2017- 2020
Consortium included Airbus, Honeywell, Fraunhofer, RIVM, VITO, TNO

Service Contract MOVE/B3/SER/2016-363/SI2.748114
Over-arching objectives from tender

- Characterization of composition and concentration of contaminants of bleed air and their impact on cabin/cockpit air quality.

- Identification of short- and/or long-term health effects (toxicological and physiological), that might evolve from exposure to cabin air.

- Strategy for simulating CAC-events.

- Toxicological risk assessment methodology for decision-support relating to cabin air quality

- Risk mitigation strategy aimed at reducing likelihood of CAQ-risk.
Set up Bleed Air Contamination Simulator BACS
Exposure variable monitored

- Pressure, temperature, relative humidity continuously
- Following compounds continuously:
  - Carbon monoxide
  - Carbon dioxide
  - Nitrogen oxides
  - Sulphur dioxide
  - Ozone
  - Formaldehyde
  - Total Volatile Organic Compounds
  - Selected Volatile Organic Compounds
  - Particulate matter 0.005 – 40 µm (UFP, PM1, PM2.5, PM10 and larger)
  - Black carbon

Compounds

- Volatile Organic Compounds (VOC, C6-C16, incl. BTXE, halogenated)
- Volatile Organic Compounds (VOC, C6-C16, incl. BTXE, Acrolein)
- Very Volatile Organic Compounds (VVOC, C2-C6)
- SVOC in suspension (phthalates, PAHs, PCBs, flame retardants, organo phosphates)
- Aldehydes/Ketones
- Carboxylic acids
- Organo-phosphates (28 incl. 10 TCP isomers)
- Dioxins and furans
- PAHs
- Odour active compounds
- Characterisation of particles
Risk assessment methodology

- **Objective (tender):** *Toxicological risk assessment methodology for decision-support relating to cabin air quality*

- Three main areas of interest identified
  - Suitability of available reference values in cabin air quality

- Development of risk assessment framework
  - For incidental fume events and normal flight conditions.
  - Flight crew as well as passengers.
  - Focus on exposure and effects via inhalation

- Flowcharts and Excel files as basis for a **CAQ III**
Toxicity testing - objectives

• **Main goal:**
  assess whether exposure to fume events contributes to neuronal effects as observed in cases of ‘aerotoxic syndrome’

• **Specific objectives**
  - **Hazard identification:** rank fume mixtures in terms of general toxicity and specific neurotoxic potency
  - **Screening for biomarkers** in test animals used in controlled exposure to characterized fumes/extracts → not performed, CAQ III will do this in vivo/mice
Toxicity testing

An air-liquid interface (ALI) exposure system for realistic inhalation exposure to fumes generated with miniBACS to assess pulmonary and neurotoxicity of simulated fume events in the aircraft cabin.

Fume generation under laboratory conditions → Air liquid interface exposure of epithelial lung cells → Exposure of primary cortical cultures to conditioned media from ALI experiment
Inhalation exposure in vitro
Diagram of mini-BACs and ALI exposure system

Mini-BACs and ALI exposure system

Lung cell exposure and fume characterization
Bleed Air Contamination Simulator (mini-BACS)

Generation of oil fume samples

Heating to 350°C or 200°C

Oil injection

Direct exposure lung cells or sampling on filters

Extraction for neurotoxicity
Lung cell modules

One for air control and several for aerosol samples. Cells are transferred into each well for exposure. Can be connected with analytical instruments: particle number concentration, size, and VOCs, etc.
Conclusions – Lung model

- 4 commonly engine oils and 2 hydraulic oils
- Almost oil samples can induce cytotoxicity at applied doses (0 - 100 mg/cm³)
- Hydraulic oil samples are more toxic than engine oil samples
Direct neurotoxicity testing

mini-BACS → Oil fume extracts → primary cortical culture

0.5 – 48 h

Neuronal function: Multi-Electrode Array Assay

Cyotoxicity: Alamer Blue Assay
Hydraulic oil-derived fumes exhibit higher neurotoxic potential than engine oil-derived fumes

### Mean Spike Rate

<table>
<thead>
<tr>
<th>Oil type</th>
<th>ID</th>
<th>Temp.</th>
<th>0.5 h exposure</th>
<th>24 h exposure</th>
<th>48 h exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Engine Oil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engine 1</td>
<td>350°C</td>
<td>121</td>
<td>86</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[85 - 233]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engine 2</td>
<td>350°C</td>
<td>39</td>
<td>45</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[33 – 47]</td>
<td>[46 - ?]</td>
<td></td>
</tr>
<tr>
<td>Engine 3</td>
<td>350°C</td>
<td>57</td>
<td>47</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[41 – 81]</td>
<td>[24 – 56]</td>
<td></td>
</tr>
<tr>
<td>Engine 4</td>
<td>350°C</td>
<td>84</td>
<td>37</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[57 – 140]</td>
<td>[43 – 64]</td>
<td></td>
</tr>
<tr>
<td><strong>Hydraulic Oil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydraulic 1</td>
<td>200°C</td>
<td>2.3</td>
<td>15</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[1.7 – 2.9]</td>
<td>[13 – 23]</td>
<td></td>
</tr>
<tr>
<td>Hydraulic 2</td>
<td>200°C</td>
<td>5.8</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[5.0 – 6.7]</td>
<td>[13 – 19]</td>
<td></td>
</tr>
</tbody>
</table>

Mean Spike Rate

IC₅₀ values [µg/mL] + CI 95%

Neuronal function: Multi-Electrode Array Assay
Neurotoxicity testing

- Fumes deriving from engine and hydraulic oils reduce neuronal activity

- Engine oil fumes-induced neurotoxicity mainly occurs after prolonged exposure whereas for hydraulic oils already acute exposure affects neuronal activity

- Fumes generated from hydraulic oils more potent in inhibition neuronal activity compared to engine oil-derived fumes
Remarks

• Simulated fume events
• Relative long and high concentration exposure
• Simplified (in vitro) models, outcome need to be interpreted with a lot of caution
• Guidance for understanding chemical component related effects and ranking potencies but not for risk assessment