National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Toxicity study (WP3)

Aim

- To investigate the toxicity of inhaled turbine engine oil fumes, generated using a Bleed Air Contamination Simulator (BACS) in mice
- Focused on assessment of neurotoxicity and neuroinflammatory effects, because of reported symptoms in humans
- To provide input for health hazard identification (WP1), and future risk assessment

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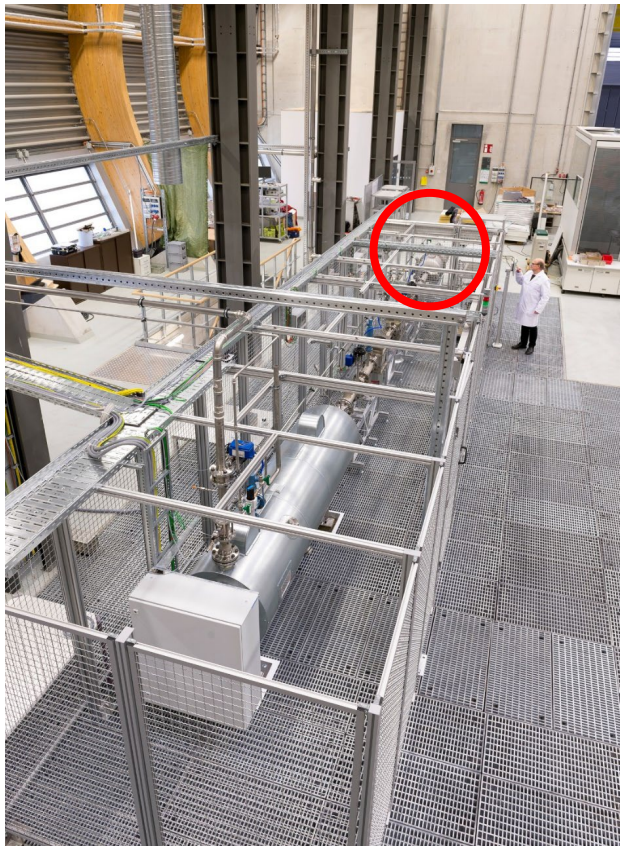
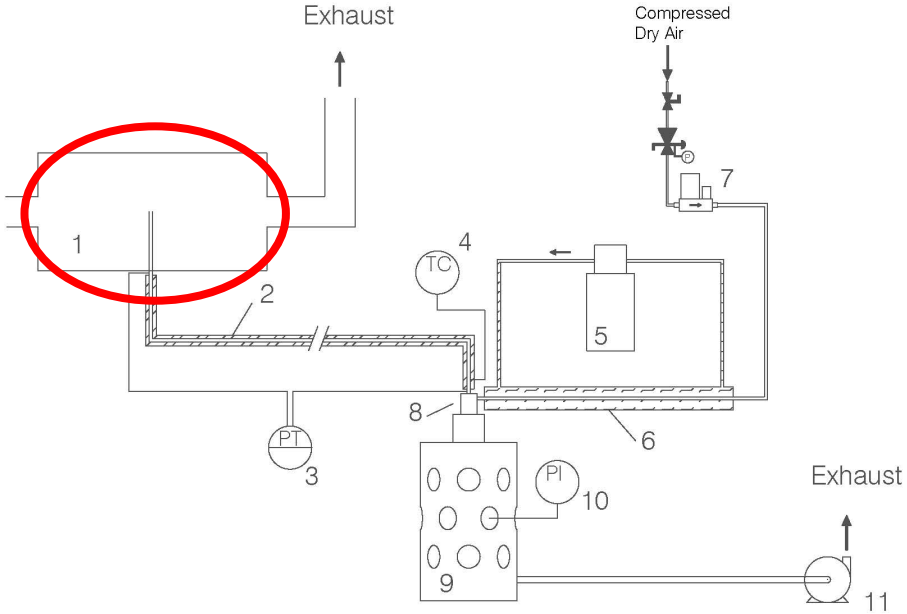
Activities

1. Connection between BACS and exposure unit
2. Setup and testing of equipment and mobile laboratories for inhalation exposure
3. Range finding (RF) study
4. Main toxicity study

Transfer line from BACS to exposure unit





Test setup BACS sample transfer line

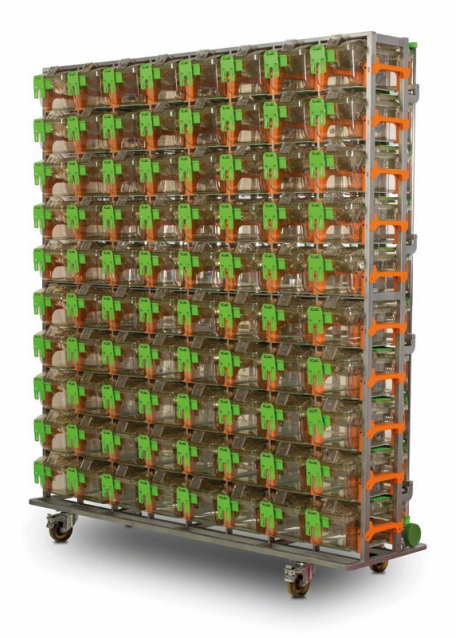
1: BACS sample vessel
2: sample line with electrical heating
3: pressure transmitter
4: temperature controller
5: cooling bath
6: heat exchanger
7: mass flow controller
8: eductor
9: inhalation exposure system
10: pressure indicator
11: pump

- Copper line (Ø 10 mm)
- Controlled at ~50°C
- Flow measurement using pressure difference
- Dilution with cooled clean compressed air (mass flow controlled) using an eductor mounted close to exposure chamber
- Based on flow rates, particles with an aerodynamic diameter up to 2.4 µm can be sampled from BACS without losses. No losses for gases

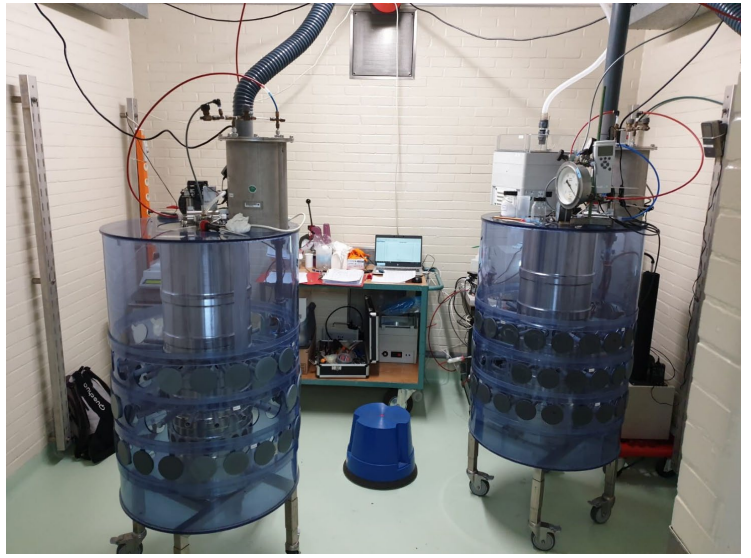
Setup of exposure equipment

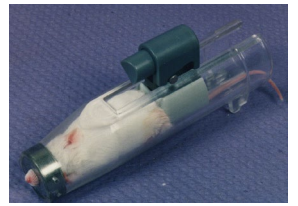
Mobile labs for animal housing and exposure

Digitally Ventilated Cages



Nose-only exposure units





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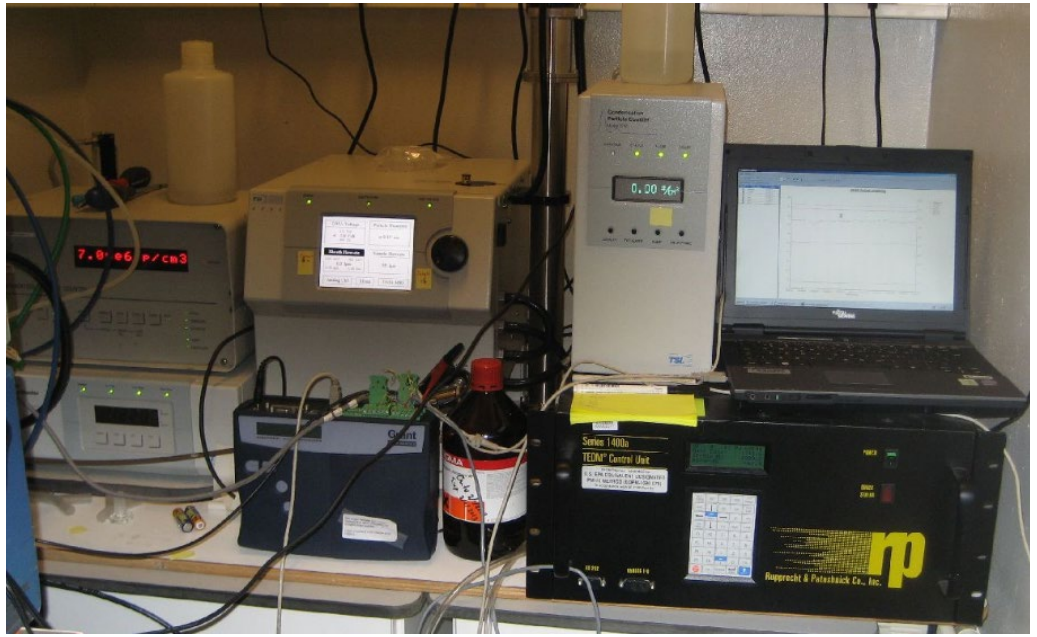
Exposure characterization

- (semi-) continuous measurements:
  - NO, NOx, NO_2, CO
  - VOC's (PTR-MS)
  - Particle number concentration (CPC)
  - Mass concentration (TEOM)
  - Temperature and relative humidity



- Time integrated measurements:
  - Particle size (APS and SMPS)
  - Mass concentration of particles (gravimetrics)

- All measurements in animals' breathing zone

Range finding study (1)

- Aim: select a suitable concentration for the main study, which is tolerated without causing severe distress or adverse effects in the lungs
- Sets of 3 mice exposed to oil fumes + 3 controls (clean air)
- 4-hour exposure, sacrifice on the next day
- Readouts:
  - clinical signs
  - body weight
  - bronchoalveolar lavage (only if no/limited signs are observed):
    - cell differentials, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity

Range finding study (2)

- Criteria for adversity:
  - Clear signs of distress
  - >100% increase in LDH or ALP, accompanied by >10% neutrophils in BAL fluid

- Starting concentration: 50 mg/m^3 (nominal)
  - Maximum achievable oil fume concentration in cabin under worst case conditions
  - Subsequent groups exposed to higher/lower concentrations based on observed toxicity
  - 3 to 10-fold steps in concentration depending on severity of observations

Range finding study (3)

- Optimize concentration using sets of 3 males + 3 controls
- Followed by (sets of) 3 females + 3 controls
  - Though no major sex differences are expected, the concentration may be adjusted slightly
- Confirmation: additional group of 3 males + 3 females (and 3+3 controls) exposed at optimized concentration
  - Acetyl- and butyrylcholinesterase (AChE / BChE) analysis as possible biomarker for exposure: pre-exposure (baseline) and directly after exposure

Main toxicity study

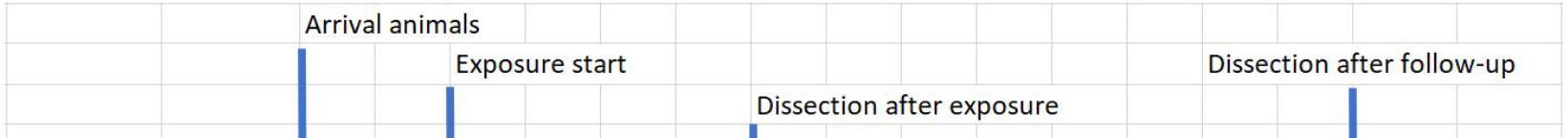
- Design based on OECD guideline 412 – Sub acute inhalation study

- Exposure (nose-only): 5 days/week for 28 days + 8 weeks follow-up
  - Oil fume (3 groups): single concentration for 25, 80 or 240 min/day
  - Control group: clean air (240 min/day)
  - Positive control group: diesel engine exhaust (DEE, 3-5 mg/m^3, 240 min/day)

- C57BL/6N mice, 10 males + 10 females per group

- Sacrifice:
  - 1 day after exposure (sub-acute effects)
  - 8 weeks after exposure (delayed effects and/or recovery of effects)

Main toxicity study – study design



Exposure	Group	Acclimatization		Exposure				Follow-up + behavioral assessment								Animal numbers male	Animal numbers female
Control	Sub-acute															10	10
Control	Follow-up															10	10
Low dose	Sub-acute															10	10
Mid dose	Sub-acute															10	10
Mid dose	Follow-up															10	10
High dose	Sub-acute															10	10
High dose	Follow-up															10	10
Pos control	Sub-acute															10	10
Pos control	Follow-up															10	10

Control: clean air, 240 min/day

Low dose: 25 min/day

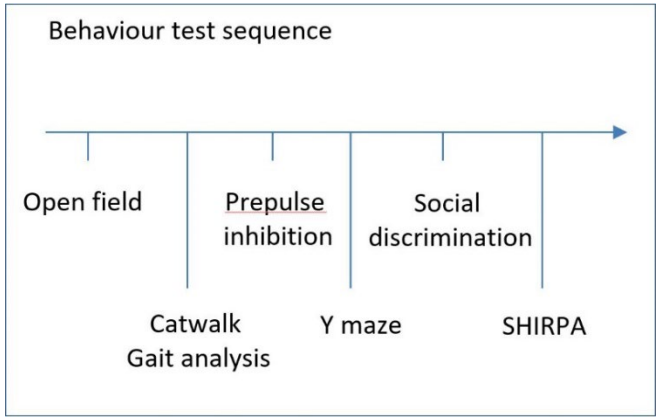
Mid dose: 80 min/day

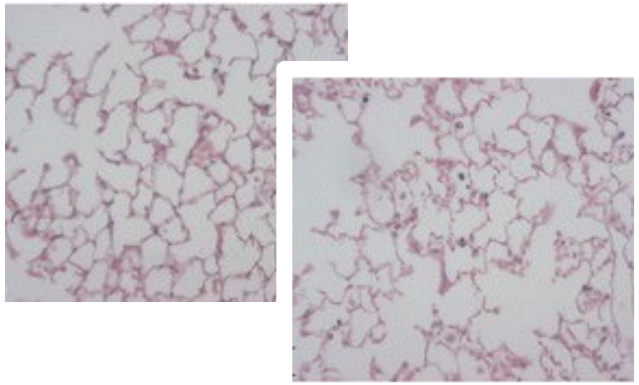
High dose: 240 min/day

Pos control: DEE, 240 min/day

Main toxicity study – parameters (1)

- Clinical observations
- Body weight
- Neurobehavioural assessment (HMGU)
- Neuroinflammation (HMGU)
  - Brain microscopy
- Lung microscopy
- Other relevant tissues will be stored for possible future analysis





Main toxicity study – parameters (2)

Blood samples (after end of exposure and end of follow-up):

- To detect small changes in metabolism, inflammatory and neurotoxic effects (Fraunhofer ITEM)
  - Targeted metabolomics
  - Quantification of up to ~1000 metabolites from >25 compound classes

- Inflammation, acute phase response (NRCWE)
  - Serum Amyloid A (SAA) mRNA in lung and liver tissue, SAA protein in blood

In summary

- The CAQ III project is intended to provide scientific evidence (data) related to the health effects of oil-related fume events

- This toxicity study will provide data for the hazard assessment of oil fumes
  - using a realistic fume mixture (complete, heated fumes)
  - generated from a commonly used engine oil (Mobil Jet Oil II)
  - for endpoints which can be related to human health symptoms
  - with information on dose-response

- … and will therefore provide valuable information for the future health risk assessment of oil-related fume events

CABIN AIR QUALITY III

Thank you for your attention!

Fraunhofer ITEM

SAFRAN

HELMHOLTZ MUNICH

GMC

InstPharmToxBw

Det Nationale Forskningscenter for Arbejdsmiljø

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