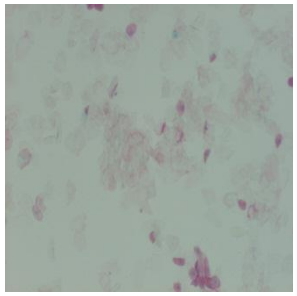
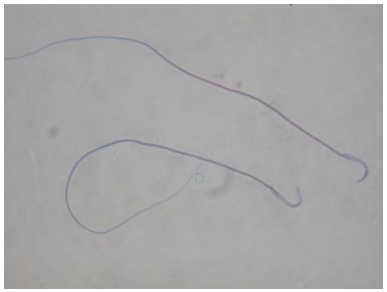
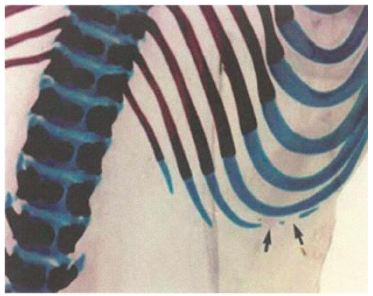
Testing and assessment of reproductive toxicity of nanomaterials

Results of the LRI-N3 project with SiO_2 and ZnO



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Evolution or Revolution – Research Priorities for Future Risk Assessment
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Introduction

- With the increased production and use of manufactured nanomaterials more people are exposed, i.e. workers, consumers and man via the environmental setting.

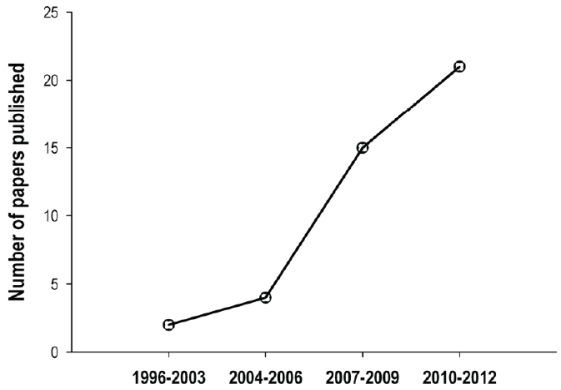
- This has raised concerns about possible implications on human health, including pregnant women.

- Recent studies show that nanomaterials are able to pass the blood-testis barrier and the placental barrier and may affect fertility and prenatal development.

Introduction

- Assessment of potential developmental and reproductive toxicity of nanomaterials is of great importance.

- The number of publications on this subject is increasing but is still limited.



Published papers on reproductive and developmental toxicity of engineered nanoparticles (Campagnolo et al; 2012)

- No studies according to international (OECD) guidelines available.

- Current guidelines on reproductive toxicity have to be evaluated for their applicability to nanomaterials.

CEFIC LRI-N3 project

'Testing and assesment of reproductive toxicity of nanomaterials'

Reproduction toxicity studies with NM-200 synthetic amorphous silica by oral exposure:

- Prenatal developmental toxicity study in Wistar rats (OECD TG 414)
- Two-generation reproduction toxicity study in Wistar rats (OECD TG 416).

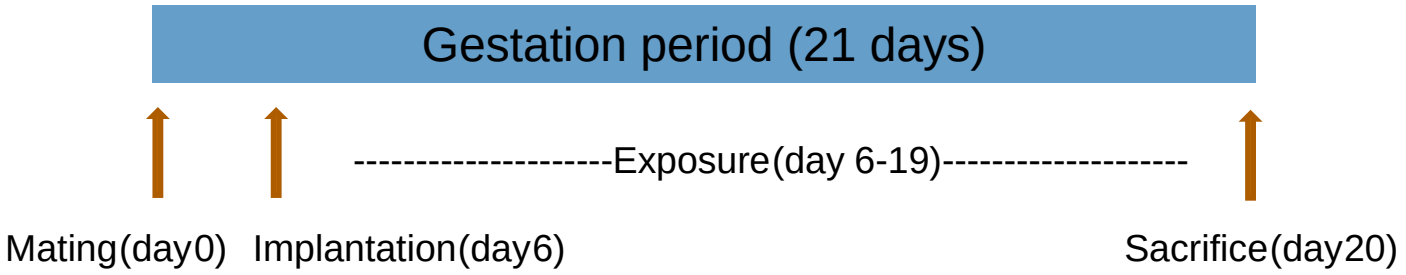
Reproduction toxicity study with Z-Cote HP 1 ZnO via inhalatory exposure:

- Prenatal developmental toxicity study in Wistar rats (OECD TG 414)

Prenatal developmental toxicity study (OECD 414)

(teratogenicity study)

Provides information on potential hazard to the unborn child which may arise from exposure of the mother during pregnancy



Prenatal developmental toxicity study

In life
- Clinical signs and mortality
- Body weight and weight gain
- Food intake

Parental necropsy
- Macroscopic examination
- Full and empty uterus weight
- Ovary weight
- Number of corpora lutea
- Implantation sites
- Early and late resorptions

Fetal observations
- Fetal weight
- Placental weight
- Sex of fetus
- Viability and abnormalities fetuses
- Visceral screening / soft tissues
- Skeletal screening (bone and cartilage)

SiO_2 used in this project:

Name

NM-200 Synthetic Amorphous Silica

Batch

PR-A-2

CAS no.

7631-86-9

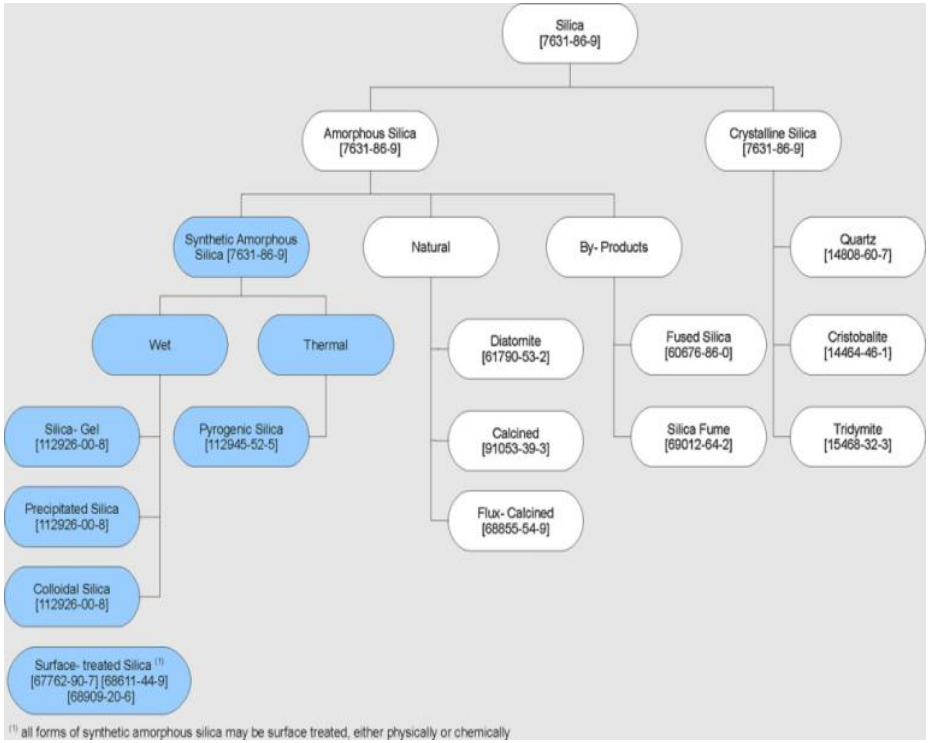
(synthetic amorphous silica)

112945-00-8

(precipitated synthetic amorphous silica)

Purity

Silicon dioxide as SiO_2 96.5%



The various silica forms (Fruijtier-Pölloth, 2012)

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Project code 10R024

Prenatal developmental toxicity study with SiO_2

Animals
25 pregnant female Wistar rats per group

Dose level
0, 100, 300 and 1000 mg/kg body weight/day

Route
Gavage (10 ml/kg body weight)

Dosing period
Gestation days 6-19

Vehicle
10% fetal bovine serum (FBS)

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TNO innovation for life

Dispersion of SiO_2 in 10% FBS

- NM-200 gently homogenized manually in highly deionzed water
- Sonification with a probe sonificator for 5 min at 100 Watt
- Vortex 10 sec.
- Sonification 5 min 100 Watt
- 10% fetal bovine serum added
- Sonification 5 min 100 Watt

- Magnetic stirring during treatment period to keep preparations homogeneous

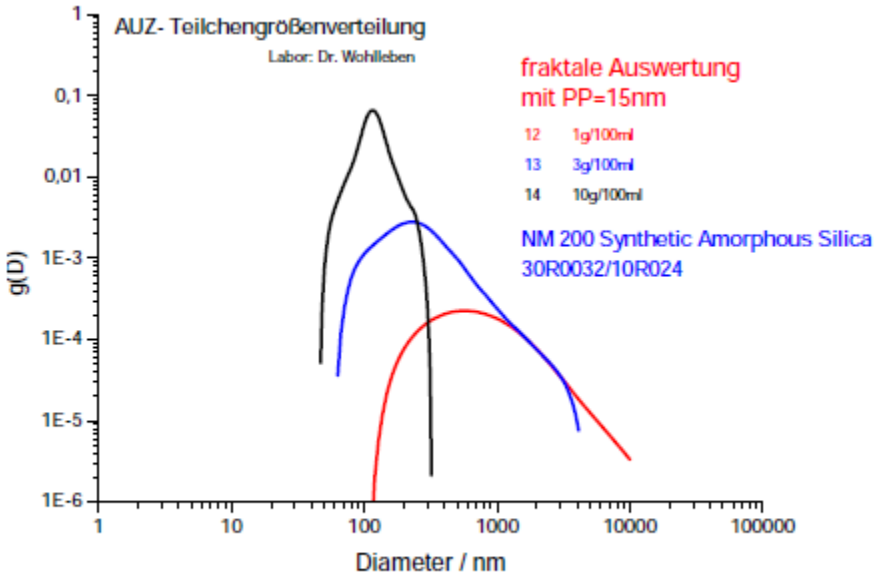
BASF
The Chemical Company

Project code 10R024

Analysis of size distribution of SiO_2 in 10% FBS

Method:
Analytical ultracentrifugation (AUC)

Mean diameter:
1 g/100 ml: ≈ 350 nm
3 g/100 ml: ≈ 230 nm
10g/100 ml: ≈ 100 nm*
*concentration too high for proper measurement, greater particles were sedimented before measurements started.



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Results of the prenatal toxicity study with SiO_2

- No effects on maternal observations

  (clinical obs., food consumption, body weights, reproductive organ weights and necropsy observations)

- No effects on reproduction data

  (conception rate, number of corpora lutea and implantations, pre- and post-implantation loss, number of resorptions)

- No effects of fetuses

  (viability, sex, fetal weights, external, skeletal- and visceral observations)

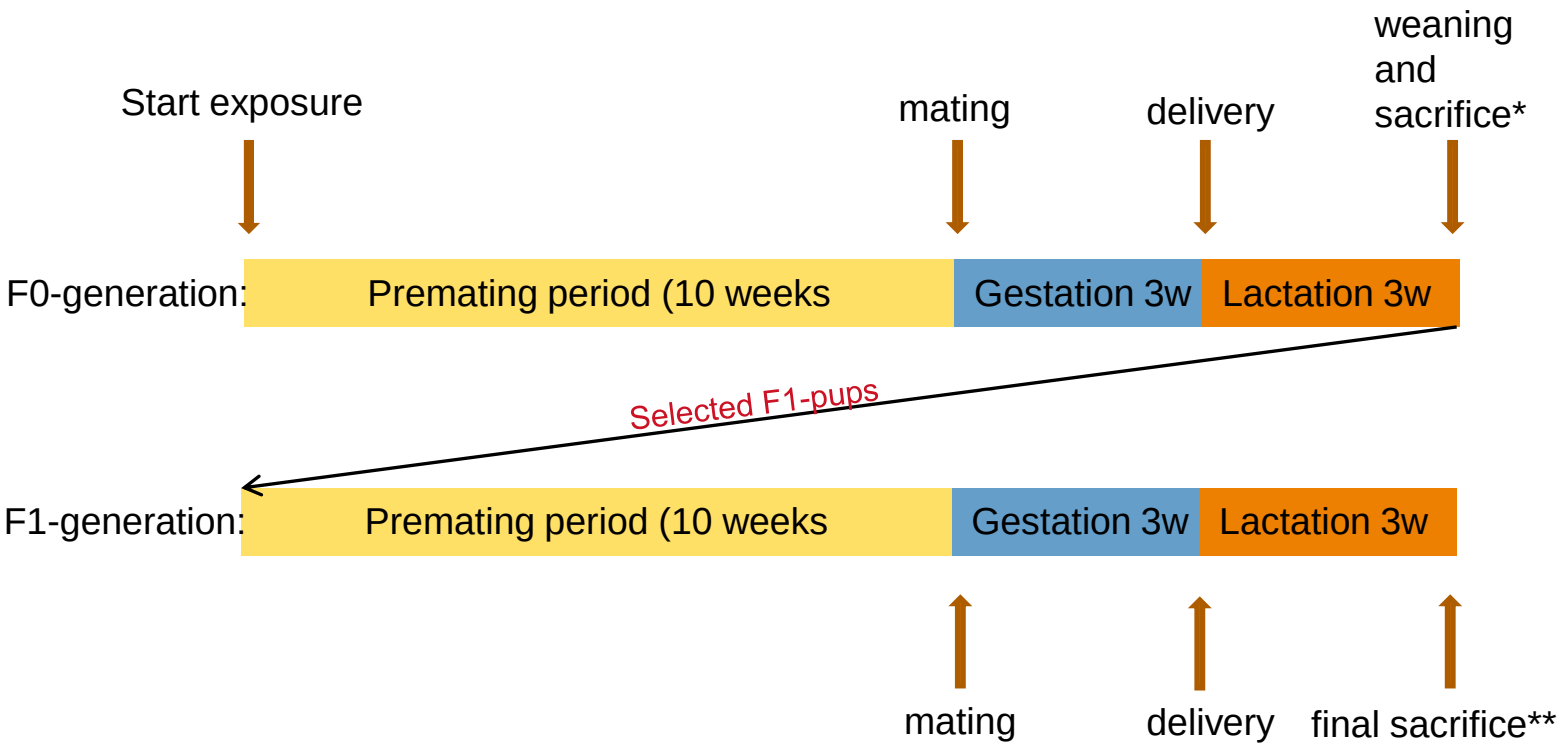
Conclusion prenatal developmental toxicity study with SiO_2

- Under the conditions of this study no test substance related adverse effects were observed at any of the tested doses.

- The No Observed Adverse Effect Level (NOAEL) for maternal and prenatal developmental toxicity is ≥1000 mg/kg body weight/day.

Two-generation reproduction toxicity study (OECD 416)

Provides information on potential hazard on reproductive performance and on the growth and development of the offspring into adulthood.



Two-generation reproduction toxicity study

In life

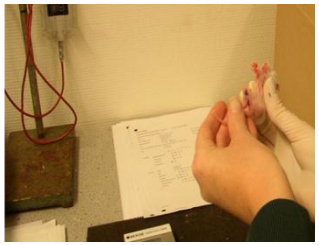
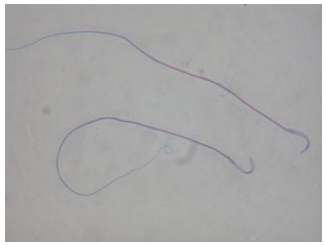
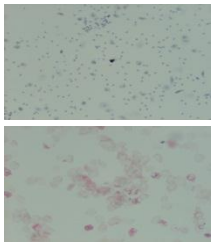
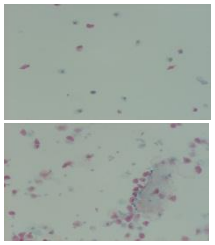
- Clinical signs and mortality
  Body weight and weight gain
- Food intake
- Estrus cycle
- Fertility indices
- Duration of gestation
- Parturition evaluation
- Dams with dead pups/litters

Pup observations

- Clinical signs and mortality
- Litter size
- Body weight and weight gain
- Physical/sexual development
- Necropsy: weight of brain, thymus, spleen
- Pups were stored for SiO_2-analysis

Parental necropsy

- Weight, macroscopy and microscopy of (reproductive) organs
- Number of implantation sites
- Sperm parameters
- Organs, blood, breast milk, urine and faeces were sampled for future quantitative and qualitative SiO_2-analysis.

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Project code 9127

Two generation reproduction toxicity study with SiO_2

Animals

28 male and 28 female Wistar rats per group

Dose levels

0, 100, 300 and 1000 mg/kg body weight/day

Route

Gavage (10 ml/kg body weight)

Dosing period

10 weeks premating, mating, 3 weeks gestation, 3 weeks lactation per generation

Vehicle

0.5% methylhydroxypropylcellulose (MHPC)

TNO innovation for life
Project code 9127

Dispersion of SiO_2 in 0.5% MHPC

- NM-200 in 0.5% MHPC*(in highly deionized water)
- Magnetic stirring (900 rpm) for at least 60 minutes before start of dosing.
- Magnetic stirring during dosing period to keep preparations homogeneous

*: methylhydroxypropylcellulose, this verhicle/method was the same as used in CEFIC-LRI N1 project after discussions with G Lewin and J Buschmann (Fraunhofer ITEM)

TNO innovation for life

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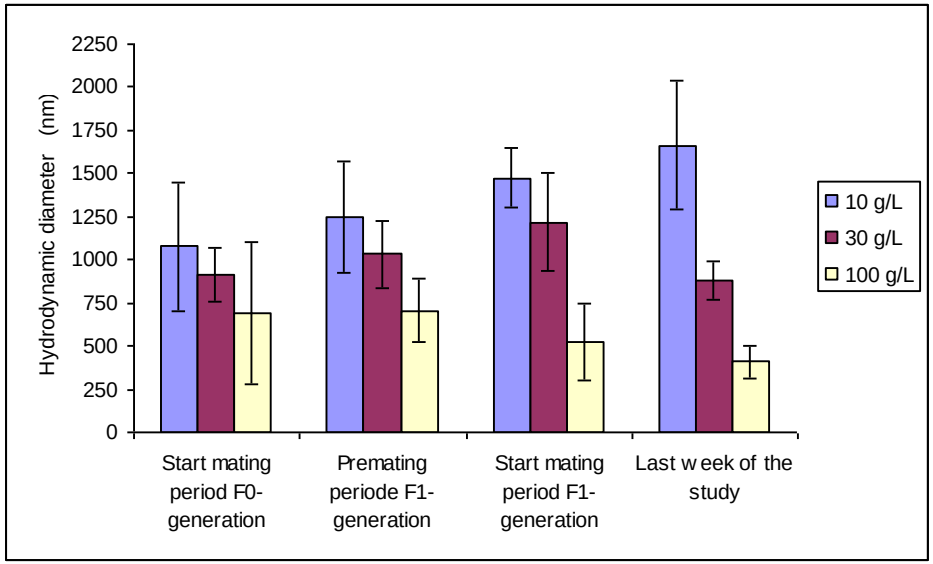
Analysis of size distribution of SiO_2 in 0.5% MHPC

Method:
Dynamic light scattering (DLS)

Mean diameter:
1 g/100 ml: ≈ 1350 nm
3 g/100 ml: ≈ 1000 nm
10g/100 ml: ≈ 450 nm*
*concentration too high for proper measurement, greater particles were aggregated and sedimented as well as multiple-scattering due to high number of particles.



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Results of two-generation reproduction toxicity study with SiO_2

- No effects on maternal observations

  (clinical obs., food consumption, body weights, organ weights, necropsy observations and pathology)

- No effects on reproduction data

  (mating and fertility parameters, oestrus cycle parameters, sperm parameters, dams with dead and or alive pups, implantation sites)

- No effects on development of pups

  (viability, sex, fetal weights, clinical- and necropsy findings)

Conclusion of the two-generation reproduction toxicity study with SiO_2

- Under the conditions of this study no test substance related adverse effects were observed at any of the tested doses on the reproductive performance of rats or on the growth and the developmental of the offspring into adulthood.

- The No Observed Adverse Effect Level (NOAEL) for maternal toxicity and for effects on fertility and development is ≥1000 mg/kg body weight/day.

BASF
The Chemical Company

Project code 06I005

Zinc oxide used in this project

Name

Z-Cote HP1

Batch

CNHK1402

Exposure

Repeated inhalation from gestation day 6-19 (6 hours/day)

Concentration

0, 0.3, 1.5 and 7.5 mg/m^3

BASF
The Chemical Company

Project code 06I005

Characterization of Zinc oxide – Z-Cote HP1

Analysis of concentration and particle size distribution in air

Target conc. mg/m³	Measured conc. mg/m³	Median diameter (nm) (WELAS-method)	Median diameter (nm) (SMPS-method)
0.3	0.24 ± 0.06	323	138
1.5	1.71 ± 0.66	310	115
7.5	7.78 ± 0.43	319	131

The targeted aerosol concentrations were met and the particle sizes of the aerosol in the inhalation atmosphere were within the respirable range.

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The Chemical Company

Project code 06I005

Results of the prenatal toxicity study with ZnO

- Maternal observations in 7.5 mg/m^3 group

  (increased lung weights (up to 47%), moderate alveolar lipoproteinosis and slight inflammation in all animals, slight haemorrhages in 30% of all animals))

- No effects on reproduction data

  (conception rate, number of corpora lutea and implantations, pre- and post-implantation loss, number of resorptions)

- No effects of fetuses

  (viability, sex, fetal weights, external, skeletal- and visceral observations)

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Project code 06I005

Conclusion prenatal developmental toxicity study with ZnO

- Under the conditions of this study histopathological adverse effects were observed in lungs of animals of the 7.5 mg/m^3 group.
- The No Observed Adverse Effect Concentration (NOAEC) for maternal toxicity is 1.5 mg/m^3
- The No Observed Adverse Effect Concentration (NOAEC) for developmental toxicity is 7.5 mg/m^3

General conclusion - 1

The studies presented are – to the best of our knowledge – the first reproductive toxicity studies with SiO_2 and ZnO performed according to OECD TG 414 and 416 (and in compliance with GLP)

Choice of vehicle and method of dispersion influences size distribution of nano-materials.

Optimal vehicle for size distribution may be different than the optimal vehicle suitable for long-term (toxicity) studies with animals.

Analysis of size distribution is of great importance (‘know your test dispersion’) and takes a lot of your resources.

Conclusion - 2

- Following OECD TG 414 and 416, NM-200 Synthetic Amorphous Silica had no effect on prenatal developmental and on the reproductive performance of rats nor on the growth and the developmental of the offspring into adulthood up to dose levels of 1000 mg/kg body weight/day

- Following OECD TG 414, Z-Cote HP1 had no effect on prenatal developmental up to concentrations of 7.5 mg/m^3

Conclusions - 3

In our experience, the existing OECD TG's 414 and 416 appear suitable for assessing safety of nanomaterials, with the following considerations:

- Dispersion of nanomaterials in a vehicle and characterization of the nanomaterials and dose solutions is essential and will take more resources than for traditional chemicals.
- Assessment of the bio-distribution of the nanomaterials may be useful to increase confidence in the outcome of the study, especially when no adverse effects are found. It should be realized that qualitative assessment is a real challenge.

These considerations may well be applicable to other OECD guidelines.

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