

Sex-specific approach in nanomaterial hazard identification

Francesca Maranghi

Centre for Gender Specific Medicine

Istituto Superiore di Sanità

Background

Sexual dimorphism in the animal kingdom has been known, described, biologically explained, and accepted by the scientific community

BUT

toxicological/pharmacological and medical research has traditionally considered male and female organisms as **equivalent**,

AND

most preclinical and clinical studies were carried out in one **sex** (mainly males) and the results extrapolated to the other sex.

In medical research, **Gender Medicine** is an approach aimed at recognizing and analyzing the differences arising from gender in several aspects: anatomical, physiological, biological, functional, social, and in the response to pharmacological treatment.

TOXICOLOGICAL RISK ASSESSMENT

- Evaluates the potential health **risks** associated with exposure to chemicals/nanomaterials
- In vivo studies performed to evaluate intrinsic toxicity of chemicals (Hazard identification) use animals of one sex (usually male) to provide a $\text{NOAEL}^*/\text{UF}^{**} = \text{ADI}$
- The ADI is expressed in milligrams of the chemical per kilogram of body weight (a standard adult person (**man**) weighs 60 kg).

- ▶ Hazard identification
- ▶ Hazard characterization
- ▶ Exposure assessment
- ▶ Risk characterization



Acceptable daily intake (ADI) is the maximum amount of a chemical that can be ingested daily over a lifetime with no appreciable health risk.

* No Observed Adverse Effect Level
** Uncertainty Factor

Definition: Sex vs Gender

Sex refers to the biological differences between males and females, such as the physiological and genetic differences (XX or XY)

Gender refers to the role of a male or female in society, known as a gender role, or an individual's concept of themselves, or gender identity.

WHO recognises that there are variations in how people experience gender based upon self-perception and expression, and how they behave

<https://www.euro.who.int/en/health-topics/health-determinants/gender/gender-definitions>

Why both sexes in the in vivo studies??



- ▶ The experimental design of in vivo studies always includes the treatment and observation of **male and female rodents** taking into account not only the potential alterations recorded in reproductive systems but also effects in other body districts significantly – and uniquely - expressed in one sex only.
- ▶ In all the studies, the recorded data were also evaluated in order to identify target organs and mechanisms showing enhanced susceptibility of male or female rodents to the test materials.

Oral, short-term exposure to titanium dioxide nanoparticles in Sprague-Dawley rat: focus on reproductive and endocrine systems and spleen

Experimental design

- Preparation and characterization (SEM/TEM) of nanoTiO₂ (anatase, primary size <25 nm) dispersion
- 7 CD rats /sex/group
- 5 days treatment
- Dose levels: 0, 1, 2 mg/kg bw di TiO₂

Endpoints – focus on reproductive and endocrine systems

- General toxicity
- Biodistribution: spleen (single-particle ICPMS and SEM/energy-dispersive X-ray)
- Serum biomarkers: E2, T, T3
- Histopathological analysis: testis; uterus; ovary; spleen; adrenal; thyroid



BIODISTRIBUTION

- ▶ in spleen, there were no differences in total Ti tissue levels between



- ▶ No general toxicity in both



- ▶ adrenal cortex and spleen alterations in

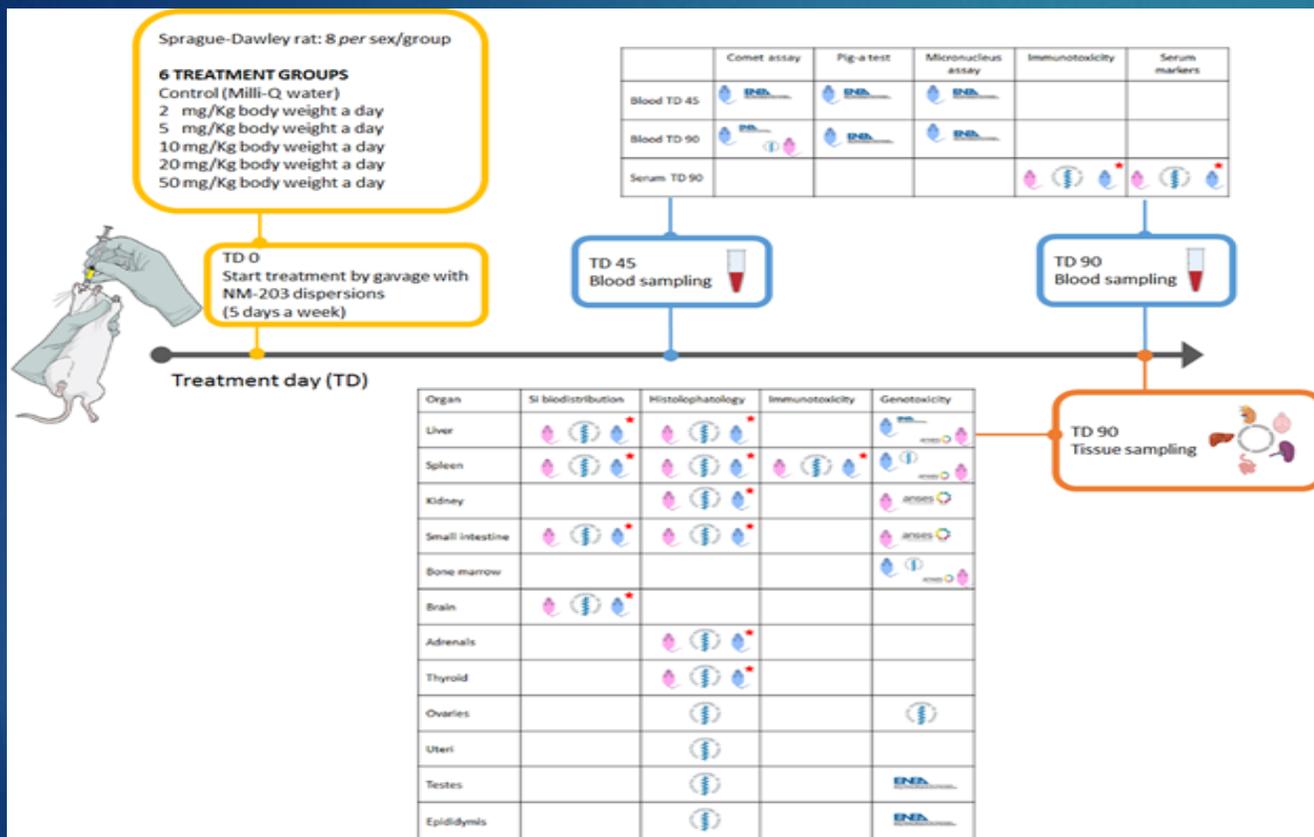


- ▶ altered thyroid tissue and function (reduced T3) in



- ▶ characterisation of sex-endocrine effects should deserve consideration in the safety assessment of nanomaterials;
- ▶ different sex-related susceptibility should be assessed as well

Hazard Identification of pyrogenic synthetic amorphous silica (NM-203) after sub-chronic oral exposure in rat: a multitarget approach



Synthetic amorphous silica (SAS) consists of agglomerates and aggregates of primary particles in the nanorange (<100 nm), food additive authorized in the European Union as E551

- OECD test guideline 408
- Dose levels selected to be as close as possible to the expected human exposure to silicon dioxide used as food additive (E551)
- Endpoints:
- dispersion characterization, tissue distribution, general toxicity, blood/serum biomarkers, histopathological and immunotoxicity

Biodistribution in tissues (brain, liver, spleen, small intestine)
 Agilent 8800 triple quadrupole inductively coupled to plasma mass spectrometer (ICP-QQQ-MS)

BIODISTRIBUTION

significant silicon accumulation was detected in SAS-50 in spleen



only, at SAS-5 in liver and SAS-

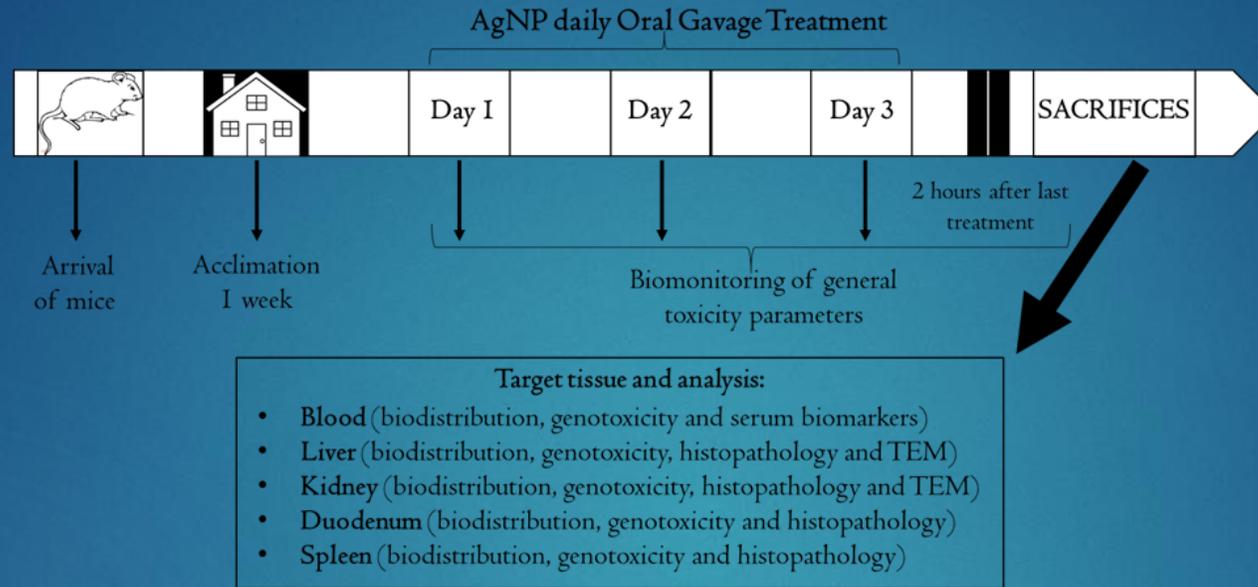


liver histopathological alterations were considered as critical effect and 5 mg/kg bw is proposed as No Observed Adverse Effect Level.



thyroid homeostasis was affected, and a Lowest Observed Adverse Effect Level of 2 mg/kg bw is suggested based on the increase of TSH and CREA serum levels in all treatment groups

Genotoxicity, biodistribution and toxic effects of silver nanoparticles after in vivo acute oral administration



OECD guideline 489



- Dose levels 50, 150, 300 mg/kg bw per day
- AgNP (20 nm) dispersions fully characterized by the manufacturer
- Biodistribution by ICPMS and TEM on liver and spleen (high dose)
- Target tissues: liver, spleen, duodenum and kidney
- Histopathological analysis and Comet assay on blood, liver, spleen, duodenum and kidney
- Micronucleus assay on spleen lymphocytes

BIODISTRIBUTION

AgNPs accumulated in duodenum as first contact site and transferred to other target tissues

No differences between



TEM showed AgNPs in liver and duodenum free in the cytoplasm or included in organelles but never in the nucleus

No genotoxic or tissue damage were recorded by both assays in all the tested tissues

No differences between



were recorded for all the endpoints analysed

- Sex/gender plays many roles in xenobiotic effects
- Gender/sex differences are still inadequately addressed in risk assessment

The results of these studies showed that it is important to test both sexes in the hazard identification of NM in order to provide reliable data for improving the knowledge

Food Chem Toxicol. 2017 Apr;102:63-75. doi: 10.1016/j.fct.2017.01.031. Epub 2017 Jan 31. PMID: 28159593

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THANKS!

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