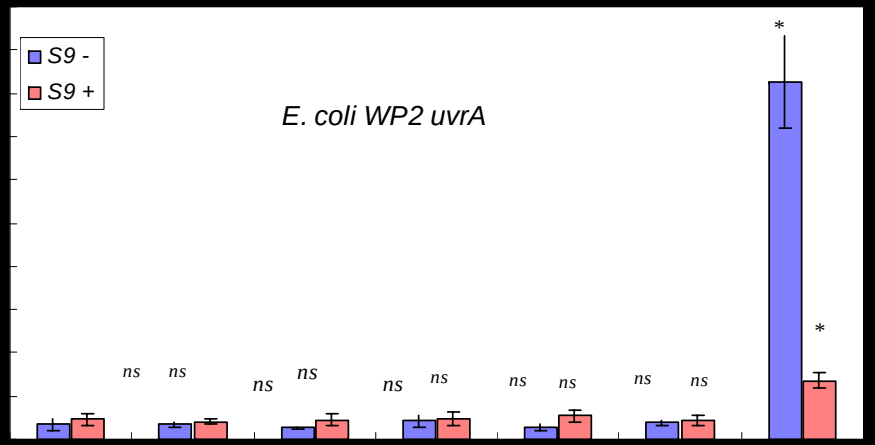
- The samples were not mutagenic in the assayed conditions





Cytotoxicity results

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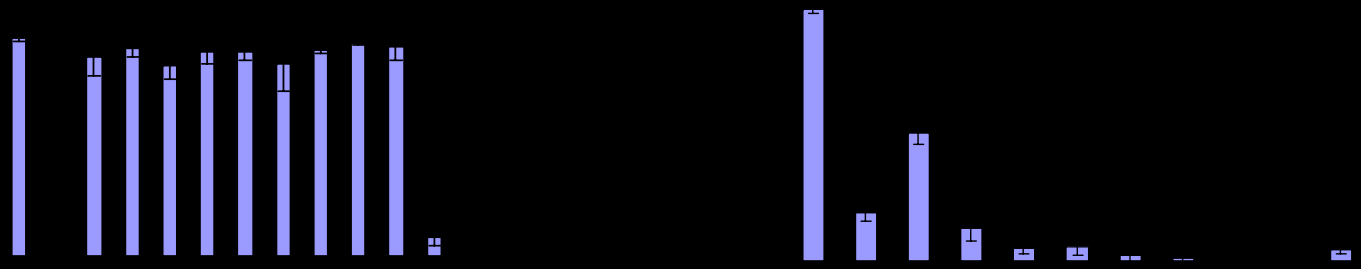
- No conclusive results could be obtained nor with LDH nor with WST-1



- Cytotoxicity will be studied using a cellular testing method which does not employ absorbance measurements (i.e: Colony Forming Efficiency Test" (CFE))

Uptake results

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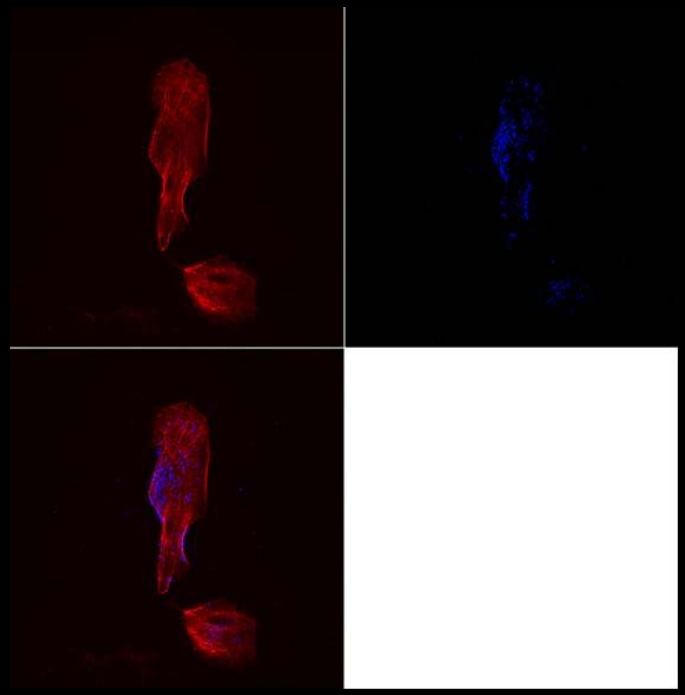
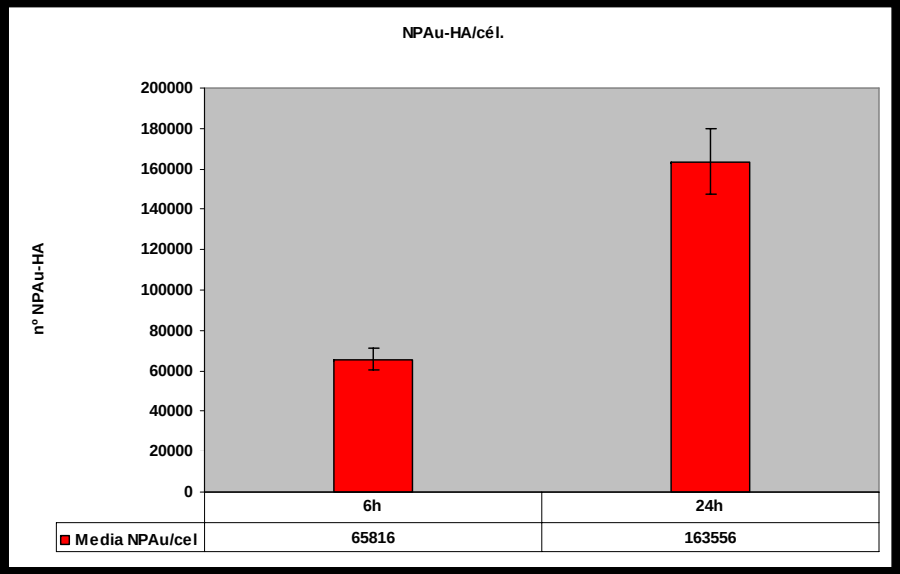


Internalization by Confocal microscopy of AuNP coated with HA



Quantification by ICP-AES

Thanks for your attention

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