Acute Inhalation Studies Part 2: Non-specific particle effects now trigger classification?

Nils Krueger – Evonik Operations GmbH

Particle & Health 20-21 October 2021

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Regulatory Background

Exposure route – dust [mg/m³]	Concentration ranges	Label
Category 1 (H330 Fatal if inhaled)	0 < Cat 1 ≤ 50 mg/m³	
Category 2 (H330 Fatal if inhaled)	50 < Cat 2 ≤ 500 mg/m³	
Category 3 (H331 Toxic if inhaled)	500 < Cat 3 ≤ 1000 mg/m³	
Category 4 (H332 Harmful if inhaled)	1000 < Cat 4 ≤ 5000 mg/L	

Acute inhalatiion studies * from hydrophobic **surface treated Synthetic Amorphous Silica (SAS)** show mortality at high particle concentration of 450 mg/m³ (4 mg/m³ = AGW-Value of the German TRGS 900 / 2006)

RAC made a proposal for classification **:

 Acute Tox Cat.2 H 330: Fatal if inhaled (and  STOT RE 2 - that will not be discussed in this presentation)

*HMDZ surface treated SAS: CAB-O-SIL® TS-530 fumed silica, Cabot 1994b; HDK SKS130, Wacker 1996; HDK SKS300, Wacker 1996
other surface treated SAS: CAB-O-SIL® TS-610 fumed silica, Cabot 2000;
** based on read-across to CAB-O-SIL® TS-610 fumed silica; Cabot 1994a (surface treated with Dimethyldichlorosilane (DDS))

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Expert statement on existing studies recommends no classification:

Existing OECD standard acute inhalation studies* with high concentrations of hydrophobic surface treated SAS show:

- **Clinical effects:** of suffocation distress and lethality
- **Macroscopical effects**: Lumps of white particles and slime in the nose, white powder in the nasal cavity and "Petechiae" (focal haemorrhages) on the lung surface (= Tardieu spots/ indicator for suffocation)

Conclusion

The clinical and macroscopical effects observed in acute inhalation studies with high concentrations are strong indicator for **physical obstruction** associated with suffocation, against this background **no classification is warranted**.

*HMDZ surface treated SAS: CAB-O-SIL® TS-530 fumed silica, Cabot 1994b; HDK SKS130, Wacker 1996; HDK SKS300, Wacker 1996
other surface treated SAS: CAB-O-SIL® TS-610 fumed silica, Cabot 2000;
CAB-O-SIL® TS-610 fumed silica; Cabot 1994a (surface treated with Dimethyldichlorosilane (DDS))

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Physical obstruction/suffocation at high particle concentrations is considered in OECD guidance

OECD Guidance 39 (2018) para 51:

"At very high concentrations, ***dry powder aerosols*** *and chemically reactive liquid aerosols (e.g., polymers)* ***tend to form conglomerates*** *in the* ***proximal nose causing physical obstruction*** *of the animals' airways (e.g., dust loading) and impaired respiration* ***which may be misdiagnosed as a toxic effect***".

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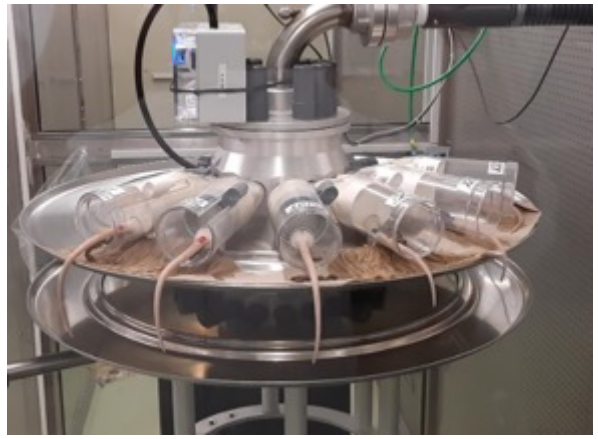
However, OECD Standard protocols for acute inhalation studies do not consider physical obstruction especially of the proximal nose/nasal cavity , they are limited to:

- Clinical observations
- Count of dead animals
- Macroscopical examination of **outer surfaces of the organs** in the abdominal and thoracal cavity



ASASP decided definitive evidence for physical obstruction is required to address OECD Guidance 39 (2018) para 51

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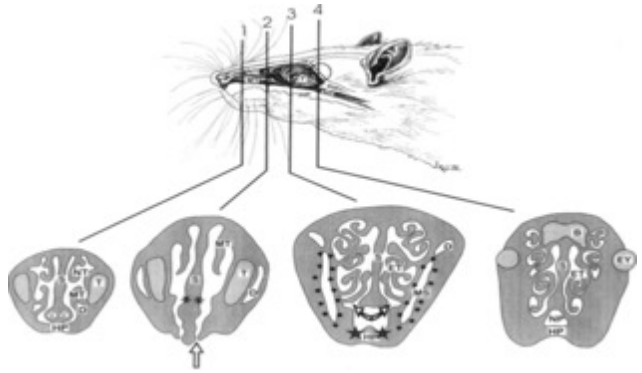
Therefore, more robust GLP acute inhalation studies were initiated, including in-depth aerosol characterization, to identify possible areas of obstruction (cooperation Fraunhofer, AnaPath & Uni Dresden)

- **Histopathology: rat is an obligatory nose breather**, examination of the entire respiratory tract incl**. proximal nose /nasal cavities)**, Larynx, Trachea and Lung
- **blood oxygen**



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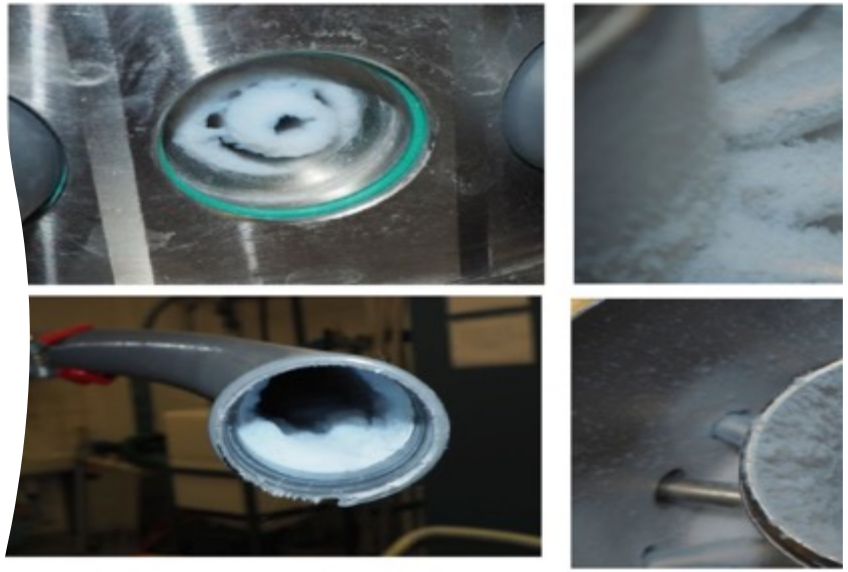
Important part of these new nose only inhalation studies is the Aerosol generation presented in Part 1 by Jürgen Nolde:

Key results from Aerosol generation at high concentration :

- Extreme coating of inhalation test unit by agglomeration
- Strong re-agglomeration in the inhalation system



500 mg/m³ the highest technically-feasible concentration without significant alterations of the aerosol characteristics were chosen **to compare** effects of **7 different forms of particulate substances, hydrophobic and hydrophilic**

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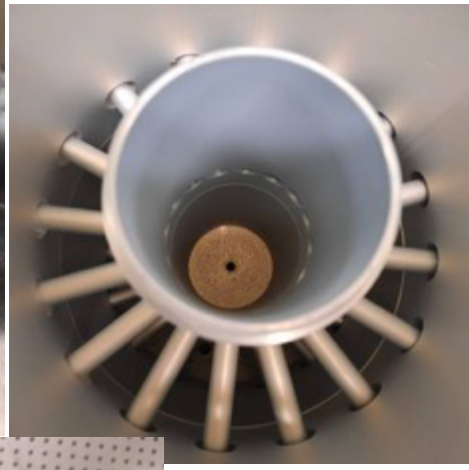
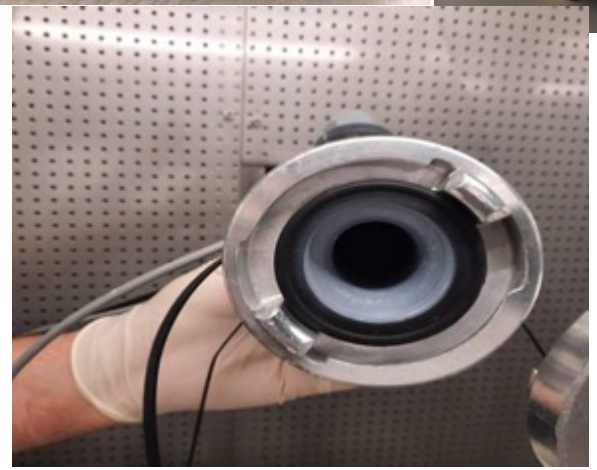
The following slides show the new results of hydrophobic HMDZ surface treated SAS only, as the results of the other 6 particulate substances of this research project are not finalized yet

New Results from acute inhalation study with HMDZ surface treated SAS

Aerosol

- a stable aerosol of 500 mg/m³was generated
- only slight coating of test unit
- No strong reagglomeration in the inhalation system observed





New Results from acute inhalation study with HMDZ surface treated SAS

Clinical observation

- No clinical observations in the first two hours in all six animals (rats did not show any stress symptoms)
- After 3 hours higher breathing frequency, first animal died, two animals died shortly afterwards
- Experiment was stopped three surviving animals were transferred into a cage, these animals showed severe signs of respiratory distress (Cheyne-Stokes leading into preterminal gasping) and were euthanized

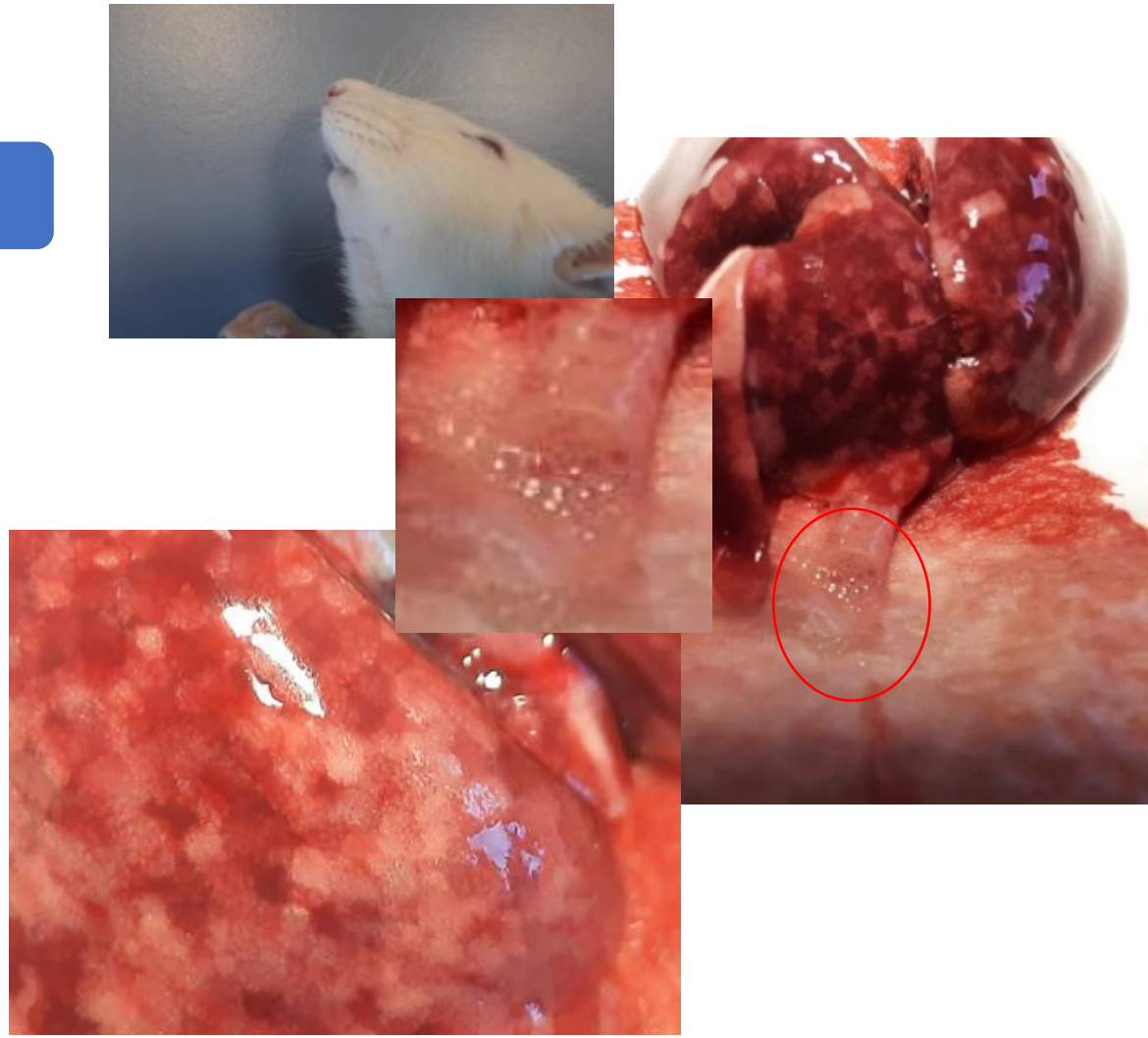
Blood oxygen

- Based on mortality or bad condition of the animals blood oxygen determination was not possible

New Results from acute inhalation study with HMDZ surface treated SAS

Necropsy

- The outer surface of the nose showed only minimal contamination with HMDZ surface treated SAS
- Lung showed congestion with edema, acute emphysema and hemorrhages/petechiae
- when opening the trachea slimy, transparent foam was observed



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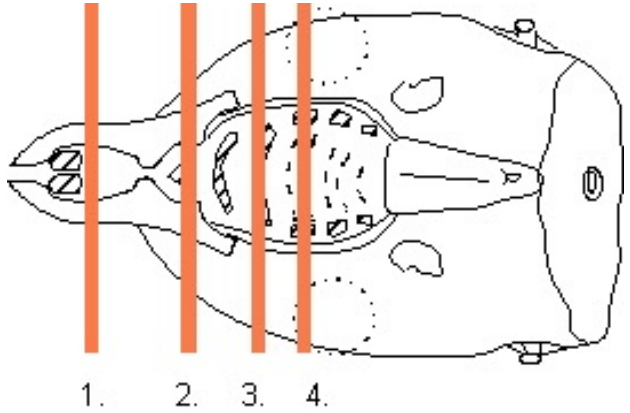
New Results from acute inhalation study with HMDZ surface treated SAS

Histopathology Processing

- Respiratory tract (lung) was fixed in formalin followed by standard processing and wax embedding for paraffin sections
- nasal cavities of three animals were kept in **frozen state to avoid loss of material** and diamond sawed and dried for EDX analysis (Platinum sputtering)
- nasal cavities of three animals were emerged in 100% ethanol and embedded in methyl methacrylate resin before being diamond sawed
- Nasal cavities were trimmed following scheme (see below) by Kittel et al. (2004)



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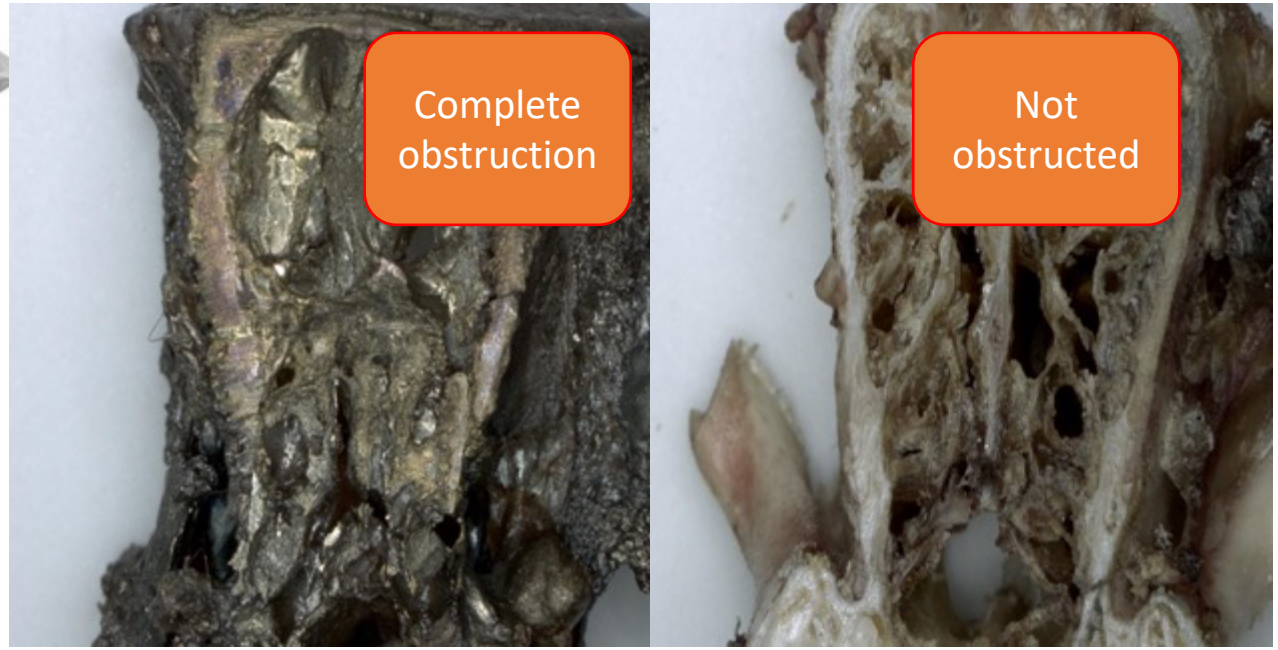
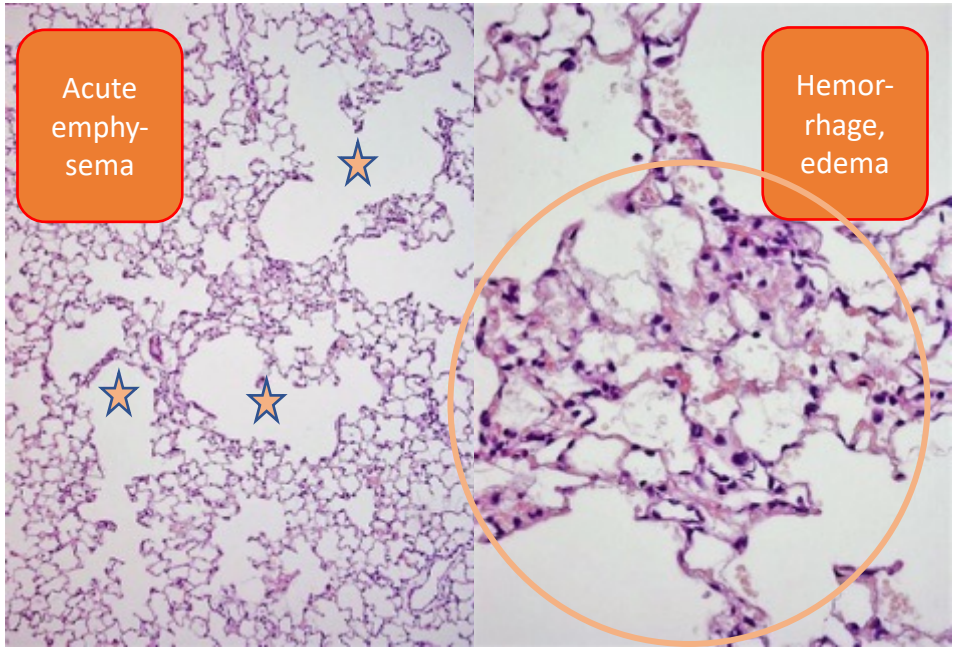
New Results from acute inhalation study with HMDZ surface treated SAS

Histopathology Results

Nasal cavity Level 4/frozen section



Lung/paraffin section



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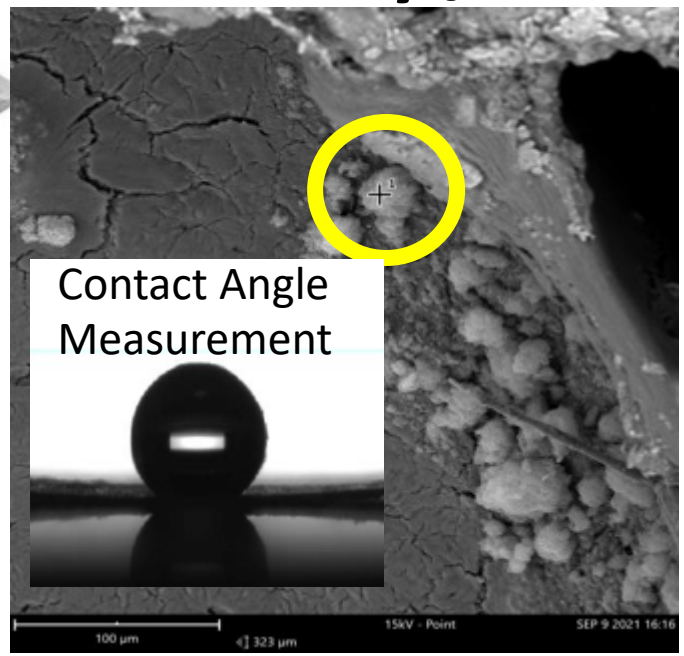
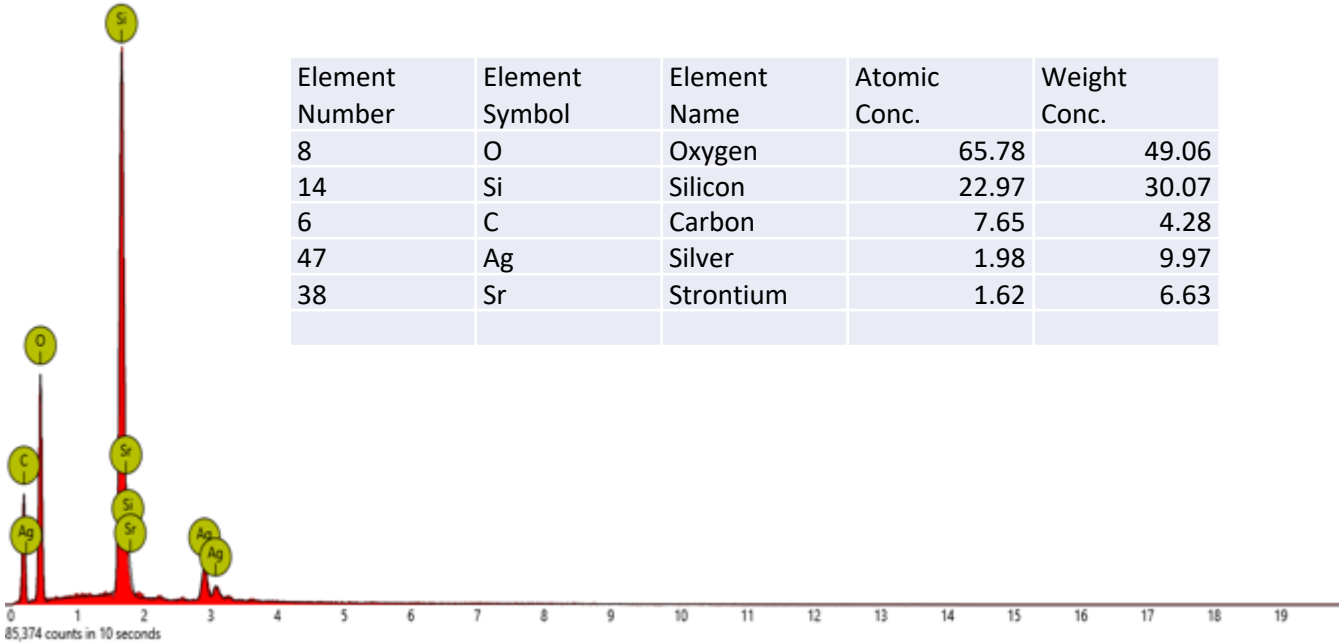
New Results from acute inhalation study with HMDZ surface treated SAS

EDX & Contact Angle Measurement

Nasal cavity /Level 2





Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	65.78	49.06
14	Si	Silicon	22.97	30.07
6	C	Carbon	7.65	4.28
47	Ag	Silver	1.98	9.97
38	Sr	Strontium	1.62	6.63

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New Results from acute inhalation study with HMDZ surface treated SAS

Discussion

Based on long term and in vitro studies no specific toxicity of surface treated SAS identified:

- Repeated dose inhalation studies (90 days) with hydrophobic surface treated SAS showed compared with plain pyrogenic SAS less severe, completely reversible lung changes (Reuzel et al 1991, Weber et al 2018).
- HMDZ surface treated SAS showed less activity in Macrophage assay compared with plain SAS (Wiemann not published)
- Results of the new acute inhalation studies clearly show that mortality is caused by complete mechanical obstruction of the nasal cavity by agglomeration of hydrophobic particulate substances.

New Results from acute inhalation study with HMDZ surface treated SAS

Conclusion

- In rat as obligatory nose breather, mortality in acute inhalation studies is caused by extreme high particle concentration (500 mg/m³) with hydrophobic particulate substances associated with mechanical obstruction of the nasal cavities and consequently suffocation by interruption of the gas exchange.
- Non-specific obstruction resulted in the observed secondary effects in the lung: congestion, edema, acute emphysema and petechiae .
- High frequency breathing/preterminal gasping in combination lung fluid resulted in foam generation in the lung.

Thank you for your attention
Any questions?

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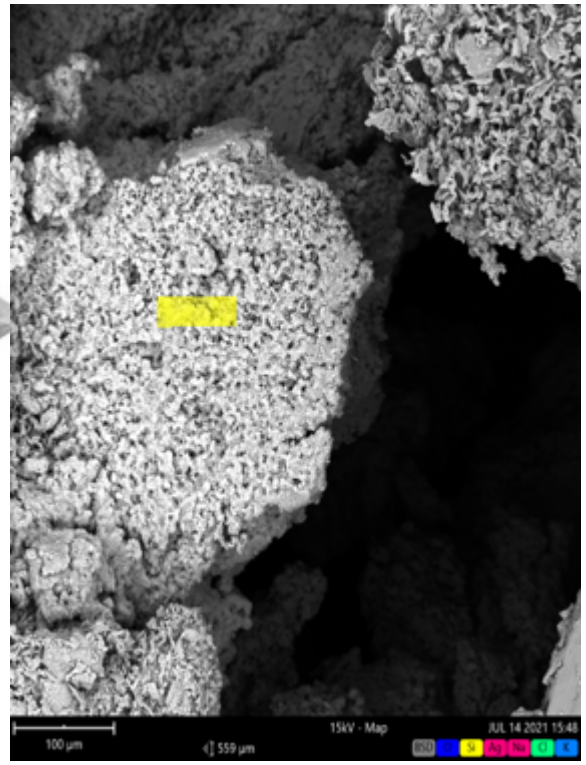
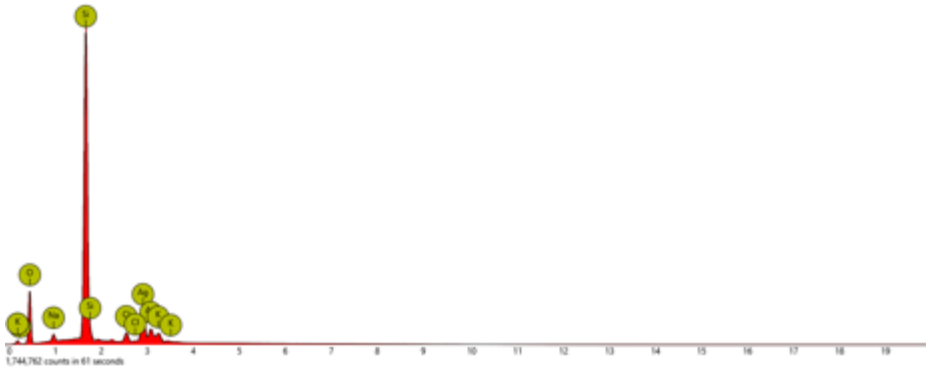


Combined map				
Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	54.82	34.70
14	Si	Silicon	35.44	39.38
47	Ag	Silver	4.64	19.82
11	Na	Sodium	2.46	2.24
17	Cl	Chlorine	1.43	2.00
19	K	Potassium	1.20	1.86

FOV: 559 µm, Mode: 15kV - Map, Detector: BSD Full, Time: JUL 14 2021 15:48



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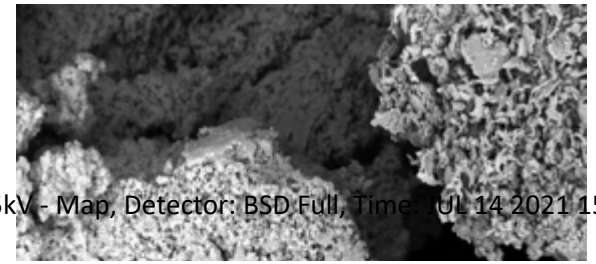
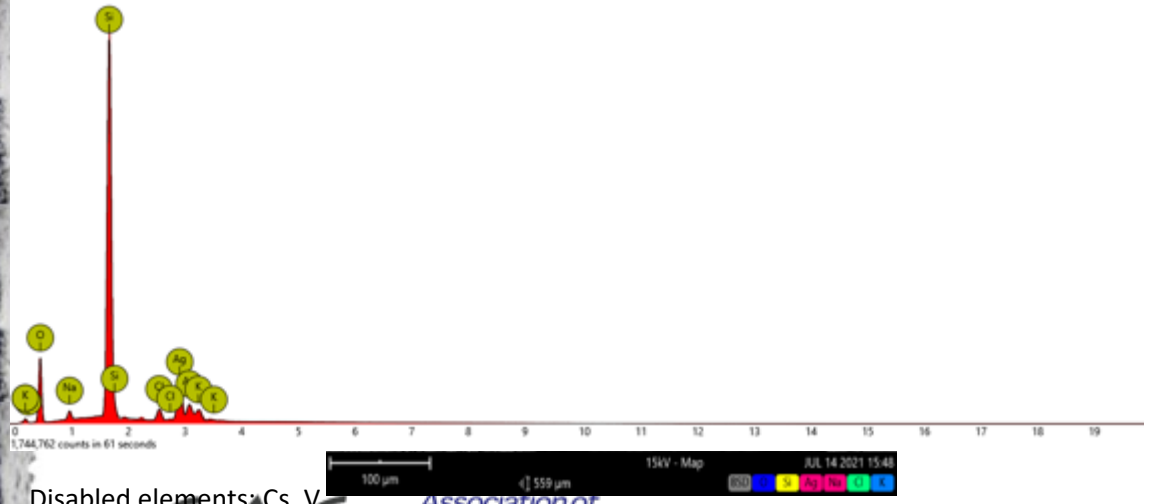
Frozen material

1. map



FOV: 559 µm, Mode: 15kV - Map, Detector: BSD Full, Time: JUL 14 2021 15:48



Disabled elements: Cs, V

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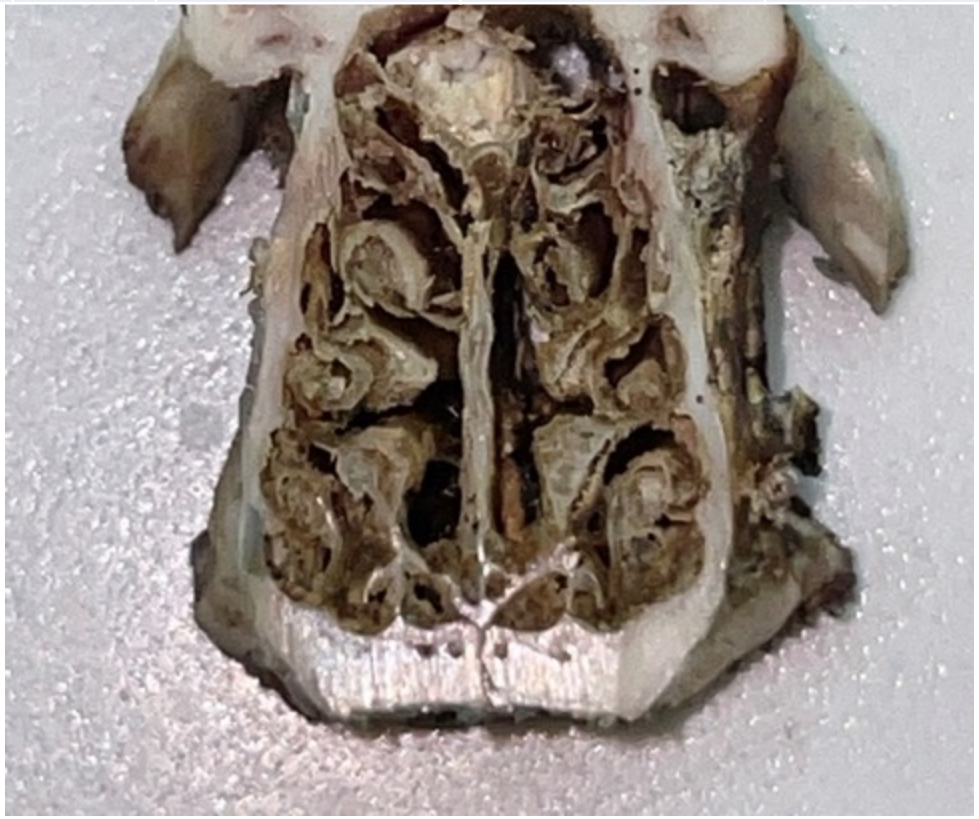

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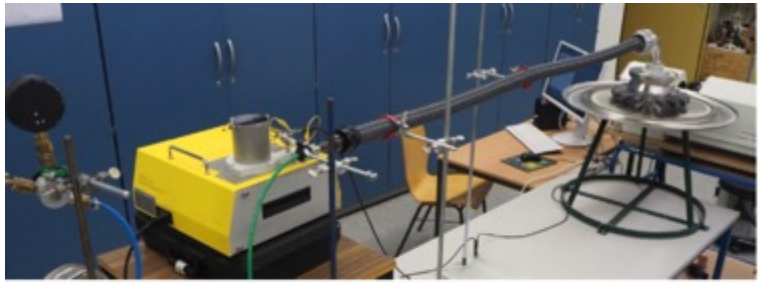
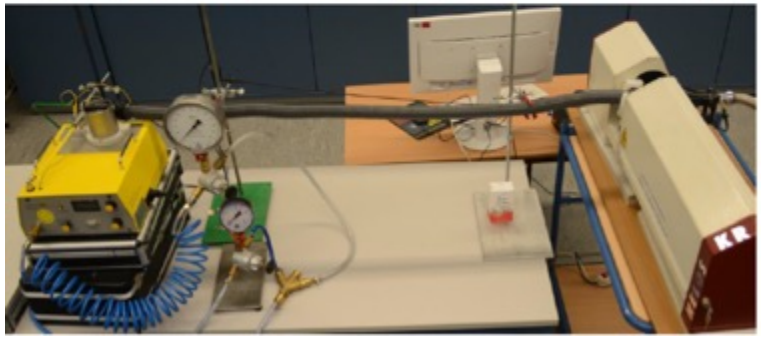
Mechanistic study

- To fill the knowledge gaps and improve understanding of in vivo behaviour of low-density powder particulate substances, ASASP members are running a "**mechanistic study".**
- The purpose of this study is to prove, **in the case of lethality**, that the mode of action can be attributed to **suffocation**, a mechanical effect, which has no biological origin.
- According to existing reviewed studies, **signs of suffocation have been identified.**
- This comprehensive study combines **physico-chemical characterization** with thorough **histopathological examination**, providing detailed and unique inputs to understand acute inhalation toxicity effects.





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ASASP urges:

- Not to include the proposed classification of HMDZ SAS in the draft 18th ATP
- Wait for the results of the "mechanistic study" Part 2 – in vivo by end of 2021 which could warrant no acute toxicity classification

OECD Guidance 39 (2018) para 69: *"At very high concentrations, dry powder aerosols and chemically reactive liquid aerosols (e.g., polymers) tend to form **conglomerates in the proximal nose** causing physical obstruction of the animals' airways (e.g., dust loading) and **impaired respiration which may be misdiagnosed as a toxic effect**".*

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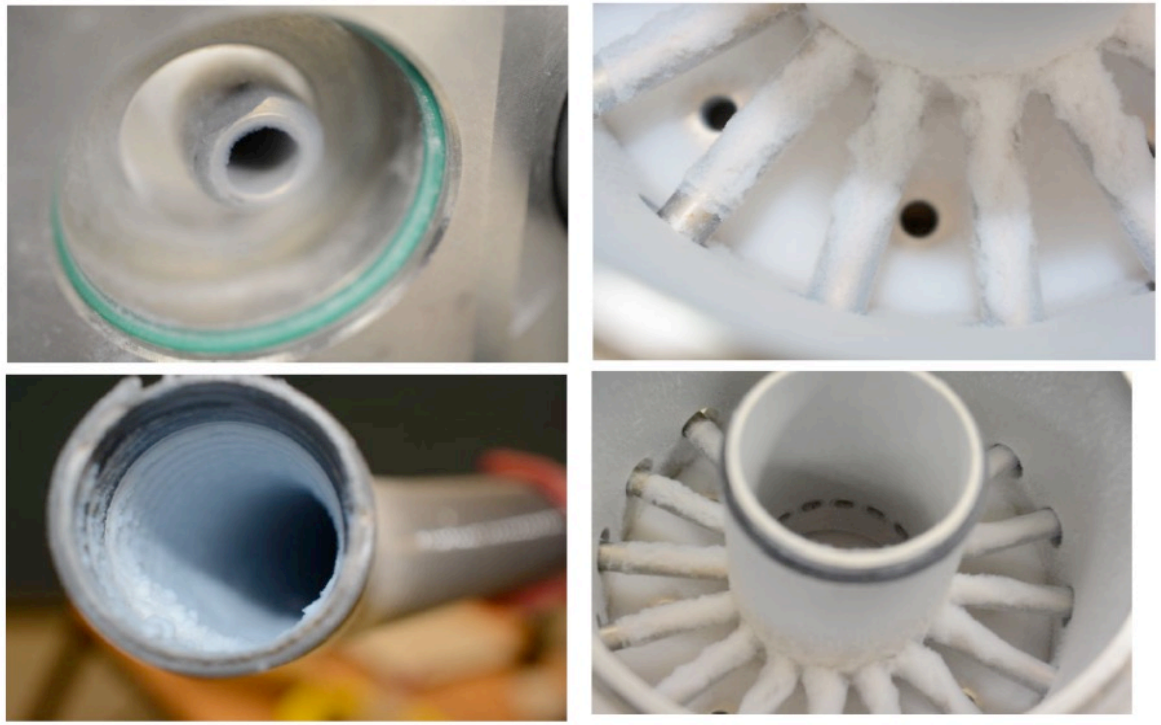
Mechanistic study:
Part 1 characterization/optimization finalised

Commissioned to the University of Dresden, Fraunhofer and Anapath (Sept 2019)

- Extreme coating of test unit by agglomeration
- Formation of larger particles with a high tendency to precipitate are causing mass loss
- Aerosol generation with acceptable altering can only be achieved in concentrations far below Guidance values (500 mg/m^3 or less)



Aerosil® R812 5100 mg/m^3 (HMDZ treated SAS)

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What is SAS, what is HMDZ-SAS?

- An **amorphous** form of silicon dioxide (SiO_2)
- **Non-hazardous** white dry powders
- **Widely used raw material** for many industrial applications & approved ingredient for food, cosmetics and pharma
- Epidemiology studies **confirmed safe handling at workplace** (no reported occupational health incidents)
- **SAS production**:
  - World: 300.000.000 tonnes
  - EU: 500.000 tonnes

Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica

or ***HMDZ-treated SAS*** is:

- **Hydrophobic** SAS through modified surface
- **Same amorphous structure** as other SAS types and forms
- **Low density powder** ("fluffy") substance form; i.e. a small quantity (mass) of powder occupies an extraordinary large volume.
- Shares properties with other hydrophobic SAS, **no toxicological concerns**

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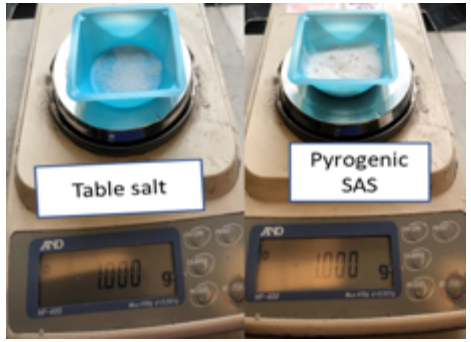

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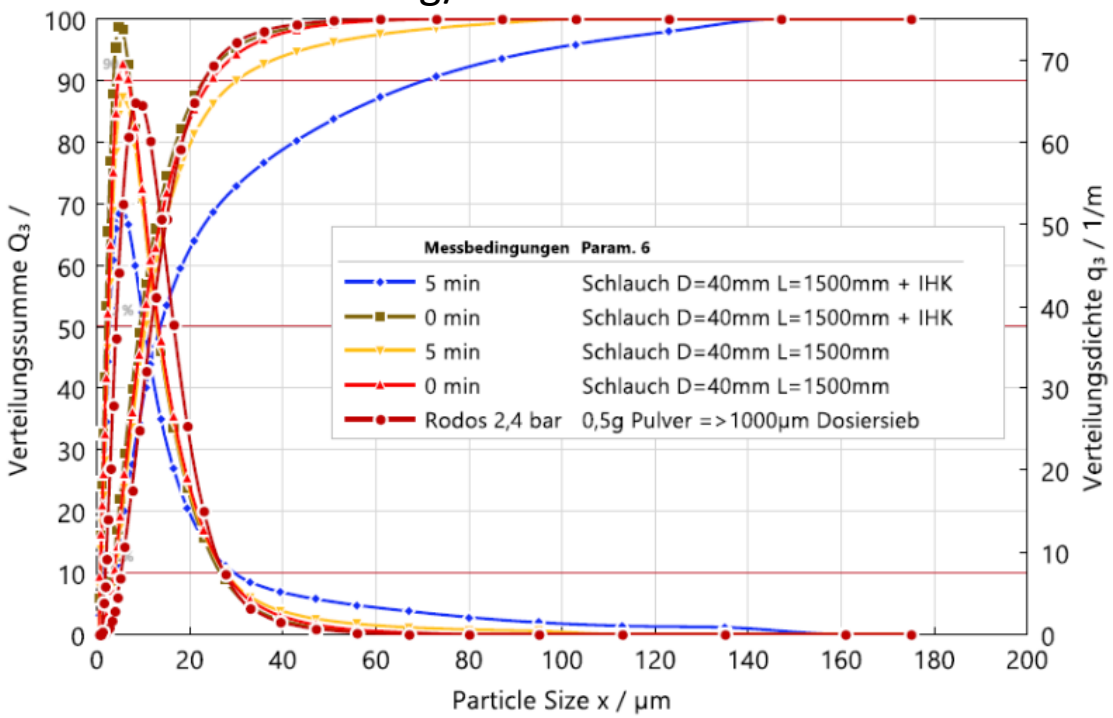
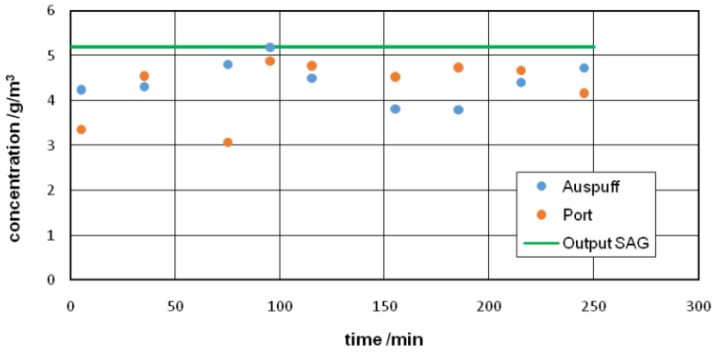
Mechanistic study: results Part 1

Aerosil® R812 5.1 g/m3



Gravimetrische Untersuchungen am IHK
Aerosil R812
SAG410 FR50 (3+1 bar)

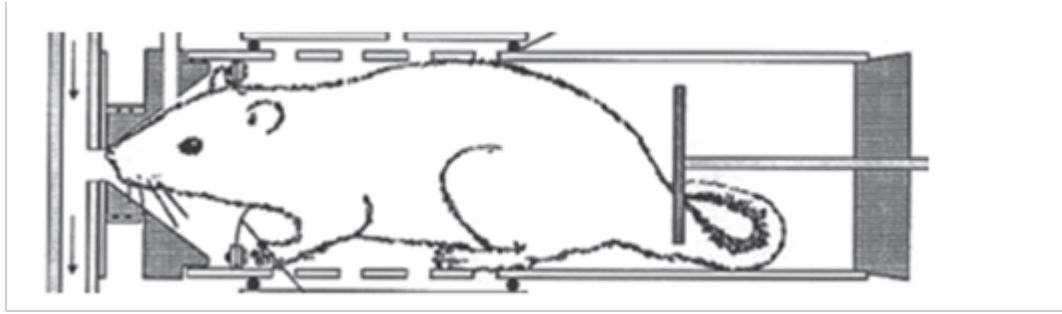


- ✓ At time zero test material forming aerosol inline with the demand of the test guideline
- ✓ Only after 5 minutes the aerosol altering process induced through the amount of particle in the air stream
- ✓ The contact with the surfaces in combination with a laminar airflow leads to agglomeration form coarse agglomerates nor respirable could cause blockage of the upper airways

Ongoing depositing of material inside the test system leads to more material entering than material leaving

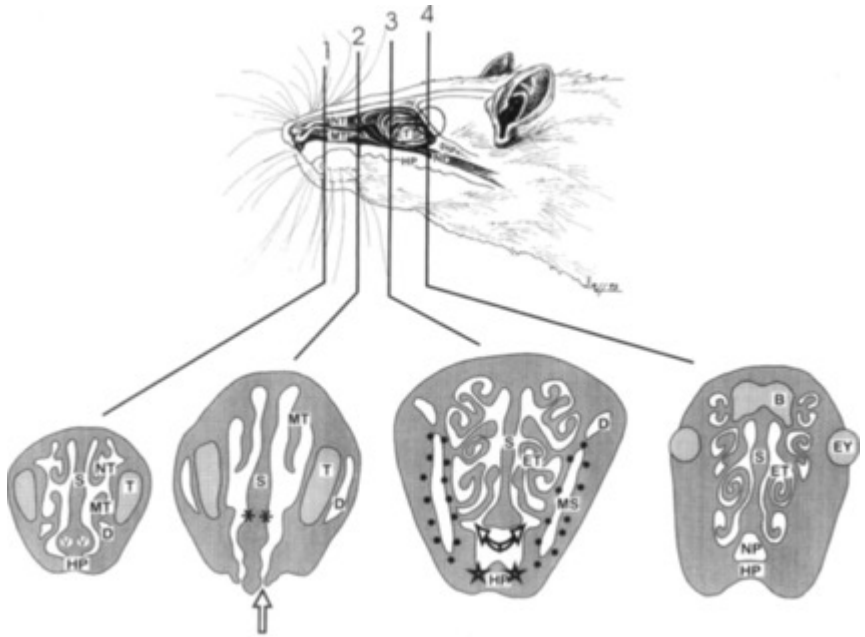
Mechanistic study: Part 2 and next steps

- **Test design Part 2 (in vivo) starts in July**
  - Rats are **obligatory nose breathers**, fixed in a tube (4 hours) - cleaning of the nose is not possible
  - **Exposure to a continuous flow of solid particles most likely causes obstruction** of the nose and/or upper respiratory tract



Generation of new data

- **"Bottlenecks" for particles** in the rat upper respiratory tract (especially nasal cavities) **were never examined** as this is not required in standard protocols for acute inhalation
- **Thorough histopathological examination** of the whole respiratory tract and **blood oxygen monitoring**



End 2021 - Publication on final results of the animal studies

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Mechanistic Study – Timeline

- Atmosphere and Aerosol Generation tests are finalized at TUD* for all test systems.
- Transfer of the inhalation test unit to Fraunhofer
- **For the week(s) 28 and/or 29 (19th to 30th July**) Dry Run Tests at the Fraunhofer set-up are scheduled without rats but with full measurement equipment from TUD and technical TUD staff
- The first two samples, untreated SYLOID® 244 FP and treated AEROSIL® R812 are scheduled for the **week 31 (2nd to 6th August)**, if feasible (but not very likely), the Calcium carbonate sample will be included
- Other samples can be run beginning end of August depending on the technical conditions and successful dry runs in coordination with TUD
- Other SAS samples to be tested: untreated Calcium carbonate, untreated pyrogenic HDK N20, Aluminium oxide (Cabot), untreated precipitated Sipernat® 2200, organic red pigment (Hofmann et all 2018)…
- Study protocol for SYLOID and AEROSIL will be up-dated and distributed
- **Analytical Slot for histopathological examination for week 33/34** to start at AnaPath has been booked

*TUD – Technical University of Dresden Prf. Dr. M. Stintz/Dr. B. Wessely

Study contracted in 2019

TECHNISCHE UNIVERSITÄT DRESDEN

2.3 The results shall be presented to the Client in the form of an interim report by 30.04.2020 and as a final report by 31.08.2020.

Fraunhofer ITEM

7. Duration of binding

Until June 30, 2020

AnaPath

Client

24.10.2019

Date

Signature

Massive delay due to COVID 19

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