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AVOIL
Characterisation of the toxicity of aviation turbine engine oils after pyrolysis

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Characterisation of the toxicity of aviation turbine engine oils after pyrolysis (AVOIL) – Final Report

February 16th 2017

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Executive Summary

There are concerns among the international governments, pilots, cabin crew and passengers and other stakeholders of commercial jet aircraft about possible health risks associated with reports of the presence of fumes in the air supplied to aircraft cabins. One particular area of concern is contamination of the cabin air after pyrolysis of jet engine fluids, not to forget the additives that are already present in the oil.

The European Aviation Safety Agency (EASA) contracted the consortium of the Netherlands Organisation for Applied Scientific Research (TNO) and the Dutch National Institute for Public Health and the Environment (RIVM) in November 2015 to perform a study on the characterization of the toxicity of aviation turbine engine oils after pyrolysis (AVOIL). This study is registered under the number EASA.2015.C16.

Aim of this work is to characterize the toxic effects of chemical compounds that are released into the cabin or cockpits of transport aircraft. The characterisation is aimed at the toxic effects of aviation turbine engine oil as a mixture of compounds, including potential pyrolysis breakdown products. Four tasks have been defined:

- Task 1. Performance of scientific literature review and selection of applicable engine/APU oils.
- Task 2. Design of a test methodology for the chemical characterization and toxic effects of these oils after pyrolysis.
- Task 3. Performance of the chemical characterization and toxic effects of the oils after pyrolysis.
- Task 4. Analysis of the human sensitivity variability factor.

This report describes the performance of the tasks, the obtained results and the drawn conclusions.

The literature descriptive review was focused on the type of effects reported in humans, measurements of oil components in an aircraft, in vitro and in vivo toxicity tests conducted with engine oil or its fumes, and composition of aviation engine oil, fumes or pyrolysis products. The findings of this descriptive review were used in the set-up of the experiments in this study. The literature showed that in time, analytical techniques for dedicated components that may be present due to the used oil, have been improved significantly. This explains probably the fact, that in older literature no components as tricresyl phosphate (TCP) were found. Further, it showed that the temperature in an aircraft engine compartment, where oil vapour and pyrolysis products may be formed, can reach temperatures above 500°C and therefore more toxic fumes (containing carbon monoxide (CO) and trimethylolpropane phosphate (TMPP)) may be generated.

Experimental work was performed using two generally used brands of oil: one typically for twin-aisle or long range flights and one for single aisle or short range flights. The latter one included also operationally used engine oil. Experimental work consisted of three distinct parts: chemical characterization of oil and oil vapours simulated by heating oil in combination with purified air, and chemical characterization of oil vapours simulated by heating oil under pyrolysis conditions. Two flight stages were simulated, i.e. ground level to top of climb and cruise. Finally, toxic effects were studied using the vapour from pyrolysis of the oil samples.

Chemical characterization of oil and oil vapours by heating oil (under normal air composition)

The experimental set-up of the chemical characterization consisted of heating the oil in different time frames and adding fresh oil drops at maximum simulation temperature in an emission chamber, simulating the cabin and the system for sampling . The experimental set-up shows a good performance for the subsequent experimental work dedicated to the emission of jet oils at elevated temperatures. The simulation test contained two stages simulating the start of the engine to top of climb (time frame of the simulation was 30 minutes) and a steady state period for 60 minutes. The simulation tests were kept under controlled and comparable conditions for each oil. At the start of the simulation test, the temperature of the oils was approx. 21˚C and within 30 minutes the oils reached a temperature of 350°C. During the next 60 minutes of the simulation test the temperature of the oils reached 375 (± 25)°C. Based on the chemical characterization of the oils itself and in the emissions of oils performed in the simulation tests for oil A (new (A_n) and used (A_u)) and oil B (new(B_n), conclusions were established and are presented below.

There was no presence of tri(o,o,o)-cresyl phosphate in the applied oils. It was found that the original oils contained the following isomers: tri(m,m,m,)-, tri(m,m,p)-, tri(m,p,p) and tri(p,p,p)-cresyl phosphate.

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The mass fraction of the TCP isomers found in the oils corresponds with the mass fraction on the fact sheets off the individual oils.
The simulation test shows that the oils heated at a steady state (375 ± 25°C) emits more TCPs compared to the simulation whereby the temperature is heated up from 20 to 350°C.

Used oil (A_u) showed lower concentrations of TCPs in the simulation test (emission chamber) compared to new oil (A_n). Oil A_n and oil B_n gave comparable results. The simulation test also shows a good correlation between the composition of TCP isomers found in the original oil compared to the oil vapours at different temperatures. Comparison of new oil (A_n) and used oil (A_u) shows no significant differences in composition of the four isomers.

It was found that naphthalene was present in the original oil B_n in a concentration of 1.9 mg/kg. The original oil A_n did not contain naphthalene as it could not be detected above the detection limit (< 0.58 mg/kg). However, both anthracene and fluoranthene were found in the applied oils.

By comparison the analytical results of the PAH presence in the vapour and in the original oils, it was found that the vapour does contain more different types of PAH. Therefore, we suggest the following hypotheses:

- PAHs found in the oil vapour may originate from small concentrations (lower than detection limit) in the original oil.
- During heating of the oil, a partial oxidation may take place (partial combustion) resulting in the formation of PAHs due to incomplete combustion.

Based on a selection of the obtained results (aromatics, ketones, and esters) it was found that the emission of both oils differ at both simulations 'ground level to top of climb (20 to 350°C) and subsequently cruise speed (375 ± 25°C). For example, the total concentration of aromatic hydrocarbons for oil A_n and oil B_n at simulation (20 to 350°C) was 36 µg/m^3 and 48 µg/m^3 respectively. These concentrations increased drastically (to 1.279 and 3.098 µg/m^3 respectively) at cruise simulation (375 ± 25°C). Similar results were observed for other various volatile compounds.

Relative high concentrations of formaldehyde and acetaldehyde were found in the oil vapours. The highest concentrations of total aldehydes were found for the new oils in the simulation test (20 to 350°C). However, the used oil A (A_u) showed the highest concentrations at 375± 25°C, indicating that the composition and behaviour of used oil differs from new oil. It has to be stated, that the sampling of aldehydes was affected by the matrix of oil vapours, resulting in breakthrough of aldehydes. Based on these observations, it can be concluded that due to matrix effects, the results of the aldehydes sampled are probably underestimated concentrations, results must be considered as indicative.

From the start of the simulation test until the oil has reached 180˚C hardly any emission of CO arises. However, it appears that due to an increase of temperature (180 to 375˚C), CO is formed and emitted due to incomplete combustion.

It was observed that the mass distribution at room temperature of the original oil A_n consists of alkane chains (mineral oil) in the range of C_{24}-C_{50}. After 30 minutes of heating from 20 up to 350°C the composition of mineral oil in the vapour remains unchanged and is comparable with the mineral oil chains found in the original oil at room temperature. After heating at 375˚C, small changes in composition of the oil are observed, i.e., an increase of relative low boiling point components. Additionally, unidentified complex mixtures (UME) are formed beneath the C_{24}-C_{50} peaks. The results of the mineral oil analysis for used oil A (A_u) gave at room temperature similar results as were found for new oil A (A_n). Based on the results, it was found that the mineral oil composition for oil B_n is comparable with the composition found for oil A_n. Also for oil B_n the alkane chains are in the range of C_{24} – C_{50}, with the exception of one peak found around C_{21}. Additionally the ratio of the alkane chains differs between the two oils.

It is evident that the particle number concentrations (PNCs) are rising as the emissions of oil increases. However, for all the tests except with the used oil A (A_u) a decrease in PNCs was found after reaching 30 - 40 minutes of heating (350 to 375˚C) despite of adding fresh oil to the reaction chamber.

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Chemical characterization of the oils after pyrolysis

The aim was to identify compounds in three oils under a variety of conditions.
In the basic oil patterns, without heating, a set of TCP isomers and 4-octyl-N-(4-octylphenyl)-benzenamine were found in all three oils. N-phenyl-1-naphthaleneamine was only found in oil B_n, albeit in low concentrations.

Heating under nitrogen led to an increase in the number of compounds found, and led to the identification of 24 compounds in the vapour, found in all oils. A number of compounds was identified unique for either oil A_n or oil B_n. In addition, used oil (A_u) appeared to contain newly identified compounds compared to unused oil (A_n), and a number of compounds originally present appeared to have disappeared during use in an engine jet. This indicates that during the lifetime of an oil, substantial changes in composition occur.

As it cannot be excluded that oxygen is present in the jet engine, the effect of oxygen addition during pyrolysis was investigated. These experiments showed that the presence of oxygen led to combustion of the oils, resulting in a major increase of the number and amount of compounds.

To permit a safety assessment of compounds originating from jet engine oils, a list of compounds identified under both nitrogen and oxygen conditions, in all oils and during different flight stages was constructed, resulting in 127 compounds. This list can be used to assess the hazard profile of these compounds using the Classification and Labelling (C&L) database of the European Chemical Agency (ECHA). As a first step, the harmonised classifications as well as the main self-classifications by manufacturers are added to the list of chemicals, as presented in Appendix 6. The classification of a substance is an indication for its toxicity.

The experiments were all performed under atmospheric pressure. In a jet engine the pressures can reach almost 10 bars. This could interfere with the formation and evaporation of organic compounds. On the basis of the physical appearance of used oil (A_u) a combustion of turbine lubrication oil is not likely to occur on a large scale. This would also result in a thick smoke and a pungent odour in the cabin during flight.

Toxic effects of the oils after pyrolysis

Toxicity of the oil vapours was investigated using an *in vitro* model of the human lung using an air-liquid-interface system, combined with an in vitro neuronal network system using primary cortical neurons grown on microelectrode arrays (MEAs). Concentrations as tested in vitro are within the broad range of concentrations reported in literature during normal flight conditions based on literature (range from 0.3 ng/m^3 to 50 µg/m^3). The results showed that acute exposure of primary rat cortical cultures to medium containing pyrolysis products derived from engine oil, following transfer to an air-liquid interface equipped with lung epithelial cells, does not induce significant changes in neuronal activity. However, a trend towards an increase in neuronal activity was observed at the highest tested concentration and it cannot be ruled out that higher concentrations may affect neuronal activity. Furthermore, exposure up to 48h resulted in a decreased neuronal activity and it is therefore possible that effects of pyrolysis products develop only following more prolonged (i.e. 48h and longer).

Analysis of the human sensitivity variability factor

The possible causes of the large variety in reported health symptoms were elucidated by exploring a) the possible role of genetic differences in metabolism and detoxification between humans and b) the possible influence of stress and/or coping strategies that may intensify or trigger health complaints. Differences in sensitivity between humans for the health effects of certain compounds can be expected for those compounds that rely on cytochrome P450 enzymes for their metabolism. However, the broad range of compounds in the cabin air, in combination with other stressors, has not been systematically mapped, making it difficult to draw conclusions on the contribution of such inter-individual genetic differences in metabolism and detoxification on the variety in reported symptoms. In view of the great variety in symptoms and the lack of specificity, it cannot be ruled out that part of the symptoms cannot be explained by actual exposure levels. The literature shows that we are dealing with symptoms that are quite common in the general population and fall within the domain of somatically unexplained physical symptoms. However, overall statements about a potential higher the prevalence of somatically unexplained physical symptoms in cabin crew are hard to make because symptoms overlap, estimates are dependent on the definitions used and

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the participants in the study. Differences in coping strategies are well-known factors to enhance stress reactions, which in their own right can lead to acute health complaints and long-term health effects. Whether or not occupational conditions are responsible for the reported complaints remains unknown until the complete set of potential chemical exposures is known, including their exposure levels, resulting internal dose levels, full spectrum of molecular targets (i.e., all different modes of action) and the related no-effect concentrations.

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1 Introduction

1.1 Introduction

The European Aviation Safety Agency (EASA) contracted the consortium of the Netherlands Organisation for Applied Scientific Research (TNO) and the Dutch National Institute for Public Health and the Environment (RIVM) in November 2015 to perform a study on the characterization of the toxicity of aviation turbine engine oils after pyrolysis (AVOIL). The AVOIL study is registered under the number EASA.2015.C16.

Sub-contractors of the consortium consists of the Institute for Risk Assessment Sciences, an interfaculty research institute within the medical faculties of Utrecht University and VU Amsterdam Institute for Environmental Studies. For additional advices and the possibility to gain operational knowhow and specific knowledge KLM and ADSE (Aircraft Development and Systems Engineering) were willing to support the consortium.

1.2 Background of AVOIL

There are concerns among the international governments, pilots, cabin crew and passengers and other stakeholders of commercial jet aircraft about possible health risks associated with reports of the presence of fumes in the air supplied to aircraft cabins. One particular area of concern is contamination of the cabin air after pyrolysis of jet engine fluids, not to forget the additives that are already present in the oil.

Despite the widespread use of jet engines, concerns have been raised over the toxicity of vaporised and pyrolysed engine fluids and their potential to cause short-and long-term health effects. The need for improved understanding of this potential source of contaminants as well as the possible influence of other sources of airborne substances (e.g. chemicals emitted by furnishing materials and ambient (outside) air contaminants) is the prime basis for the current need for in-depth expertise of the toxicity of these materials during a cabin air contamination (CAC).

The cabin (flight deck and passenger cabin) air supply is controlled by a system called the Environmental Control System (ECS). The air supply in most commercial jet aircraft is bleed-air from the engines that is drawn from the compressor stage of the engine into the ECS where the air is conditioned to meet the temperature required for the cabin/flight deck environment. If seals within the engine are not performing effectively, oil and possibly thermal degradation products of oil can result in contamination of the bleed air. Besides contaminated bleed air, the ECS itself and the ducts can also be a secondary source of contaminants.

Several studies have considered air quality in aircraft and have found a variety of differences with those of other indoor environments, including those of homes and offices. For example, low humidity and reduced pressure can affect passenger's well-being and their sensory perception and the high level of occupancy means the passengers themselves are a notable source of carbon dioxide and a variety of organic chemical compounds. "Fume events", while rare, have been reported by passengers and crew and may be associated with elevated exposure to airborne contaminants, the nature of which will depend upon the source. Laboratory studies have shown that vapours and aerosols released from heated aircraft oils can contain hazardous substances and therefore may be of concern if present at sufficient concentration during a 'fume event' to cause health effects.

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2 Aims and Objectives

The objective of the study is to characterize the toxic effects of chemical compounds that are released into the cabin or cockpits of transport aircraft. The characterisation is aimed at the toxic effects of aviation turbine engine oil as a mixture of compounds, including potential pyrolysis breakdown products. Toxic effects of the mixture will be characterized at the pulmonary and neuronal level, considering the primary routes of exposures and mode of toxicity. Additionally, identification of suspected toxic individual compounds is provided to adequately assess inter individual susceptibility. The overall aim is to integrate these aspects based on already available material and experimental results, to provide a solid basis for steps towards recommendation of Threshold Limit Values for the identified chemicals.

The Tender proposal describes the requested tasks in EASA consists of the following tasks:
Task 1. Performance of scientific literature review
Task 2. Selection of applicable engine/APU oils
Task 3. Design of a test methodology for the chemical characterization and toxic effects of these oils after pyrolysis
Task 4. Performance of the above mentioned test
Task 5. Analysis of the human sensitivity variability factor

During the kick-off meeting with EASA on the 10th of December 2015 the approach presented by the consortium was discussed and no major amendments regarding the methodology were made. The following changes and comments on the project tasks were suggested by the consortium and agreed: Task 2 - it is suggested to combine this with Task 1 and Task 3. The oil selection will be included in Task 1, as it is based on available information that shall be gathered. The conditions of the pyrolysis are defined in Task 3, where the pyrolysis products and the chemical composition of the oil vapour are analysed.

Due to minor changes mentioned in the above text, the project tasks are as follows:
Task 1. Performance of scientific literature review and selection of applicable engine/APU oils
Task 2. Design of a test methodology for the chemical characterization and toxic effects of these oils after pyrolysis
Task 3. Performance of the chemical characterization and toxic effects of the oils after pyrolysis.
Task 4. Analysis of the human sensitivity variability factor

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3 Task 1: Descriptive literature review and selection of oils

3.1 Introduction

The objective of this task is to conduct an inventory of former studies related to aviation turbine engine oil toxicity. It was suggested to focus the descriptive literature review on several areas of interest, e.g.:

- Characterization of aviation turbine engine oil, its vapour or pyrolysis products.
- Air cabin measurements during flight operations in airplanes of aviation turbine engine oil vapour or assumed compounds and pyrolysis products.
- Surveys of health complaints related to potential exposure of individuals to aviation turbine engine oil or its breakdown products.
- Experimental toxicological studies of aviation turbine engine oil in cell cultures and experimental animals focussed on neurotoxicity and inhalation toxicity.
- Studies relating to specific toxicity (e.g. neurotoxicity) or a mode of action of aviation turbine engine oil or its pyrolysis products.

The aim of Task 1 is to perform an analysis of relevant existing studies related to aviation turbine engine oil toxicity to obtain usable lessons for the other tasks of the overall study. To ensure an optimal inventory of relevant studies, search profiles need to be as specific as possible. The areas of interest presented above are reformulated into four subcategories aimed to support the information specialist in determining a search profile. The link of the subcategory with the other task in this study is described:

1. Type of effects found in humans and possible mechanistic explanations
    - This links to Task 4 where an explanatory chapter will be written discussing the various factors that could explain the variability in health complaints observed
2. Order of magnitude of measurements of oil compounds found in a realistic setting in an airplane
    - This links to the outcome of Task 3 where exposures in realistic settings could be compared with effect levels found in this study
3. *In vitro* or *in vivo* toxicity tests conducted with aviation engine oil or its fumes
    - This links to Task 3 to identify any usable lessons for this study
4. Composition of aviation engine oil, fumes or pyrolysis products
    - This links to Task 2 to characterize the engine oil pyrolysis products

3.2 Methodology

3.2.1 Search profile

An information specialist, located at the RIVM, was asked to compose a search profile for each subcategory based on the defined subcategories and consultation with a RIVM team member (Table 3.1).

Table 3.1 Search profiles for each category

Category	Search profile
1. Type of effects found in humans and possible mechanistic explanations	**Medline** Database: MEDLINE 1950 to present, MEDLINE In-Process & Other Non-Indexed Citations Search Strategy: ---------- 1 (aircrew* or air* crew* or flight crew* or flightcrew* or air* pilot* or cabin crew* or crewmember* or (air* adj5 passenger*)).tw. (3130) 2 Aircraft/ (7790) 3 Aviation/ (5664) 4 1 or 2 or 3 (14841) 5 inhalation exposure/ or occupational exposure/ (49947) 6 *Environmental exposure/ae (7160)

	7 Air Pollution, Indoor/ae, an [Adverse Effects, Analysis] (7168) 8 *Air Pollution/ae, an [Adverse Effects, Analysis] (6292) 9 (bleed air or cabin air or cockpit air or (contamin* adj air*) or air quality or exposure or (health adj effect*) or (health adj complaint*) or neurologic* or neurotox* or (neuro* adj2 tox*)).tw. (888018) 10 Neurotoxicity Syndromes/ or Syndrome/ (111874) 11 5 or 6 or 7 or 8 or 9 or 10 (1020200) 12 cresols/ or tritolyl phosphates/ (4052) 13 (tricresyl phosphate* or tricresylphosphate* or tri-ortho-cresyl phosphate* or organophosphate*).tw. (8091) 14 (((engine or hydraulic or turbine or lubricat* or jet or pyroly*) adj2 oil) or (hydraulic adj fluid)).tw. (618) 15 Fuel Oils/ (1261) 16 Oils/ae, po, to [Adverse Effects, Poisoning, Toxicity] (1111) 17 Smoke/ae, to [Adverse Effects, Toxicity] (2666) 18 (fume* or odour* or odor*).tw. (27416) 19 12 or 13 or 14 or 15 or 16 or 17 or 18 (44760) 20 aerotoxic syndrome.tw. (15) 21 4 and 11 and 19 (63) 22 20 or 21 (68) **Scopus** ((((TITLE-ABS-KEY((aircrew* OR air*-crew* OR flight-crew* OR flightcrew* OR air*-pilot* OR cabin-crew* OR crewmember* OR crew-member* OR (air* W/5 passenger*)))) OR (TITLE(aircraft OR aviation))) AND (TITLE-ABS-KEY((bleed-air OR cabin-air OR cockpit-air OR (contamin* W/1 air*) OR air-quality OR exposure OR (health W/1 effect*) OR (health W/1 complaint) OR (adverse-effect*) OR neurologic* OR inhalat* OR (indoor W/1 air) OR neurotoxic* OR (neuro* W/2 tox*))))) AND ((TITLE-ABS-KEY((tricresyl-phosphate OR tricresylphosphate OR tri-ortho-cresyl-phosphate OR organophosphate))) OR (TITLE-ABS-KEY(((engine OR hydraulic OR turbine OR lubricat* OR jet OR pyroly*) W/2 oil))) OR (TITLE-ABS-KEY((hydraulic W/1 fluid))) OR (TITLE-ABS-KEY((fume OR odour* OR odor* OR neurotox*))))) OR (TITLE-ABS-KEY(aerotoxic-syndrome))
2. Order of magnitude of measurements of oil compounds found in a realistic setting in an airplane	Database: **MEDLINE** 1950 to present, MEDLINE In-Process & Other Non-Indexed Citations --- 1 (airplane* or aircraft* or aviation or inflight or in-flight or bleed air or cockpit air).tw. (12587) 2 Aircraft/ (7795) 3 Aviation/ (5665) 4 1 or 2 or 3 (20861) 5 (((engine or hydraulic or turbine or lubricat* or jet or pyroly* or fume*) adj2 oil?) or (hydraulic adj fluid?) or (lubrication adj additive?) or (oil? adj additive?)).tw. (989) 6 Fuel Oils/ or Oils/ or Lubrication/ (13534) 7 5 or 6 (14286) 8 cresols/ or tritolyl phosphates/ or exp Organophosphates/ (27236) 9 (tricresyl phosphate* or tricresylphosphate* or tri-ortho-cresyl phosphate* or organophosphate*).tw. (8101) 10 Volatile Organic Compounds/ or Hazardous Substances/ (12057) 11 (volatile organic compound? or semi-volatile organic compound? or VOC? or SVOC? or hazardous substance? or hazardous compound?).tw. (9124)

	12 8 or 9 or 10 or 11 (50734) 13 4 and 7 and 12 (36) 14 exp Chemistry Techniques, Analytical/ or Environmental monitoring/ (1920781) 15 (method* or measure* or detect* or spectrometr* or quantif* or characteri* or investigat*).tw. (9280153) 16 14 or 15 (10097693) 17 13 and 16 (26) **Scopus** TITLE-ABS-KEY(airplane OR aircraft OR aviation OR bleed-air OR cockpit-air) AND TITLE-ABS-KEY(((engine OR hydraulic OR turbine OR lubricat* OR jet OR pyroly* or fume*) W/2 oil) OR (hydraulic W/1 fluid) OR (lubrication W/1 additive?) OR (oil W/1 additive?)) AND TITLE-ABS-KEY(tricresyl-phosphate* OR tricresylphosphate* OR tri-ortho-cresyl-phosphate* OR organophosphate OR volatile-organic-compound? OR semi-volatile-organic-compound? OR VOC? OR SVOC?)AND TITLE-ABS-KEY(method* OR measure* OR detect* OR *spectrometric* OR quantif* OR characteri* OR investigat*)
3. *In vitro* or *in vivo* toxicity tests conducted with aviation engine oil or its fumes	Database: **MEDLINE** 1950 to present, MEDLINE In-Process & Other Non-Indexed Citations --- 1 (airplane* or aircraft* or aviation or inflight or in-flight or bleed air or cockpit air or aerotox*).tw. (12595) 2 Aircraft/ (7795) 3 Aviation/ (5665) 4 1 or 2 or 3 (20865) 5 (((engine or hydraulic or turbine or lubricat* or jet or pyroly* or fume*) adj2 oil?) or (hydraulic adj fluid?) or (lubrication adj additive?) or (oil? adj additive?)).tw. (989) 6 Fuel Oils/ or Oils/ or Lubrication/ (13534) 7 5 or 6 (14286) 8 (toxicity adj2 test*).tw. (8497) 9 exp Toxicity Tests/ (94283) 10 ((in vitro or in vivo) and (toxic* or neurotoxic* or cytotox* or intoxic* or test* or study or studies or assay*)).tw. (833926) 11 8 or 9 or 10 (913224) 12 4 and 7 and 11 (13) **Scopus:** TITLE-ABS-KEY(airplane OR aircraft OR aviation OR bleed-air OR cockpit-air OR aerotox*) AND TITLE-ABS-KEY(((engine OR hydraulic OR turbine OR lubricat* OR jet OR pyroly* or fume*) W/2 oil) OR (hydraulic W/1 fluid) OR (lubrica* W/1 additive) OR (oil W/1 additive?)) AND TITLE-ABS-KEY (((in-vitro OR in-vivo) AND (*toxic* OR test* OR study OR studies OR assay)) OR (*toxic* W/2 (test* OR study OR studies OR assay)))
4. Composition of aviation engine oil, fumes or pyrolysis products	**Scopus**: (TITLE(aircraft OR aviation OR bleed-air OR cockpit-air OR aeroengine* OR jet)) AND (TITLE-ABS-KEY(lubricat* OR lubricant* OR hydraulic-fluid* OR engine-oil* OR jet-oil*)) AND (TITLE(chemical OR composition OR analy* OR identificat* OR spectromet* OR investigat* OR pyroly* OR characteri* OR identificat* OR measure* OR monitor* OR assess* OR study OR toxic*))

This search yielded 197, 45, 25 and 167 references respectively per category. A personalized search was performed by the information specialist in Embase and Toxcenter to obtain additional results not found using the

abovementioned protocol. This generated 5, 8, 10 and 13 more references respectively. The Endnote files are provided as separate documents accompanying this report.

3.2.2 Selection of relevant papers

The references found by the information specialist were screened. The first assessment was performed based on the title. Two scientists, one based at IVM and one based at RIVM, have independently screened all the titles and decided whether the article is relevant for this literature review or not. Next, the abstract of the selected article was read and subsequently it was decided whether the article was still relevant. In the end, all articles that were included by only one researcher were jointly discussed for inclusion or exclusion.
The work presented here is a descriptive literature review describing the key points of each study.

3.2.3 Grey Literature

Besides peer reviewed literature also grey literature reports (non-peer reviewed literature which are not controlled by commercial publishers) were identified as potential relevant by the consortium (Table 3.2).

Table 3.2. Grey literature identified at the start of the project.

References	Title
Crump et al. (2011).	Aircraft Cabin Air Sampling Study. Cranfield University, UK, Institute of Environment and Health. Cranfield Ref No YE29016V
EPAAQ (2011).	Contamination of aircraft cabin air by bleed air – a review of the evidence. Document reviewing evidence up to September 2009. Adelaide, Australia, Expert Panel on Aircraft Air Quality
Occupational Health Research Consortium in Aviation (OHRCA) (2014)	Cabin Air Quality Incidents Project Report
Solbu (2011)	Airborne organophosphates in the aviation industry. Sampling development and occupational exposure measurements. Dissertation Kasper Flatland Solbu. National Institute of Occupational Health and University of Oslo, Oslo, Norway
TNO (2013b)	Investigation of presence and concentration of tricresyl phosphates in cockpits of KLM Boeing 737 aircraft during normal operational conditions. TNO 2013 R11976, 12 December 2013
Committee on toxicity of chemicals in food consumer products and the environment (COT) (2007)	Statement on the review of the cabin air environment, ill-health in aircraft crews and the possible relationship to smoke/fume events in aircraft
United Kingdom Parliament 2000	Select Committee on Science and Technology – Fifth Report
United Kingdom Parliament 2007	Select Committee on Science and Technology – First Report update
Professor Michael Bagshaw. (2014)	Health Effects of Contaminants in Aircraft Cabin Air. Summary Report v2.7
German Federal Bureau of Aircraft Accident Investigation (2014)	Study of reported occurrences cabin air quality in transport aircraft
IOM (2012)	Cabin Air – surface residue study Report
Parliament of the Commonwealth of Australia (2000)	Air Safety and Cabin Air Quality in the BAe 146 Aircraft

During the interim meeting with EASA (23 February 2016) and subsequent correspondence via e-mail additional grey literature was provided which is summarized in Appendix 1. Some of the reports were already listed in Table 3.2 and therefore excluded from the list. In total 29 documents, links or references were provided and screened for relevance.

3.3 Results

3.3.1 Reference library

The 260 references retrieved by the information specialist for category 1 and 2 were screened on title and abstract. In total 28 references were directly included and three references were jointly discussed (see Table 3.3). This resulted in a total of 28 papers; full text articles were obtained from 26 articles. For two articles no full text could be retrieved by the consortium without substantial costs. The matching endnote file is provided as a separate file accompanying this report.

Table 3.3. Selection process of the retrieved references

	Category-1		**Category-1 extra[a]**		**Category-2**		**Category-2 extra[a]**	
Total references	202		5		45		8	
Reviewer	I	II	I	II	I	II	I	II
Not selected	133	118	5	5	29	27	4	4
Selected by title	69	84	2	2	16	18	4	4
Selected after summary	20	21	0	0	7	7	1	1

[a] A personalized search was performed by the information specialist in Embase and Toxcenter to obtain additional results not found using the abovementioned protocol.

The 215 references retrieved by the information specialist for category 3 and 4 were also screened on title and abstract. In total 28 references were directly included and 17 references were jointly discussed (see Table 3.4). This resulted in a total of 38 papers; full text articles were obtained from 20 articles. For 18 articles, mostly conference papers, no full text could be retrieved by the consortium without substantial costs. The matching endnote file is provided as a separate file accompanying this report.

Table 3.4. Selection process of the retrieved references

	Category-3		**Category-3 extra[a]**		**Category-4**		**Category-4 extra[a]**	
Total references	25		10		167		13	
Reviewer	I	II	I	II	I	II	I	II
Not selected	21	14	2	1	130	123	9	7
Selected by title	4	11	8	9	37	44	4	6
Selected after summary	3	10	8	9	14	23	3	5

[a] A personalized search was performed by the information specialist in Embase and Toxcenter to obtain additional results not found using the abovementioned protocol.

3.3.2 Grey literature

Beside the scientific papers, several reports provided by the EASA and consortium members listed in Table 3.2 and Appendix 1 were considered relevant for the literature review of category 1 and 2. The justification for the selection is given in Table 3.2 and Appendix 1. The report from EPAAQ (2011): *Contamination of aircraft cabin air by bleed air – a review of the evidence. Document evidence up to September 2009* is regarded most relevant containing useful information for subcategory 3 and 4 and was included in the analysis.

Literature provided by EASA and consortium members: in total four reports are considered relevant for subcategory 3 and 4 and included in the analysis. The justification for the selection is provided in Appendix 1.

3.3.3 Google search

To screen for any missing relevant document related to subcategory 4, a Google search was performed with the following keyword:
Search 1: Pyrolysis, jet engine oil, air quality
Search 2: Pyrolysis, turbine oil, cabin air
Search 3: Fume event, engine oil, contaminated air

The first 30 hits of each of the three google searches were screened for relevant information. The scientific papers and reports that were included so far were selected by title and abstract. The following 3 scientific paper/reports were selected and discussed between the reviewers:

1. Michaelis, S.. Contaminated aircraft cabin air. Journal of biological Physics and Chemistry, vol 11, 2011, p 132-145 (Michaelis, 2011)
2. Cabin air quality Civil Aviation Authority (CAA) ISBN 0 86039 961 3. Published February 2004 (https://publicapps.caa.co.uk/docs/33/CAPAP2004_04.PDF)(CAA, 2004)
3. Hildre, T. T., Jensen, J. K., Fume Events in Aircraft Cabins. Master thesis, NTNU, Trondheim, June 2015. (http://brage.bibsys.no/xmlui/bitstream/handle/11250/2353003/12960_FULLTEXT.pdf?sequence=1&isAllowed=y) (Hildre and Jensen, 2015)

After discussing these results, it was decided to include these three paper/reports in the analysis for subcategory 4.

3.3.4 Analysis

For category 1 and 2 an extensive meta-analysis of the reported symptoms or substance concentrations is not foreseen, instead a table is generated with descriptive elements (type of study, number of participants or measurement, symptoms or type of substance and study conclusions) of the selected studies.

For category 3 and 4, the descriptive review was focussed on information considered useful for the experimental set-up in the other tasks of this project. These include the generation of vapours and pyrolysis products at relevant conditions. In order to extract useful lessons for these experiments, the analysis of the selected articles was focussed on those topics deemed useful for next steps in the study.
The coordinators of these experiments were requested to provide specific subjects that shall be taken into account in the literature review and that may contribute to their experimental set-up.
Summarized, information was requested on:

- The temperature in an aircraft engine compartment where oil vapours and pyrolysis products may be formed.
- Any specifics on the conditions during a fume event, e.g. temperature, pressure, amount of oxygen. This can refer to the conditions in the engine compartment where vapours and pyrolysis products may be formed, but also to conditions in the cabin where people may be exposed.
- The type of products that may be formed during the heating and/or pyrolysis process, e.g. vapours, aerosols.
- Any indications on the time period of the pyrolysis.
- *In vitro* experiments performed with vapours or pyrolysis products of aviation engine oils.

It is noted that information on these subjects by the experiments coordinators was also obtained via other information routes, including own literature databases and information obtained from ADSE.

3.4 Main findings and usable lessons learnt

3.4.1 Analysis subcategory 1: type of effects found in humans.

The 19 selected papers were screened for effects found in humans exposed to “contaminated” aircraft cabin air. The symptoms found were summarized in the Table 3.5 as well as study design, data set and conclusion of the study.

Table 3.5. Study design, information regarding the data set studies, and general conclusions according to the authors were summarized for each study.

Study design	Data set	Symptoms	Conclusion[a]	Reference
This study presents the exposure of Westland Sea King helicopter crew to engine exhaust fumes, clinical signs of CO intoxication, and levels of carboxyhemoglobin saturation (SpCO) after standard operation training flights.	69 completed surveys of crew specialists pilots (n=18), system operators (n=14), engineers (n=11), rescuers (n=11), flight physicians (n=15) were collected over a 2 week period.	The median duration of the training session was 80 minutes. 64% reported subjective exposure to engine exhaust during the training sessions. 8.6% reported clinical symptoms such as; exhaustion (n=4), headache (n=1) and nausea (n=1).	Exposure to engine exhaust fumes in common, whereby 8.6% reported clinical symptoms mainly during open cargo door operations. Toxic SpCO levels were not reached however, 29% showed SpCO levels outside the normal range (>4%).	Busch (2015)
General practitioner (GP) M. Somers has seen 39 flight crew members (7 pilots and 32 flight attendants) and one passenger who reported symptoms in relation to exposure to fumes in the cabin of mainly the BAe 146.	In this study, data files were collected over a period of 6 years from 36 flight crew members who came to see GP M. Somers because of their health concerns regarding to the exposure to contaminated cabin air.	The most common symptoms observed after long and short-term exposure where: nausea, headache, mucous membrane irritation, lethargy and cognitive dysfunction. Cognitive impairment were also reported such as unable to speak, poor memory, felt drugged, unable to think clearly, disorientation, trips over words, couldn't put dates in order etc. All symptoms reported are listed in Table A.1 in Appendix 2.	Symptoms were reported during taxiing, take-off, climbing, top of descent, descending and landing. Correlation between fume events and technical faults were observed. Overall there is a need for a reporting system that is objective and independent of the operator. Exposure to contaminated cabin air has influences on the worker's health, finances, future work capacity and may also affect the safety of the aircrew and passengers.	Somers (2005)
In this epidemiological study, the health effects of aircrew members were investigated through a questionnaire survey. The aircrew monitored were flying with the BAe 146 and/or A320 aircraft.	The collected data set consisted of 50 Australian aircrew members (72% female) in the age range of 26 to 59 years. 70% of the respondents were cabin crew and 30% flight crew.	Adverse health symptoms related to exposure to oil fumes or odours were reported by 94% of the respondents. 96% reported adverse symptoms immediately after flying whereby 74% also experienced symptoms for at least 6 months after exposure. Symptoms reported by the 50 corresponding aircrew were related to eyes (76%) and skin irritation (58%), nausea (58%), neuropsychological symptoms including intense headache (86%), dizziness and disorientation (72%), and exhaustion(78%) etc. All symptoms reported are listed in Figures A.1-A.7 of Appendix 2.	The symptoms reported by the 50 Australian aircrew were compared to 18 US aircrew and showed significant similarity in symptoms. This study showed that contaminated cabin air can result in adverse health effects. Further investigation regarding the finding of neurological impairment, respiratory system effects, reproductive dysfunction and long-term effect is needed according to Winder et al.. According to the authors of the study, there is still an issue on reporting health symptoms by aircrew; they are worried about job security after filling a complaint.	Winder et al. (2002b)
This study investigated a self-selected group of affected commercial aircrew and document their symptoms and treatment in relation	The aircrew members studied consisted of 39 pilots and 19 flight attendants. 51% of the respondents works in the UK, 37% in Australia, 10% in the US and 2% in Egypt.	The most common symptoms reported by the aircrew were: neurological symptoms (impaired concentration, dizziness, difficulty thinking, altered depth perception) followed by headache, fatigue and	Exposure events were most frequently detected in the BAe 146 aircraft and during take-off and ascent. Aircrew reported that health problems were underestimated and undertreated. According to the author of the study, there is still	Harper (2005)

Study design	Data set	Symptoms	Conclusion[a]	Reference
to contaminated cabin air.		mucous membrane irritation. For more than 50% of the respondents the symptoms became chronic and persistent for months or years. All symptoms reported are listed in Figure A.8 of Appendix 2.	an issue on reporting health symptoms by aircrew they are worried about job security after filling a complaint. In general, the symptoms occur with odour or fume event.	
In this study the health symptoms in aircrew were investigated while flying on the BAe 146 aircraft.	21 flight crew members were included in this survey consisting of 19 pilots and 2 flight attendants. The responded were in the age range of 30-50 years. 20% of the responded crew were female. All pilots had more than 500 flight hours per year.	A wide range of symptoms were reported such as headaches, irritation and respiratory problems and disorientation. However also coordination or memory effects were reported which could influence the flying safety. Most respondents considered that the symptoms were related to flying with the BAe 146 aircraft. All symptoms reported are listed in Table A.2 of Appendix 2.	Due to small sample size and removal of questionnaires by one of the airlines, this survey cannot be considered representative. However the small data set does report health problems by aircrew members which may be related to the contaminated air in the BAe 146 aircraft.	Cox and Michaelis (2002)
A survey of health symptoms in pilots from the British Airline Pilots Association (BALPA) flying with the Boeing 737, 757 and the airbus A320.	From the 600 questionnaires that were sent out, 106 pilots responded. 104 of the 106 respondents were male.	96 respondents reported that they experienced smoke or fume smell during flights. 93 of the B757 pilots believed that the fumes were caused by oil contamination of the air supply. The most common symptoms reported where irritation, headaches and fatigue, followed by confusion, memory impairment, diarrhoea and nausea. Improved health was observed after duty or on days off. All symptoms reported are listed in Figure A.11,Appendix 2.	According to the author of the study, leak events occur and are underreported by pilots. According to the author, this study shows that contaminated cabin air causes toxic exposure and adverse health effect in aircrew.	Michaelis (2003)
In this study a neuropsychological assessment was performed using a battery of tests on aircrew members that were exposed to jet oil emission while flying on the BAe 146 aircraft.	Neuropsychological assessment was carried out on 8 BAe 146 aircraft crew members who had been exposed to engine oil emission. All aircrew members were female in the age range of 24-56 years who flew on the BAe 146 for 2 to 12 years.	Significant impairment was observed on test of reaction time, information processing speed and fine motor skills. These findings could indicate a serious aviation safety problem.	Neuropsychological impairment has been observed on a number of neuropsychological measures by the BAe 146 aircrew who have been exposed to engine fumes. However, due the small sample size and no control group of individuals workers in the same field not exposed to BAe 146 jet engine oil emission, no strong conclusions could be drawn. Based on the findings the author would recommend to conduct a wider-scale study of BAe 146 aircrew.	Coxon (2002)
This study investigated the incapacitation of an aircraft pilot on a military C-130A aircraft after exposure to aerosolized or vaporized engine oil.	A 34 years male pilot in good health, observed adverse health effects while flying a military C-130A aircraft.	The following symptoms were observed by the pilot: headache, slight dizziness, nausea, vomiting, incoordination, and diaphoresis. 80 min after the first symptoms he was examined in the hospital. He was observed	After exposure to vaporized aerosolized or vaporized engine oil a 34 years pilot on a military C-130A aircraft observed neurological impairment and gastrointestinal distress. His clinical status returned to normal within 24 hr. Further investigation into the potential	Montgomery et al. (1977)

Study design	Data set	Symptoms	Conclusion[a]	Reference
		to be lethargic, mild dysarthria and had depressed deep tendons reflexes and anisocoria (unequal size of the eyes' pupils).	hazards from exposure to jet engine oils is highly recommended.	
The goal of this study was to raise awareness among physicians of short and long term health effects that may appear after exposure to pyrolyzed engine oil. Question: are the symptoms observed a psychosomatic disorder or neurological injury?	A single case study was used, a middle aged pilot who had been flying for 11 years on a particular type of airplane, to illustrate some of the issues regarding causation and diagnosis.	The symptoms observed by the pilot were cognitive impairment for nine-month and a 10 years history of skin ulcerations and gastrointestinal problems. During his flights he often smelt oily fumes which he supposed to find normal for this type of airplane.	General practitioner, a dermatologist and a surgeon were not able to diagnose the cause of pilot symptoms. The pilot license was taken by the aviation authority on psychiatric grounds, however the pilot was not suffering from mood disorder and has never been examined by a psychiatrist. After neurological examination, it was most likely that the symptoms of the pilot were related to exposure to engine oil fumes. According to the authors, medial protocols should be created to prevent misdiagnosis of aircrew.	Mackenzie Ross et al. (2006)
The study includes a physical examination, neuropsychological examination and a PET-scan of the brain of flight attendants.	26 North American airline attendants who were exposed to toxic fumes, emanated from the Auxiliary Power Unit (APU) to the aircraft cabin air.	Neurological abnormalities were detected in 58% of the flight attendants. Cognitive impairment such as impaired balance and coordination and movement disorder was observed. 12 of the 26 attendants were subjected to PET functional brain scan and abnormalities were observed such as occurrence of hypofrontality (decrease frontal and increase posterior brain function), and increased function in some limbic areas, especially the extended amygdala region. All symptoms reported are listed in Table A.4 in Appendix 2.	According to the author, the neurological abnormalities observed in the studies groups were most certainly caused by the exposure to chemical fumes. The symptoms observed by the attendants were often misdiagnosis by physicians. Therefore, medial protocols should be created to prevent misdiagnosis.	Heuser et al. (2005)
This study investigated the health complaints by flight crew as well as the finding regarding air quality measurements taken during test flight conditions on two BAe 146 aircraft that experienced oil seal failures. The findings were compared with two BAe 146 aircraft and a Dash 8 aircraft that never had been associated with any complaints.	Health symptoms were reported over a period of 4 months involving 5 airplanes, 35 flight, 112 individuals of a total of 200 individuals reported symptoms.	Symptoms reported by the 112 flight crew were mainly burning trout (n=48), headache (n=29), burning eyes (n=27), disorientation (n=16). The crew also complained about sharp odour in cabin, assault by toxic fumes, heavy exhaust smell, de-icing smell, soapy smell, detergent smell, dirty sock smell during take-off, oven cleaner smell, peroxide smell, acrid noxious fumes filling cabin on descent, aircraft filled with heavy blue haze, and strong smoke odor. All symptoms reported are	Most of the symptoms resolve within 24 hours. The oil smell in the BAe 146 aircraft was caused by oil leaking by one of the seals. The reported symptoms headache, nausea, disorientation could be caused by low levels of CO. Other symptoms like, burning eyes and throat, watery eyes and sinus congestion, could be caused by smoke or VOCs. Whereas neurological symptoms could be caused by hexane, octane or neurotoxic additives present in the engine oil like TCP isomers. However, no TCP isomers were detected in the air above the LOD of 80 µg/m^3.	Van Netten (1998)

Study design	Data set	Symptoms	Conclusion[a]	Reference
		listed in Table A.5 in Appendix 2.		
Evaluation of an odour incident in the cockpit of an Airbus A319 passengers aircraft in relation to 'aerotoxic syndrome'.	The evaluation was based on two pilots who reported an odour incident in the cockpit and were flying further using oxygen masks.	Both pilots reported nausea and progressive cognitive deficits. The pilot and co-pilot were sent to the hospital and after 2 hours they were sent home again. After the incident the pilot did start working again after 5 days. However the co-pilot did not work for 7 months and was diagnosed with a post-traumatic stress disorder. The odour incident was not reported as a fume event.	The German "Bundesstelle fur The German "Bundesstelle fur "Fluguntersuchungen" was not able to provide evidence of toxins in the cabin air based on toxicological studies of the incident. According to the authors, systematic investigation on health effect in relation the contaminated cabin air is lacking. Such investigation is needed to find scientifically evidence that there is a relation between contaminated cabin air and adverse health effect.	Schwarzer et al. (2014)
Case study, analysis of reported contaminated air events at one major US airline over a period of two years involving 8 types of airplanes (A319, A320, A321, B737, B757, B767 and E190.	The focus of this study was on oil fume events that were reported by the aircrew over a period of 2 years. Only some of the reported events involved also hydraulic fluid fume events. Exposure related to de-icing fluid, exhaust fumes and malfunctioning galley equipment were excluded from this study.	87 fume events were reported on 47 aircraft fleet wide. In all 8 types of aircraft fume events were reported. However, the A319, B767 and E190 appeared to be overrepresented. The most common inflight symptoms were headache, nausea, coughing and disorientation. The most post-flight symptoms were difficulties to concentrate and problems with word recall, fatigue, headache and memory deficits. Some of the pilots developed chronic neurological symptoms post-flight and lost their license. In 83 of the reported events unusual odour was reported described as "dirty socks".	On 41 of the 87 events mechanical records confirmed that oil contaminated the air supply. In 68 of the 87 reported events flight attendants reported symptoms and in almost half of those events the symptoms were so serious that emergency medical care was needed. Flight attendants were less protected to fume events than pilots: they are trained to use oxygen masks if events occur, flight attendants are not trained for that. According to the author, air crew need to be better trained to recognize and respond to events and maintenance workers need to be better trained to identify and solve the problem. Filters and/or monitoring equipment are needed to detect events and prevent them.	Murawski et al. (2011)
In this study symptoms from seven case studies, from aircrew in four airlines operating in four countries and in three aircraft models (B747, Fokker 100, BAe 146) were investigated.	Seven case studies consisting of aircrew from four airlines operating in four countries and in three aircraft models.	A broad range of symptoms have been reported by the aircrew after exposure to contaminated cabin air. Symptoms from single or short term exposure: 1) neurotoxic symptoms such as tunnel vision, disorientation, shaking, loss of balance. 2) neuropsychological symptoms, memory impartment, headache, confusion and feeling intoxicated. 3) gastrointestinal symptoms. 4) cardiovascular symptoms. (5) irritation of eyes, nose and upper airways. Symptoms from long term	According to the authors, exposure to fume event is known to be toxic and if happened to pilots it could influence the flight safety. According to the authors factual evidence is available that aircrew and passengers can be directly exposed to airborne chemicals on aircraft in sufficient concentration to cause acute, immediate to long-term symptoms.	Winder and Balouet (2001)

Study design	Data set	Symptoms	Conclusion[a]	Reference
		low level exposure: 1) neurotoxic symptoms, paresthesia, numbness fingers, lips and limps. 2) neuropsychological symptoms, memory impairment, lack of coordination. 3) gastrointestinal symptoms, 4) respiratory symptoms, breathing difficulties. 5) cardiovascular symptoms. 6) skin symptoms. 7) irritation of eyes, nose and upper airways. 8) sensitivity, immunosuppression, multiple chemical sensitivity. 9) general, weakness and fatigue. All symptoms reported are listed in Table A.6 in Appendix 2.		
Summary of aircraft air quality incident, symptoms, exposures and possible solutions.	Symptoms were reported by aircrew members flying for an unreported airline company on a MD-80 aircraft and by aircrew from another unreported airline company flying on a mixed fleet of aircraft.	Exposure to contaminated bleed air varied within an aircraft due the differences of air supply in the cockpit (100% fresh bleed air) compared to the flight deck (60/40 recirculated/fresh bleed air). Central nervous system symptoms were predominantly followed by gastrointestinal and respiratory system problems.	According to the author, most of the aircraft air quality incidents can be traced to contamination of the bleed air with jet engine oil and/or hydraulic fluid. The symptoms reported by the aircrew may be related to CO and TCP. Long term chronical effect are difficult to trace back to exposure events. Because the symptoms are a results of exposure to low levels of contaminated bleed air for a long period of time.	Van Netten (2005)
This study investigated if there is a link between exposure to contaminated bleed air and neuropsychological impairment. Comparing an “exposed” group flying on the BAe 146 and B757 to an “unexposed” group flying on the B737.	Questionnaires were sent to 1500 pilots; only 70 questionnaires were returned. Randomly 29 pilots were asked to undergo neuropsychological assessment. 15 pilots were in the exposed group flying the BAe 146 and B757 and 14 pilots were in the unexposed group flying the B737.	Physical and neurological symptoms were identified among airline pilots. Despite the weaknesses (exposed versus unexposed by air type flown and relative small sample size) the findings warrant further investigation.	No significant differences were identified between the two groups on the neuropsychological tests or mood state measures. However, it was observed that the air type flown is not a reliable indicator of exposure history because in both groups multiple fume events have been reported. The profile of the cognitive performance was different than that of an normal population and comparable to that of framers exposed to organophosphates. Cognitive performances are showing dips in test on attention, psychomotor speed and visual sequencing.	Mackenzie Ross et al. (2006)
This study investigated cognitive impairment in aircrew by an extensive neuropsychological test battery, and if a neurobiological substrate could be found for their	12 aircrew members (10 male, average age of 44, 8130 flight hours) compared to matching control group consisting of 11 race car drivers (10 male, average age of 43 years, 233 flight hours).	The aircrew groups reported complaints regarding cognitive impairment.	Aircrew reported significant more self-reported cognitive complaints and depressive symptoms compared to the control group. However, the differences did not reach statistical significance. Small brain regions in which brain white matter microstructures were affected and higher	Reneman et al. (2015)

Study design	Data set	Symptoms	Conclusion[a]	Reference
complaints by using MRI techniques.			cerebral perfusion values in the left occipital cortex were observed. The extent of cognitive impairment was strongly associated with white matter integrity, but extent of estimated number of flight hours was not associated with cognitive impairment nor with reductions in white matter microstructure.	
5 'aerotoxic syndrome' cases documented by the Federal Institute for Risk Assessment (BfR) were investigated regarding possible risks for fume events associated with TCP-contamination cabin air.	5 'aerotoxic syndrome' cases in Germany.	Health impairment however no specific symptoms are reported.	In none of the 5 'aerotoxic syndrome' cases studied, documented by the Federal Institute for Risk Assessment (BfR), causality between possible inhalation exposure and the health impairment that occurred was found. According to the authors, TCP poisoning is unlikely.	Hahn et al. (2013)
This study investigated the association between neurological deficits and elevated levels of autoantibodies in flight crew.	12 healthy controls were compared with 34 flight crew (pilot and attendants) who experienced adverse health effects after exposure to bleed air and had medical attention. Also 1 case study of a single pilot was included who was followed for 21 months and suffered health problems after exposure to contaminated cabin air.	The most common symptoms reported by the 34 aircrew members were memory deficits (78%), headaches (62%), fatigue (53%), muscles weakness (42%) and imbalance (35%). All symptoms reported are listed in Figure A.9 in Appendix 2.	The limitation of this study was the small sample size and the lack of identification and quantification of the chemicals to which the flight crew were exposed to. However, according to the authors this study supports an association between self-reported neurologic deficits and levels of autoantibodies against neuro- and glia-specific proteins in sera form 34 flight crew member compared to a control group. Development of biomarkers as reported in this study may help to detect chemical-induced central nervous system injury.	Abou-Donia et al. (2013)
A health survey on 4011 flight attendants from two airline companies was compared to data from a health survey of a general population (data used from the National Health and Nutrition Examination Surveys (NHANES)	Addresses were randomly chosen from union provided lists of flight attendants. The mean age of the flight attendants were 47 years. 20% was male. 41% had more than 20 years of working experiences. 9% were current smokers and 22-30% were former smokers.	The largest number of work related injuries were musculoskeletal (33%), respiratory (23%), neurological problem (17%), psychological problems (14%). Significantly elevated standardized prevalence ratios (SPR) were observed for chronic bronchitis, cardiac disease, diagnosed sleep disorders, fatigue and depression for the flight attendants compared to the general population. Health symptoms reported by the flight attendants are listed in Table A.7 in Appendix 2.	Almost 50% of the flight attendants reported one or more work related injuries. This compared to 4.2% for all industries and 10.2% for the transportation (BLS statistics).	OHRCA (2014)
This study measures residues on the internal surfaces of aircraft and control environment to	86 wipe samples were collected using ethanol-moistened glass fibre filters. Wipe samples were	The mean levels observed in the aircraft ranged up to 3×10^4 ng/m^2 for TCP, to 10^6 for butyl diphenyl phosphate (BDPP), to 7 x	Estimated maximum airborne concentrations were calculated for tri (o,o,o)-cresyl phosphate (0.001-0.0006 µg/m^3) and TnBP (10-40 µg/m^3). The levels are in	IOM report (2012)

Study design	Data set	Symptoms	Conclusion[a]	Reference
further investigate fume events.	collected from 6 sites, consisting of 4 airports and 2 control sites; divided over 5 types of airplanes, 2 types of vehicles and 2 offices.	10^5 for DBPP and to 9 x 10^4 for TnBP. Tri-n-butyl phosphate (TnBP), BDPP and DBPP[b] were in general higher in the wipes collected in the cockpit compared to the passengers area. The levels of TnBP, BDP and DBPP were also higher in wipes from aircraft and airport based vehicles than in offices. The TCP levels were higher in planes than in other places, with may indicate that TCP originate from aircraft sources.	line with those reported in literature.	
This study is based on investigation and prevention of accidents and incidents, which have been reported by the German Federal Bureau of Aircraft Accidents Investigation (BFU) between 2006-2013. The goal of this study was to prevent future accidents and incidents.	663 fume events are investigated in this report, in 460 cases smell and in 188 cases smoke developed. In 180 reports health impairment was reported. However, in only 15 cases health impairments may possibly have a conjunction with cabin air quality.	Health impairment reported by 66 pilots were light-headedness (n=1), tremor of hands (n=1), nausea (n=3), eye irritation (n=6), headache (n=7), dizziness (n=8), other (n=8) and multiple (n=32). Health impairment reported by 105 cabin crew were: light-headedness (n=1), eye irritation (n=4), nausea (n=6), dizziness (n=6), others (n=7) headache (n=17) and multiple (n=64). Heath impairment reported by 21 passengers were: dizziness (n=1), headache (n=1), nausea (n=5), other (n=6) and multiple (n=8).	Fume events occurred and resulted in contaminated cabin air. According to the authors, contaminated cabin air has led to health impairments in occupants and cabin crew. Identification of toxic compounds (such as TCP) in the cabin air was not performed in the fume events the BFU investigated.	German Federal Bureau of Aircraft Accident Investigation (2014)

[a] NB: The conclusions were reported by the authors from each study; these conclusions were not reviewed or evaluated by the authors of this report.

[b] TBP: tri-n-butyl phosphate, BDPP: butyl diphenylphosphate, DBPP: dibutyl phenyl phosphate. Analysis of subcategory 2: measurements of oil compounds in an airplane.

3.4.2 Analysis subcategory 2: measurements of oil compounds in an airplane.

The 9 selected papers were intensively screened for compounds found in realistic settings in an airplane and summarized in the Table 3.6.

Table 3.6. Study design, information regarding the data set studies, and general conclusions by the authors of the studies were summarized for each paper.

Study design	Data set	Concentrations	Conclusion	Reference
In this study methods were developed to measure the exposure to organophosphates	4 airline companies in Norway were included in this study. In total 40 aircraft were sampled consisting of jet engine airplanes,	95 within-day OP samples were collected from cabin air in 47 flights (jet airplanes, propeller airplanes and helicopters). TCP[1] (sum of tri(m,m,m,)-	TCP levels were an order of magnitude higher in the air samples collected from the cabin during a ground experiment on an airplane that experienced turbine oil leakage compared to	Solbu et al. (2011)

[1] TCP comprises 10 isomers, based the arrangement of the three cresyl groups (ortho, meta or para arrangement).

Study design	Data set	Concentrations	Conclusion	Reference
(OPs) from jet engine oils and hydraulic fluids among aircrew members. The sampling methods included within-day air sampling for OPs using Chromosorb 106, and VOCs using Tenax-A 60/80 mesh as well as passive long term methods by deposition of OPs using wipe surface area and activated charcoal cloths (ACC). All samples were collected under normal flight conditions.	propeller airplanes and helicopters. The samples were collected in the cockpit and the passengers cabin.	cresyl phosphate, tri(m,m,p)-cresyl phosphate, tri(m,m,p)-cresyl phosphate and tri(p,p,p)-cresyl phosphate was only detected in 4% of all within-day samples, only in propeller airplanes, with levels range from <75 ng/m^3 to 290 ng/m^3. No ortho-isomers of TCP were detected. Triphenyl phosphate was only detected in one propeller airplane with a concentration of 110 ng/m^3. TnBP was detected in all jet engine and propeller airplanes and in 58% of the helicopters sampled with levels range from 24 to 4100 ng/m^3. BDPP was only detected in the jet engine and propeller airplanes with levels range from <75 to 310 ng/m^3. Passive long term sampling was only performed in jet engine and propeller airplanes using wipe (n=56) and ACC (n=56). The OP levels detected were summarized in Table A.8 of Appendix 3. Overall, in the wipes the TCP levels range from <0.05 to 8.3 ng/dm^3 per day, TPP levels range from <0.05 to 15 ng/dm^3 per day, DBPP levels range from <0.05-20 ng/dm^3 per day. TnBP levels range from <0.05-19 ng/dm^3 per day. And the less frequently detected tri-iso-butyl phosphate (TiBP) levels range from <0.05 to 0.42 ng/dm^3 per day. In the ACC the TCP levels range from <0.9 to 270 ng/dm^3 per day, TPhP levels range from <0.05 to 7.6 ng/dm^3 per day, DBPP levels range from 1.7- 970 ng/dm^3 per day. TnBP levels range from 56-16000 ng/dm^3 per day and the TiBP levels range from 5.6 to 390 ng/dm^3 per day. In all 6 HEPA filters from the jet engine airplanes TCP was detected with levels range from 1.1 to 4.3 ng/g per hour. Again no ortho-isomer of TCP was detected.	"after engine replacement". Ortho-isomers of TCP were not detected in any of the samples collected in this study. Wipe sampling is in general favors sampling of non-volatile OPs (TCP and TPhP) whereas ACC sampling resulted in high recovery for all alkyl OPs. Still, for the long term sampling, wipe samples were preferred over ACC samples for the non-volatile OPs due lower LOQs and higher extraction recovery. There was no differences in concentration observed between sampling the cockpit versus the passengers cabin.	

Study design	Data set	Concentrations	Conclusion	Reference
In this study the presence of VOCs in cabin air of aircraft were studied to identify possible emission sources. A receptor model using positive matrix factorization was used to couple the measured VOC levels with information related VOC sources to identify the major VOC sources in the aircraft cabin.	84 air samples (Tenax-TA tubes) were collected during 14 flight on a B737-800, The duration of sampling ranged from 80 to 190 minutes.	In total 19 VOCs were detected in air samples collected during the 14 domestic flights. All compounds and median and mean levels are reported in Table A.9 of Appendix 3. Highest levels were found for d-limonene (median of 31 µg/m^3), followed by decanal (median of 24.43 µg/m^3), nonanal (18 µg/m^3), toluene (13 µg/m^3) 5-hepten-2-one, 6-methyl-styrene (11 µg/m^3) and benzene (10 µg/m^3).	29% of the total VOC emission was attributed to service of humans, followed by chemical reactions (15%), fuels (13%), materials (12%), combustion (12%), non-fuel oil (9%), cosmetics and perfumes (5%), and cleaning agents (4%). Benzene (69%) followed by acetic acid (11%) and octanal (10%) attributed to the total VOC concentration related to non-fuel oils.	Wang et al. (2014)
The materials research laboratory was requested to identify the chemical composition of the odorous vapours in the environmental control system of the Royal Australian Air Force (RAAF) Hercules C-130 aircraft.	Bleed air samples were collected (using Porapak Q adsorbent tubes) from the bleed air adducts from the cargo/cabin compartment, while the engine was operating in a variety of situations. 4 aircraft were sampled during the fight. One of the four was also sampled on the ground. From each airplane 5 samples were collected. Additional pyrolysis experiment were performed with Avtur jet fuel at 25 °C, and with the 2 hydraulic fluids (MIL-L-23699C, NATO 0-146, MIL-H-5606E NATO H515) at 100 and 200 °C. The Exxon 2380 was also pyrolysed at 450 °C.	TCP was not detected in any of the air samples collected. However some trace levels of organophosphorus compounds, particularly TCP was found in the air filter bags. Avtur jet engine leakage from the fuel nozzle produces a continuous background of hydrocarbon vapours (0.1 -0.5 PPM). No evidence of any vapour contamination, other than avtur, from in-flight air samples was found. Trace levels of the neurotoxic trimethylolpropane phosphate (TMPP) was detected during the laboratory pyrolysis experiment. However there was no evidence that this compound is present in samples taken from the aircraft.	No evidence was found that neurotoxic bicyclophosphorus compound derived from the oil additive are present in the cabin air. The authors recommend that additionally to the normal maintenance, the use of charcoal cloth filters need to be further investigated to absorb the noxious odours.	Kelso et al. (1988)
This study investigated the presence of TCP in cabin air from two airplanes. The focus in this study is on the mono-, di- and tri-ortho-TCP.	90 air samples were collected during 26 flight on two airplanes which had two Rolls-Royse turbine engines. Samples were taken during the take-off (25 min) and during the total flight (5h) with 2 L/min.	In the engine oils (Mobil jet oil II) the ortho-TCP levels were < 20 µg/kg). In 15% of the samples o,o,o-TCP was detected with levels ranged from 2 to 65 ng/m^3. This was during normal flight conditions. The total TCP concentration in the air samples ranged from 17 to 167 ng/m^3.	Strong correlation (R^2 = 0.81) between the tri(o,o,o)-cresyl phosphate levels in air of the cockpit and air collected in the passenger cabin was observed. The total TCP concentration is higher during take-off compared to the samples taken over the entire flight.	Rosenberger et al. (2013)
The aim of this study was to develop a procedure to monitor TCP in cockpit and cabin air of an aircraft. By using this	3 different airplanes from the ADF were sampled: the fighter trainer (FT), cargo transport (CT) and fighter bomber (BF).	Mono, di or tri(o,o,o)-cresyl phosphate were below LOD in all collected samples. The following TCP isomers (mmm, mmp, mpp and ppp) were	The highest level observed was 51.3 ug/m^3 during this flight smoke and odour was reported. However, the report of smoke and odour did not necessarily correlate with TCP	Denola et al. (2011)

Study design	Data set	Concentrations	Conclusion	Reference
method the TCP concentration in Australian defence force (ADF) aircraft were measured in order to assess potential risks for the exposure to TCP.	Long duration air sampling was performed with 0.06 g of Porapak-Q glass tube with 2 L/min. Short term air sampling was performed with metricel filters (GN; 0.8 µm) at 36 L/min.	reported in some of the samples and were reported as total TCP. In only 11 of the total of 78 samples the total TCP levels were just above the LOQ with total TCP levels range from 0.12 to 4.99 µg/m^3. In only 2 samples the total TCP levels (21.7 and 51.3 µg/m^3) were higher than 10 times the LOQ. Other peaks were present in the gas chromatogram with similar retention times as TiBP, TnBP and TPhP.	concentrations in other incidents. In two flights smoke was reported however no TCP was detected above the LOQ. The results of this study indicate a low health risk from TCP exposure.	
This study investigated the health complaints by flight crew as well as the finding regarding air quality measurements taken during test flight conditions on two BAe 146 aircraft that experienced oil seal failures. The findings were compared with two BAe 146 aircraft and a Dash 8 aircraft that never had been associated with any complaints.	Aircraft 1: complaints about air that made flight crew ill. BAe 146 was flying on Castrol 5000. In the evening the oil was replaced with Exxon 2380. VOCs were sampled using charcoal absorbents tubes (0.1 L/min) and higher molecular weight hydrocarbons with filter cassette (2 L/min). Aircraft 2: complaints about air quality during 2h flight. CO and CO_2 measurements were performed. Aircraft 3: BAe 146 no complaints monitored for 3 h flight. Aircraft 4: no complaints monitored for 4 h with charcoal filter. Aircraft 5: monitored for 3h.	Aircraft 1; BAe 146 sampled for 1.5 h. 2 min after take-off oily smell. During test flight number of ascent and descents were made to simulate take-off and landing. VOCs detected in the air were long chain hydrocarbon derivatives, 3,7-dimethy-1,3,6 octatriene, 3-isopropoxy-1,1,1,7,7,7 hexamethyl-3,5 and 5 siloxane derivatives. No clear differences between VOC detected in the cockpit compared to the rear end of the aircraft, with the exception of hexadecamethyl heptasiloxane which was only found in the rear end of the aircraft. The sources of these compounds were not identified in this study. Major oil compounds could not be detected in the air of aircraft 1. They may be filtered out by the APU or condensed in the ventilation system. In all airplanes the CO_2 levels increased after landing of the passengers and before take-off, and decreased until landing. In aircraft 2 the CO_2 levels ranged from 528 to 900 ppm. The CO_2 levels in aircraft 3, 4 and 5 ranged from 800-2300 ppm. CO was not detected in any of the airplanes above the LOD of 1 ppm. O_2 levels decreased after take-off and increased prior to landing. The average O_2 percentage in the airplanes was 21.7 %.	TCP was not detected in the air of the aircraft that suffer from smoke odour and oil leakage. In flight oil seal failure in jet engines of BAe 146 aircraft was traced as the source of smoke in the cabin.	Van Netten (1998)
Summary of aircraft	Symptoms were	In this study no	The symptoms reported by	Van Netten (2005)

Study design	Data set	Concentrations	Conclusion	Reference
air quality incident, symptoms, exposures and possible solutions.	reported by aircrew members flying for an unreported airline company on a MD-80 aircraft and by aircrew from another unreported airline company flying on a mixed fleet of aircraft.	measurements were performed on oil compounds in air planes.	aircrew members appear very consistent with the symptoms of CO and TCPs. Exposure measurement in airplanes during air quality incident is rare but is needed to connect the symptoms observed by aircrew members to those that have been reported for these agents in the literature.	
Investigation of the presence and concentration of five TCP isomers (ooo-TCP, mmm-TCP, mmp-TCP, mpp-TCP and ppp-TCP) in the cockpit of 737 Boeing aircraft under normal flight conditions and during the operation of the auxiliary power unit (APU) only.	80 air samples were collected from 4 B737-700s, 3 B737-800s, 3 B737-900s and from 2 B737-700s and 800 while running the APU on the ramp. Sampling was performed using Chromosorb 106 in glass tubes in combination with glass filters. Wipe samples were taken before and after the flight.	No events were reported during any of the flight. In 37 of the 80 air samples measureable TCP-isomers have been detected. The levels ranged from (the lowest detectible level) 0.5 ng/m^3 to 155 ng/m^3, with an average of 6.9 ng/m^3. Highest TCP levels were observed during climb and descent. The TCP levels observed in the wipes ranged from 0.01 to 0.06 ng/cm^2.	According to the authors, it is likely that the emission of particles containing TCP isomers in the cockpit is discontinuous. No detectible ooo-TCP was found in the air samples, wipe samples or oil samples analysed in this study.	TNO report (2013b)
Analysis of cabin air for VOCs, SVOCs, particles and CO under normal flight conditions and during fume or air quality events.	100 flights in 5 different aircraft (B757 cargo, B757, A320/1, BAe 146 and A319) were monitored in this study. Sorbent tubes containing Tenax TA were used for the sampling. Besides total VOCs and ultrafine particles numbers the following target compounds were included in this study: ooo-TCP, the other TCP isomers, TnBP, toluene, meta+para-xylenes, limonene, tetrachloroethylene (TCE) and undecane.	Concentrations of toluene, limonene, xylenes, undecane and TCE in cabin air were comparable with levels observed in homes in developed countries. Total VOCs levels were mostly below 2 ppm. Higher levels were reported during air quality events. Levels of CO are in some cases even higher in homes than in the aircraft cabin and were mostly below 2 ppm. In more than 95% of cabin air samples no total TCP of ooo-TCP was detected. Highest ooo-TCP level of 22.8 µg/m^3 was observed during climb of the aircraft (overall mean 0.07 µg/m^3). The overall mean total TCP levels was 0.14 µg/m^3 (with a maximum of 28.5 µg/m^3). The highest TnBP levels recorded was 21.8 µg/m^3 with an overall mean of 1.07 µg/m^3.	No fume events occurred that triggered the airline's protocols for formal reporting of incidents. However during 38 flights, fumes/smell events were reported in the post flight questionnaires. Samples collected during air quality event did not contain elevated levels of any of the target compounds included in this study.	Crump et al. (2011), part 1

3.4.3 Analysis subcategory 2: In vitro or in vivo toxicity tests conducted with aviation engine oil or its fumes

Temperature ranges for pyrolysis experiments on jet engine oils.

The pyrolysis experiments described in the selected papers were performed at various temperature ranges. Examples are summarized below.

Crane et al. (1983) used temperatures ranging from 300 to 600°C for the evaluation of thermal degradation products from aircraft engine oil temperatures ranging from 300 to 600°C. No specific information was given on the selection of the temperature range, only that measured carbon monoxide (CO) occurred at 306°C and doubled in concentration when the temperature increased from 350 to 533°C (up to 10600 ppm). Crane et al. (1983) concluded that the temperature of 400°C is an adequate model for thermal degradation in the turboprop engine. Crane et al. (1983) also concluded that none of the formed pyrolysis products generated was more toxic to rats than the quantity of CO that was formed.
Porvaznik et al. (1987) evaluated the acute dermal toxicity of a thermally decomposed military specification synthetic aircraft lubricant. The temperature of the pyrolysis experiment ranged from 300 to 700°C. Again no specific information was given for temperature range used in this paper. However, the formation of trimethylolpropane phosphate (TMPP) was evaluated showing that it was formed at temperature around 440°C, and increased significantly in concentration with temperature. Provaznik et al. (1987) concluded that TMPP was formed during pyrolysis of the engine oil if the temperature was higher than 400°C. The engine oil contained trimethyl propane triheptanoate (TMP) and tricresyl phosphate (TCP) or triaryl phosphate (TAP).

Van Netten et al. (2000, 2001) and van Netten and Leung (2000) used a temperature of 525°C to perform pyrolysis experiments on various engine oils. Bleed air was diverted from a location just prior to the engine combustion chamber at a temperature around 500°C. Van Netten et al. (2000, 2001) and van Netten and Leung (2000) also mentioned that 525°C is the optimum temperature for the formation of TMPP.
The overview of Chaturvedi et al. (2010) specified that oil leaks related to dysfunctional seals can be subjected to temperatures of 500°C and higher.
Hildre and Jensen (2015) observed that bleed air from the high compression stage ranges from 350 to 600°C, whereas it ranges from 100 to 300°C when taken from the low stage compressor (Spittle, 2003).
Michaelis (2011) reports that fogs formed during pyrolysis of the jet engine oil (MIL-L-7808) at temperature ranged from 204 to 288°C were less toxic than those formed at 315°C (Treon et al., 1954, Treon et al., 1955).
Michaelis (2011) reports that typically high-stage engine compressor temperatures can vary from 300 to 650°C (Spittle, 2003) or from 450-600°C (AT&H. 2000) and go up to 650°C in a B767 at take-off power (Hunt et al., 1995).
In the report of National Research Council (2002) much lower temperatures were reported. Those do not exceed temperatures higher than 350°C (Table 3.7).

Table 3.7. Typical conditions from bleed air of an aircraft engine (Table is copied from National Research Council (2002)).

Mode of Operation	Temperature, °C (° F)	Absolute Pressure, kPa (psi)	Extraction Stage
Takeoff—maximal power[a]	350 (660)	1170 (170)	Low pressure
Top of climb	310 (590)	690 (100)	Low pressure
Cruise	250 (480)	340 (50)	Low pressure
Initial descent	185 (365)	200 (29)	High pressure
End of descent (ground level)	230 (445)	460 (67)	High pressure
Switchover from high to low pressure[b]	280 (535)	480 (70)	High pressure
Ground operations	170 (340)	—	Auxiliary power unit

[a]Some engines (e.g., Avro/4GRJ) have only one bleed port; during takeoff, temperatures might exceed values given.
[b]Maximal temperature and pressure for high-pressure stage occur just before bleed air system automatically switches from high-pressure port to low-pressure port.
Source: Responses from Boeing to committee questions, July 13, 2001 .

Overall, it can be concluded that the temperature in an aircraft engine compartment, where oil vapour and pyrolysis products may be formed, can reach temperatures above 500°C. It was also observed that more toxic fumes (containing CO and TMPP) were generated at temperature higher than 400°C.

3.4.4 The analysis of subcategory 4: Composition of aviation engine oil, fumes or pyrolysis products.

Composition of the aviation engine oils.
Porvaznik et al. (1987) reports that military specification L-23699 synthetic aircraft lubricants contains trimethyl propane phosphate (TMP), pentaerythritol monobutyrate triheptanoate (PE), tricresyl phosphate (TCP), or triaryl phosphate (TAP).

Van Netten (1999) analyzed jet engine lubricant oils for toxic element. However no toxic elements (such as lead, mercury and thallium) were identified in any of the engine oils (Exxon 2380, Mobil and Castrol 5000).

Van Netten et al. (2000, 2001) and van Netten and Leung (2000) measured the composition of two commercially available jet engine oils (Castrol 5000 and Exxon 2380). Various compounds were detected in the oil samples which are listed in Table 3.8. It is noted that the identified compounds in Table 3.9 were not all confirmed with appropriate standards.

Winder and Balouet (2002a) examined the ingredients in jet engine oils and indicated that at least two ingredients are hazardous: N-phenyl-1-naphthylamine and tricresyl phosphate (TCP). Other compounds listed on the material safety data bulletin (MSDB) from Mobil jet oil are listed below.

- Synthetic esters (mixture of 95% C5 – C10 fatty esters of pentaerythritol and dipentaerythritol.
- 3% tricresyl phosphate
- 1% phenyl-α-naphthylamine (N-phenyl-1-naphthylamine) (PAN) (CAS No. 90-30-2)
- Benzamine (4-octyl-N-(4-octylphenyl) CAS No. 101-67-7)

Winder and Balouet (2002a) report that the commercial product of N-phenyl-1-napthylamine is 99% pure, however, can contain the following six impurities N-phenyl-2 naphthylamine (500 to 5000 ppm), 1-naphthylamine (below 100-

500 ppm), 2-naphthylamine (below 3-50 ppm), aniline (below 100-2500 ppm), 1naphthol (below 5000 ppm) and 1,1 dinaphthylamine (below 1000 ppm).
De Nola et al. (2008) screened jet engine oils for the presence of 10 isomers of TCP. De Nola et al. (2008) were able to separate 9 of the 10 isomers by GC-MS/MS (only the omm-TCP and oop-TCP were coeluting). The ortho isomers of TCP observed in the jet engine oils consist almost exclusively of mono-ortho-isomers in a concentration range of 13-150 mg/L.

The mmp-, mmm- and mpp- TCP isomers were dominating in the jet engine oil. The omm- and omp-TCP isomers were found at low concentrations while the opp-, oox- and ooo-TCP isomers were not detected. The ratio of the m/z observed for the isomers are different. This helps identifying and separating the isomers. Whereby m/z 165 fragment ion was the base peak for all the six ortho-isomers. m/z 168 was the base peak for the four isomers containing only p- and m- substituents. An m/z 243 fragment was also characteristic of the xxx-isomers (where x= m or p). Also m/z 277 was more abundant in the o-isomers compared to the m- and p- isomers. However, the intensity may vary with instrument operating conditions.

Other compounds that have not been mentioned above, which were summarized in the following review report (Expert Panel on Aircraft Air Quality, 2011) are listed below.

On the MSD of Mobil jet oil II from Australia and Canada is written that it contains <2% alkylated diphenyl amines.

NYCO SA produces Turbonyciol 600, which contains triphenylphosphate (TPhP) rather than TCP.

OHRCA (2014) describes the analysis of four isomers of TCP (ooo-TCP, mmp- TCP, mpp-TCP and ppp-TCP) in nine jet engine oils. In three of the nine engine oils (Aeroshell 560, BP 2389 and BP2197), ooo-TCP was detected with a concentration of 0.01%, just at the detection limit. Overall the TCP isomer patterns observed in the nine engine oils were comparable, with mmp-TCP as the most dominant isomer followed by mmm-TCP, mpp-TCP and ppp-TCP with an relative concentration of 49%, 29%, 22% and 0.2%, respectively. In six of the nine jet engine oils (Mobil II,BP 2380, used BP2380, Mobil 291, Exxon O-156 and Mobil 245) the total-TCP concentration was around 5%, which is higher than the 3%, which is often referred to in the MSDS for these oils. OHRCA (2014)(OHRCA, 2014) also reports that in NYCO jet engine oil, TCP was replaced by triisopropyl phenyl phosphate (TIPP). Whereas others have reported that in the NYCO jet engine oils, TCP is replaced by TPhP.

The compounds observed in jet engine oils and hydraulic fluids by Hildre and Jensen (2015) are listed below.
- N-phenyl-1-naphthylamine (CAS No. 90-30-2)
- Alkylated diphenylamine (CAS No. 122-39-2)
- Butyl diphenyl phosphate (CAS No. 2752-95-6)
- Dibutyl phenyl phosphate (CAS No. 2528-36-1)
- Isopropylated triphenyl phosphate (CAS No. 68937-41-7)
- Phenol, dimethyl-, phosphate (3:1) (CAS No. 25155-23-1)
- Tributyl phosphate (CAS No. 126-73-8)
- Tricresyl phosphate (CAS No. 1330-78-5)

Note that the compounds listed above could be present in jet engine oils or in hydraulic fluids. No distinction was made in this report.

Pyrolysis and thermal degradation products formed during pyrolysis of aviation engine oils.
Crane et al. (1983) concluded that during pyrolysis of six jet engine oils (including Exxon 2380, 2389 and Mobil II jet oil) none of the products generated a smoke component that is significantly more toxic to rats than the quantity of CO produced. CO was the only pyrolysis product identified in this paper.
Provaznik et al. (1987) only quantified TMPP in the condensate of the pyrolysis engine oils at temperatures higher than 440°C.

Van Netten et al. (2000, 2001) and van Netten and Leung (2000) performed pyrolysis experiments on two commercially available jet engine oils (Castrol 5000 and Exxon 2380). The pyrolysis experiments were performed for 1 min at a temperature of 525°C. Various pyrolysis products were identified and are listed in Table 3.9. It is

noted that the identified compound in Table 3.9 were not all confirmed with appropriate standards). No evidence was found for the generation of NO_2 and HCN but the CO_2 and CO levels increased in time. However, TMPP was not detected in this study. Some of the volatilized and pyrolysis produces generated in this study may not reach the cabin air because they may condensate onto the ducts of the aircraft ventilation system. This indicates that through condensation some of the pyrolysis products accumulated onto the ducts of the ventilation system. This was also observed by Rubey et al. (1996) for TMPP, which was not observed it the gaseous phase during incineration of jet engine oil but in the scrapings of the boiler walls.

Therefore, it would be unlikely that TMPP produced during pyrolysis would end up in cabin air, unless the temperature of the ducts for some reason would become elevated.

Winder and Balouet (2002b) examined the ingredients of jet engine oils and suggested that the following chemical may release after pyrolysis of jet engine oil;

- Combustion gases such as carbon dioxide and carbon monoxide
- Other irritating gasses, such as oxides of nitrogen
- Partially burnt hydrocarbons (including irritating and toxic by products, such as acreolein and other aldehydes)
- TCP and TCP thermal degradation products (TCP boils at 420°C)

Ramsden et al. (2013) suggested that the higher ortho-TCP/TCP ration observed in bleed air compared to the ration observed in the engine oil may be related to isomerization of the TCP within the engine during operation. Investigation on the isomerization of cresol at 380°C using a solid phase catalyst resulted in an equilibrium composition of 36% ortho, 48% meta and 16% para.

The National Research Council (2002) reports that formaldehyde, acetaldehyde and acreolein could be found in engine oil or could be formed during thermal decomposition of engine oil (Nagda et al., 2001).

CAA (2004) reports that jet engine oil breakdown products could contain over 40 different chemicals, and that most of them have no published toxicity data. Various organic acids were observed in the pyrolysed jet engine oils. Most likely the short-chain organic acids such as valeric and pentatonic acid may be responsible for the “old sock” odor observed in airplanes. Furthermore, differences in composition were noticed between used and new oil both for unpyrolysed oil as well as for pyrolysed oil (at 350°C; 350°C high humidity and 450°C). It also analysed the various compounds observed in the environmental control system ducts from BA 146 aircraft. Figures 3.1-3.4 show the results.

Overall, TCP isomers and TMPP were the most frequently reported compounds in the engine oil or formed during pyrolysis. It is obvious that in most earlier studies, TCP isomers and TMPP were the compounds of high interest. However, many other compounds are present in the oil and even more can be formed during pyrolysis of the jet engine oil. The compounds listed above and listed in Table 3.8 and 3.9 can be used to generate a suspect compound list to screen the engine oils tested in this study. During pyrolysis many compounds may be formed. The resulting products formed are highly dependent on the conditions under which the pyrolysis experiment is achieved. Besides the TCP-containing turbine engine oils, nowadays TCP-free engine oils are available that contain TPhP or TIPP instead of TCP. It would be interesting for the pyrolysis experiment performed in our study to see whether the pyrolysis product profile would be different between TCP-free and TCP-containing turbine engine oils.

Table 3.8. Various compounds detected with GC-MS in two jet engine oils, Castrol 5000 and Exxon 2380. (Table is copied from Van Netten and Leung (2000).

Bulk oil analysis			
Ret. time	Compound	Fit	Cas #
	Castrol 5000		
1049	8-Benzylquinoline	972	28748-19-8
	2-naphthylamine, N-phenyl-	963	135-88-6
1274	Phosphoric acid, tris(3-methylphenyl)ester	989	563-04-2
1287	Phosphoric acid, tris(3-methylphenyl)ester	986	563-04-2
1301	Phosphoric acid, tris(3-methylphenyl)ester	984	563-04-2
1316	Phosphoric acid, tris(4-methylphenyl)ester	968	78-32-0
	Phosphoric acid, tris(3-methylphenyl)ester	966	563-04-2
1328	1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-,trans-	892	34315-34-9
1378	3,3-dimethyl-5-(2,2-dimethylpropyl)tetrahydrofuran-2-one	978	0-00-0
1436	Gallium, tetraethyl-di.mu.-1-piperidinyldi-	899	42777-03-7
	Silane derivative	845	56771-62-1
1504	1,8-Dihydroxyanthraquinone	897	7336-68-7
1590	Decanoic acid, 1,2,3-propanetriyl ester	864	621-71-6
1690	Decanoic acid, 1,2,3-propanetriyl ester	813	621-71-6
1807	1,8-Dihydroxyanthraquinone	891	7336-68-7
1957	8-methoxy-2-(p-methoxyphenyl)-1,2,4,5-tetrahydro-1-benzazocine-3,6-dione	903	90732-26-6
	Decanoic acid, 1,2,3-propanetriyl ester	837	621-71-6
2132	4-hydroxyanthraquinone-2-carboxylic acid, di-TMS	868	0-00-0
2615	Methanone, (4-ethoxy-3-methoxyphenyl)(6-methyl-1,3-benzodioxol-5-yl) -	863	52828-42-9
3639	Octadecanoic acid, 8,9,11,12-tetrakis[trimethylsilyl)oxy]-, methyl ester	840	35437-04-8
	Exxon 2380		
1051	8-Benzylquinoline	973	28748-19-8
	3-Benzylquinoline	970	37045-16-2
	2-naphthylamine, N-phenyl-	958	135-88-6
1275	Phosphoric acid, tris(3-methylphenyl)ester	987	563-04-2
1288	Phosphoric acid, tris(3-methylphenyl)ester	987	563-04-2
1303	Phosphoric acid, tris(3-methylphenyl)ester	984	563-04-2
1318	Phosphoric acid, tris(4-methylphenyl)ester	968	78-32-0
	Phosphoric acid, tris(methylphenyl)ester	966	1330-78-5
1330	1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-,trans-	890	34315-34-9
1379	3,3-dimethyl-5-(2,2-dimethylpropyl)tetrahydrofuran-2-one	975	0-00-0
1438	Gallium, tetraethyl-di.mu.-1-piperidinyldi-	898	42777-03-7
	Silane derivative	845	56771-62-1
1506	1,8-Dihydroxyanthraquinone	900	7336-68-7
1591	Decanoic acid, 1,2,3-propanetriyl ester	851	621-71-6
1693	Decanoic acid, 1,2,3-propanetriyl ester	824	621-71-6
1809	1,8-Dihydroxyanthraquinone	924	7336-68-7
1958	8-methoxy-2-(p-methoxyphenyl)-1,2,4,5-tetrahydro-1-benzazocine-3,6-dione	882	90732-26-6
	Decanoic acid, 1,2,3-propanetriyl ester	827	621-71-6
2133	Naphthalene, 2-(1,1-dimethyl)decahydro-4a-methyl	873	54934-96-2
	4-hydroxyanthraquinone-2-carboxylic acid, di-TMS	871	0-00-0
2615	Methanone, (4-ethoxy-3-methoxyphenyl)(6-methyl-1,3-benzodioxol-5-yl) -	887	52828-42-9
3639	Octadecanoic acid, 8,9,11,12-tetrakis[trimethylsilyl)oxy]-, methyl estert	843	35437-04-8

Table 3.9: Various compounds detected with GC-MS in two pyrolysed jet engine oils, Castrol 5000 and Exxon 2380. (Table is copied from Van Netten and Leung (2000).

Pyrolyzed oil analysis			
Ret. time	Compound	Fit	Cas #
	Castrol 5000		
737	diethyl phthalate	967	84-66-2
793	hexane, 1,1′-oxybis	815	112-58-3
958	hexane,2,2,3,4,5,5-hexamethyl-, meso	848	55258-16-7
1059	3-benzoquinoline	937	37045-16-2
1098	2-t-butyl-2,3-dimethyl-3-buten-1-o1	906	0-00-0
1144	5,6-decanedione	847	5579-73-7
1166	Butanimidamide, N-(1-chloro-2-methyl-1-butenyl)-2-monochlorid e	938	40645-73-6
1189	5-decen-1,o1 (Z)-	977	51652-47-2
1269	anthraquinone, 1-p-tolyl	943	20600-74-2
1288	Phosphoric acid, tris(methylphenyl)ester	937	1330-78-5
1301	Phosphoric acid, tris(methylphenyl)ester	961	1330-78-5
1317	Phosphoric acid, tris(methylphenyl)ester	942	1330-78-5
1349	dodecane,1-isocyanate	790	4202-38-4
1375	piperazine, 1-(aminoacetyl)-	868	77808-88-9
1409	3,3-dimethyl-5-(2,2-dimethylpropyl)tetrahydrofuran-2-one	947	0-00-0
1478	Silane, methyltriphenoxy	801	3439-97-2
1562	1,8-Dihydroxyanthraquinone	818	7336-68-7
1664	1,1,3-Tri(alloxy)propane	822	0-00-0
1789	Phenylethylamine, N-Methyl-.beta.,3,4-tris-(trimethylsiloxy)	824	10538-85-9
1933	Benzo[g][I]benzothiopyrano[4,3]-indole	752	10023-23-1
	Exxon 2380		
739	diethyl phthalate	969	84-66-2
794	hexane, 1,1′-oxybis	844	112-58-3
958	hexane,2,2,3,4,5,5-hexamethyl-, meso	902	55258-16-7
1056	3-benzoquinoline	970	37045-16-2
1099	2-t-butyl-2,3-dimethyl-3-buten-1-o1	913	0-00-0
1145	5,6-decanedione	872	5579-73-7
	2-nitro-2-methylcyclohexanone	954	0-00-0
1268	anthraquinone, 1-p-tolyl	927	20600-74-2
1286	Phosphoric acid, tris(methylphenyl)ester	817	1330-78-5
1301	Phosphoric acid, tris(methylphenyl)ester	859	1330-78-5
1317	Phosphoric acid, tris(methylphenyl)ester	946	1330-78-5
1350	1-piperidinecarboxaldehyde	897	2591-86-8
1407	3,3-dimethyl-5-(2,2-dimethylpropyl)tetrahydrofuran-2-one	977	0-00-0
1475	Silane, methyltriphenoxy	755	3439-97-2
1559	1,8-Dihydroxyanthraquinone	845	7336-68-7
1665	1,1,3-Tri(alloxy)propane	750	0-00-0
1789	Phenylethylamine, N-Methyl-.beta.,3,4-tris-(trimethylsiloxy)	677	10538-85-9
1931	Benzo[g][I]benzothiopyrano[4,3]-indole	764	10023-23-1

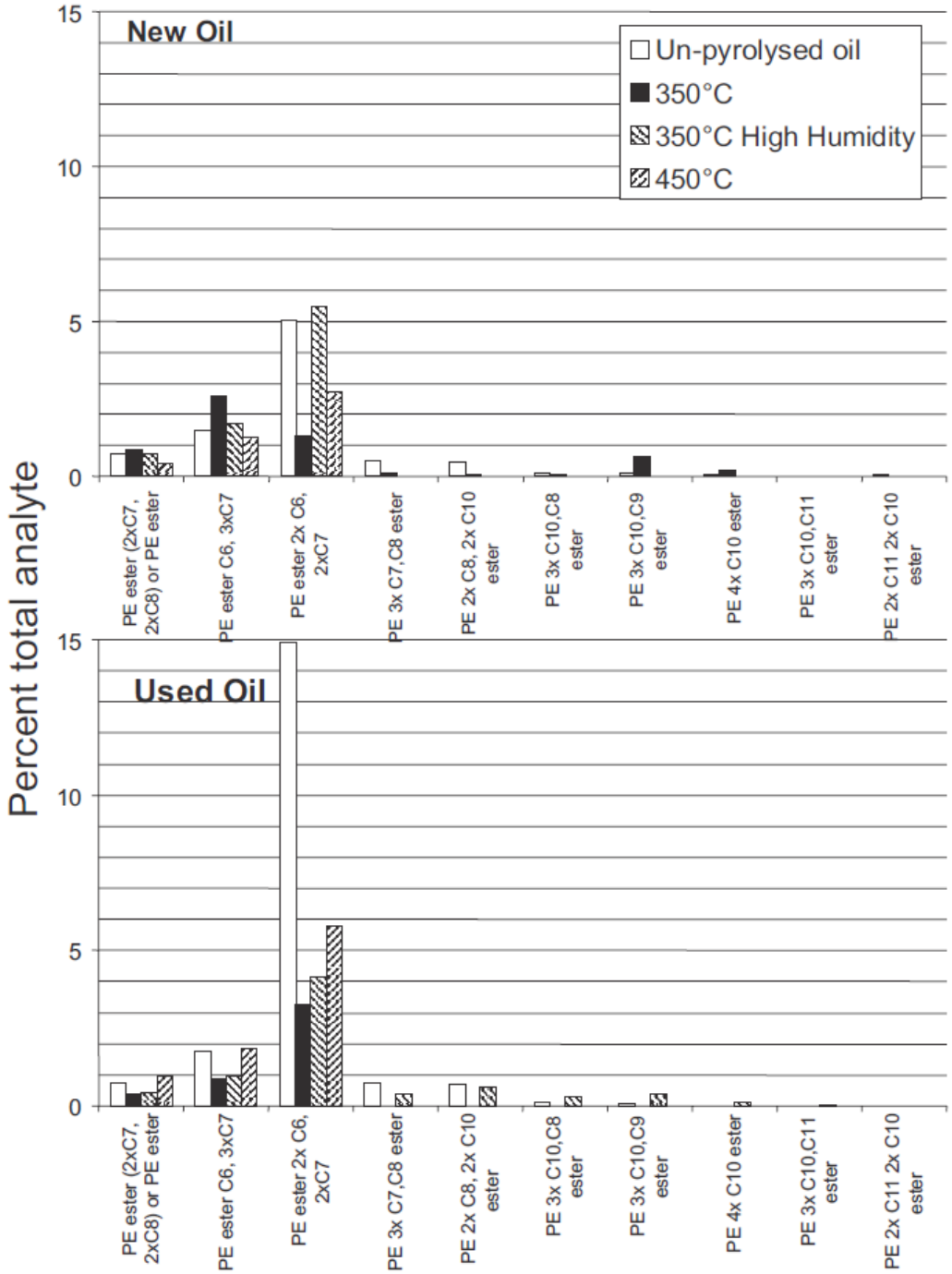

Figure 3.1. Pentaerythritol (PE) esters identified by GC-MS in pyrolysis products of new and used oils. Details of methods of pyrolysis and analysis are given in Marshman (2001) (Data used is the property of BAE Systems) (Figure is copied from CAA (2004)).

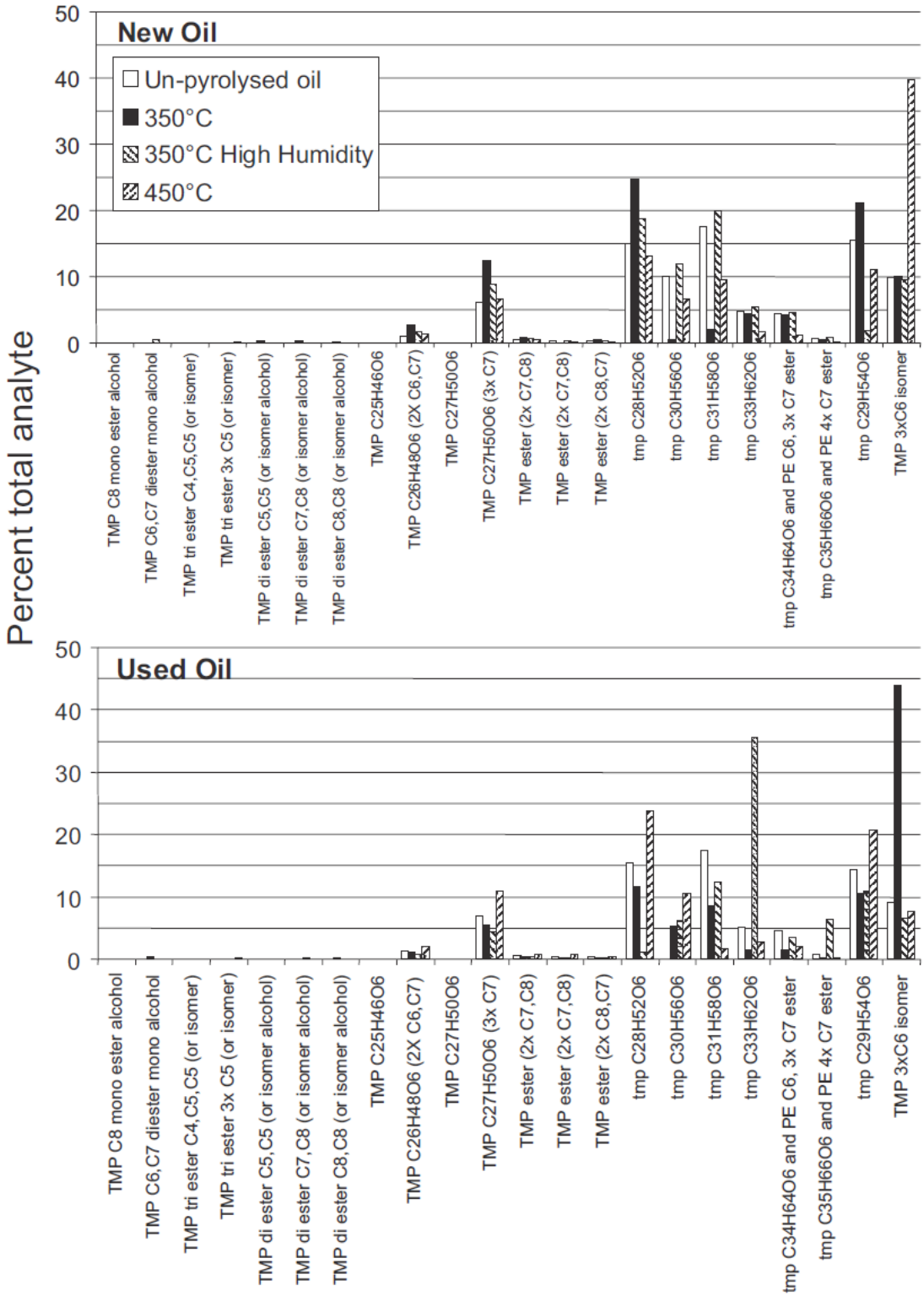

Figure 3.2. Trimethylpropane (TMP) esters identified by GC-MS in pyrolysis products of new and used oils. Details of methods of pyrolysis and analysis are given in Marshman (2001) (Data used is the property of BAE Systems) (Figure is copied from CAA (2004)).

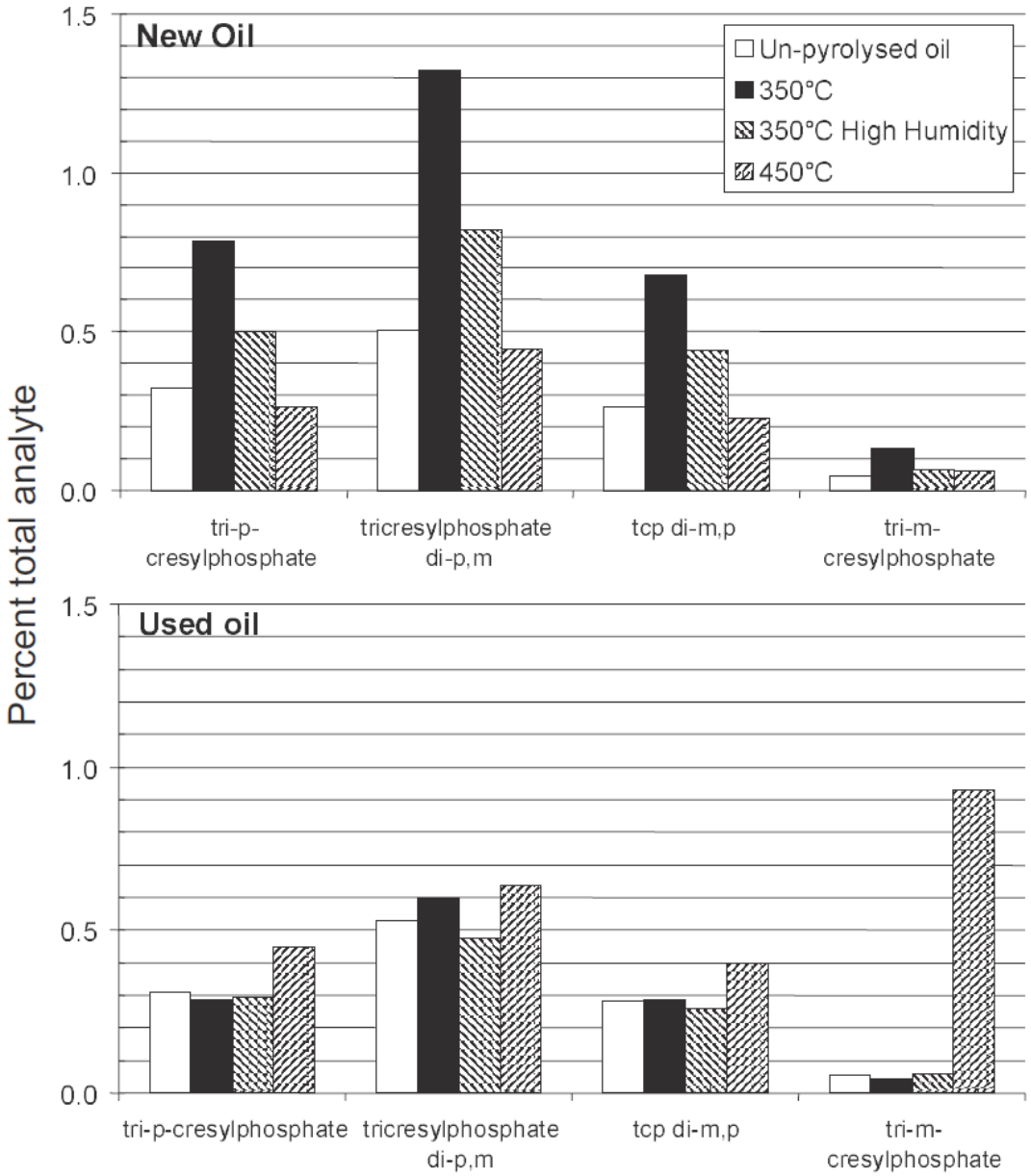

Figure 3.3. Tricresyl phosphate (TCP) esters identified by GC-MS in pyrolysis products of new and used oils. Details of methods of pyrolysis and analysis are given in Marshman (2001) (Data used is the property of BAE Systems) (Figure is copied from CAA (2004)).

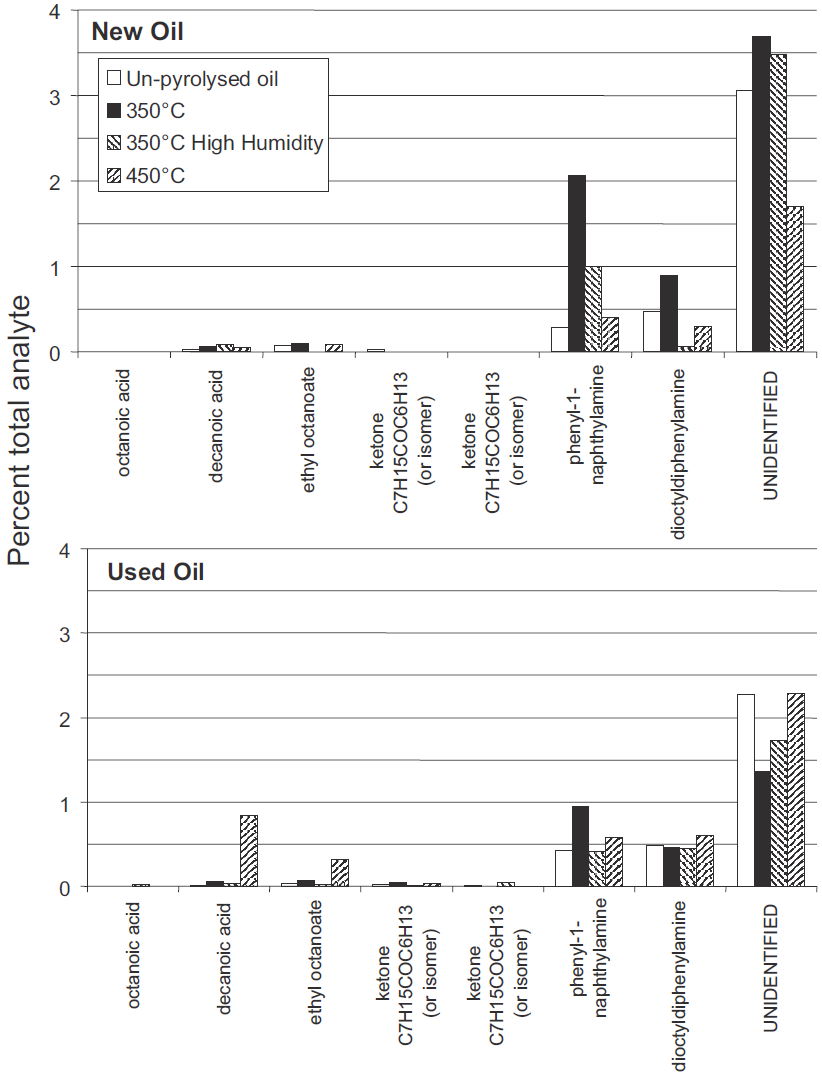

Figure 3.4. Organic acid, ketone and amine contaminants identified by GC-MS in pyrolysis products of new and used oils. Details of methods of pyrolysis and analysis are given in Marshman (2001) (Data used is the property of BAE Systems) (Figure is copied from CAA (2004)).

3.4.5 Selection of oils

In the tender a proposal was made by EASA for selection of several (at least two) aviation turbine engine/APU oil brands present on the market, in order to purchase and use them in the frame of this study. The selection shall consider oil brands which are widespread among commercially operated large transport airplanes.
In close consultation between the consortium and EASA, a selection was made of two most commonly used brands of oils for different aircraft types covering the European main fleet.
The selection contains the following candidate oils:

- Unused engine oil typically for category twin-aisle/ long range (B747/A340)
- Unused engine oil typically for category single aisle/short range (B737/A320)
- Used engine oil typically for category single aisle/short range (B737/A320)

KLM provided the consortium the above mentioned engine oils. For each oil brand three one litre new oil cans of same batches were delivered to TNO. KLM provided the used engine oil in six different cans, which were mixed and homogenized by TNO and put into one batch.

4 Task 2: Chemical characterisation of jet oil

4.1 Introduction

Pyrolysis is a thermochemical decomposition of organic material at elevated temperatures in the absence of oxygen. It may involve the simultaneous change of chemical composition and physical properties and is irreversible.

It is a fact, that simulations in a laboratory environment are not a 1 to 1 representation of the exact engine circumstances that occur during real flight conditions. For example, important combustion gases like nitrogen oxides, emitting from engines, and ozone, which is a constituent of ambient air, may cause reactions with uncontrolled emissions of oil. For the AVOIL study the reactions with ozone and nitrogen oxides were not included in the experiments due to the absence of sufficient valid data of typical concentration ranges for these gases in the troposphere and in between ground level and cruise height. For ozone counts that concentrations in the troposphere vary enormous for different tropospheric flight areas and therefore it would cost a lot of time to get well verified data on these concentrations. Thus for both ozone and nitrogen oxides, it is difficult to define a certain concentration in order to make a proper design for experimental conditions. In advance, we recommend therefore to study the chemical interactions between nitrogen oxides and ozone with possible oil emissions in a separate research project.

4.2 Design of a test methodology for the chemical characterization of oil vapours

4.2.1 Design of a simulation test set-up

A simulation for bleed air was developed including a sampling system. Within the developed simulation system the temperature and all in and out going flows are adjusted and controlled.
The simulation system is schematically shown in Figure 4.1. How it was build is presented in Figure 4.2 'the laboratory simulation system'. The different parts are described in details in the coming paragraphs.

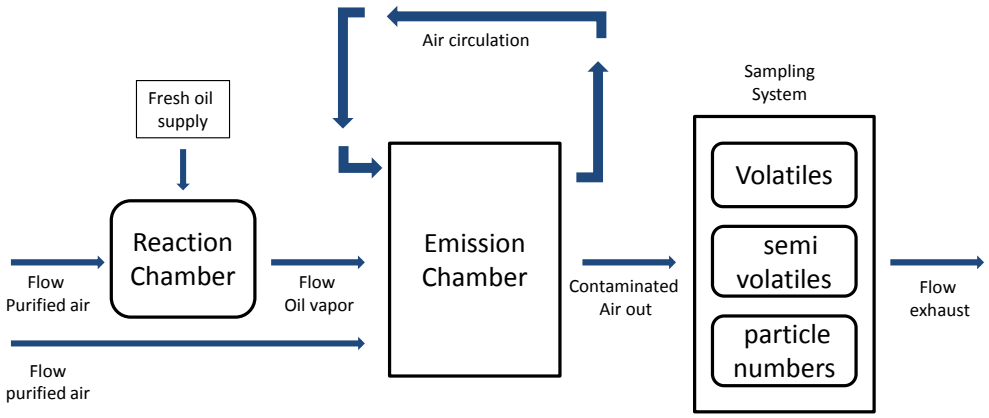

Figure 4.1. Experimental set-up of the simulation system. Purified air is led over the heated jet oil in the reaction chamber. Additionally a stream of purified air is led into the emission chamber to ensure that the overall flow out is similar to the flow in. The air is mixed in the emission chamber, causing a dilution compared to that of the indoor air in cabins. Here also a dedicated system takes care for air circulation. Contaminated air is pumped out of the emission chamber and is led through a sampling system.

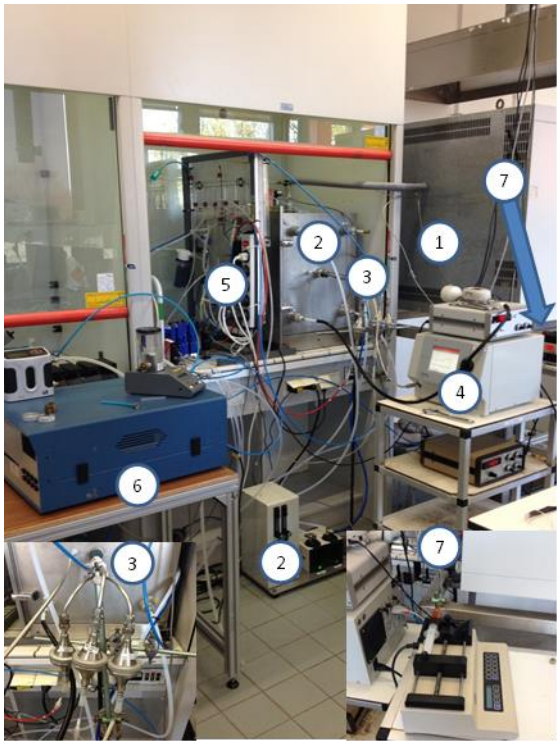

Figure 4.2. The laboratory simulation set-up. The following elements can be seen:

1. *Reaction chamber (programmable temperature controlled oven)*
2. *Stainless steel emission chamber and recirculation pumps*
3. *Stainless steel sampling devices for (semi)volatile compounds*
4. *Particle number monitor*
5. *Glass manifold sampling device for volatile compounds*
6. *Carbon monoxide monitor*
7. *Automated syringe pump device*

4.2.2 Reaction chamber

The reaction chamber (Figure 4.2; no. 1), consists of a temperature controlled (programmable) oven in which the glass reaction vessel with the oil is placed. The reaction vessel consists of a three stainless steel channel system (see Figure 4.3).

1) The input channel is for adding fresh purified air into the vessel to create a transport flow of oil vapour.
2) The output channel is for streaming the oil vapour to the emission chamber.
3) The supply channel is used to supply fresh oil into the reaction channel. For this purpose an automated syringe device was directly coupled to this channel.

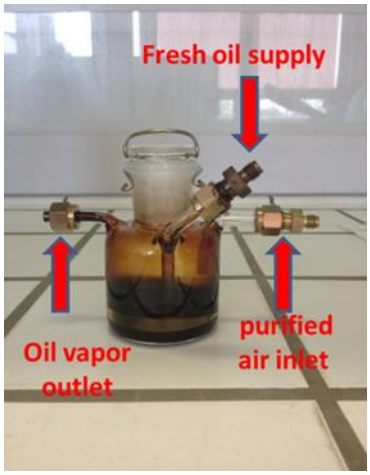

Figure 4.3. The reaction vessel with the three channels for the supply of fresh oil, supply of purified air and to stream the contaminated air out of the vessel to the emission chamber.

4.2.3 Emission chamber

The second section is the emission chamber which simulates the cabin air.
The simulation of a cabin air indoor environment is not easy as the cabin air is not only dependent of the type of aircraft and its use of ventilation system but also on other parameters e.g. number of passengers. In general a cabin can be seen as a box, where also air will enter, circulate and leave the cabin.

Samples of the diluted oil vapours were directly taken from the emission chamber.
This chamber has the possibility for ventilation and recirculation (including setting the air velocity over the surface).
The emission chamber is made of stainless steel, its dimensions are 43 * 45 * 52 = 100,620 cm^3 = 0.1 m^3.

This chamber gives the following possibilities:
- To reach quickly a homogeneous mixture of the polluted air stream.
- To dilute high concentrations of oil vapour
- To sample under room temperature conditions under inert conditions
- To use the chamber as a functionality for collecting oil condensation
- To apply easily different ventilation rates and recirculation.
- Flexibility in inputs and outputs for sampling devices.

Figure 4.4 shows the inside of the stainless steel emission chamber and the different flow connections on top and on the right side of the chamber. On the top one connection where the oil vapour enters the chamber and on the right side 5 connections for establishing sample points and flow in/outputs.

Figure 4.4. The stainless steel emission chamber and its connectors for in- and output of flows.

4.2.4 The sampling system

Two important boundaries for sampling have been defined and therefore a novel dedicated sampling system was developed. The defined boundaries were:

- Sampling should not affect the total flow including air speed in the emission chamber.
- If there is no sampling, the flow should behave as a bypass, and thus not affecting the total flow including the air speed in the emission chamber.

Leak tests
Leak tests were carried out before each test. In order to check certain increase or decrease in flow during the tests, the total flow out of the simulation system was measured continuously.
All flows of the individual sampling devices were controlled by mass flow units and before start and end of each simulation test, flows were measured with a calibrated flow device.

Preventing losses caused by adsorption
In order to prevent losses caused by adsorption to surfaces of semi volatiles and for compounds with relative low vapour pressure, the inlet of the sampling devices for polycyclic aromatic hydrocarbons (PAHs), mineral oil and organophosphate esters (OPEs) were directly connected to the emission chamber. The inlet of the particle number monitor was positioned in the middle of the emission chamber. This is shown in Figure 4.5 ‘the sampling devices for PAHs, mineral oil and OPEs’.

Figure 4.5. The sampling devices for PAHs, mineral oil and OPEs, coupled directly to the emission chamber.

The glass manifold

The volatile compounds like VOCs[2], aldehydes and carbon monoxide were directly measured from the glass manifold which is connected via a short Teflon tube to the emission chamber (see Figure 4.6).

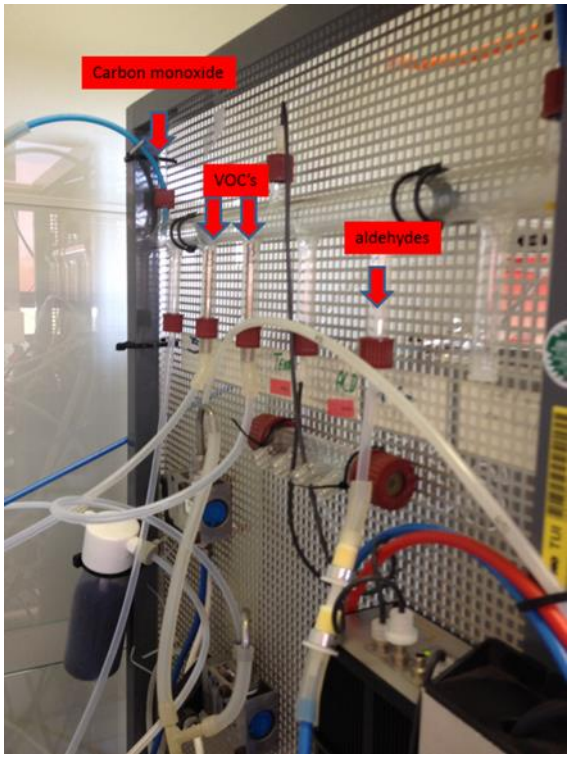

Figure 4.6.The sample points connected to the glass manifold. Carbon monoxide was sampled via the blue line (left), VOCs were sampled using two Tenax™ GR tubes (middle), and aldehydes were sampled via 2,4-dinitrophenylhydrazine (DNPH) cartridges (right).

Advanced observation

In advance, the following observation have been made. During the oil simulation tests some condensation of oil took place (see Figure 4.7) near by the oil vapour outlet of the emission chamber and at the front of the glass manifold.

[2] VOCs are defined as organic volatiles, which boil between 50 and 260°C.

Figure 4.7. Left the condensation of oil nearby the vapour inlet can be seen, while on the right condensation of the oil inside of the emission chamber after a simulation run can be seen.

In order to minimize losses of semi volatiles (compounds with relative low vapour pressure) during sampling, the inlet of the sampling devices for polycyclic aromatic hydrocarbons (PAHs), mineral oil and organophosphate esters (OPEs) were positioned directly in the emission chamber. Due to condensation of oil in the chamber, concentrations of PAH, mineral oil and OPE's might be slightly underestimated.

Realising having leakages of oil under real flight conditions, the same physiological effect will take place as in the simulation experiment.
In case of real oil leakage, evaporation of the oil takes place due to high temperatures in the compression chamber, and shall condensate because of temperature drops downstream of the compression chamber towards the packs and cabin. Condensation of oil in the simulation test will gave some losses of semi volatile compounds. It must be emphasized that the reported measured concentrations of semi volatiles are therefore underestimated results due to condensation. As already mentioned before, condensation of oil will also be the case in real flights when leakage of oil takes place.

4.3 Performance of the simulation tests

4.3.1 Experimental

The simulation tests were carried out using the equipment and analytical procedures as described in section 4.2. In summary a reaction vessel with the oil was placed in the oven. A flush of purified air was applied to transport the oil vapour produced at the given temperature. This flush of contaminants was mixed in the emission chamber with purified air. As the door of the emission chamber is provided with a rubber seal no contaminants can leave the emission chamber other than by the sampling system. The contaminated air was led through the different samplers and analysed afterwards. Particle number concentrations, carbon monoxide and temperature were continuously monitored during the whole simulation tests. In between the oil experiments blank system (without oil) experiments were carried out before each new simulation test.
The simulation set-up was placed in a laboratory environment, the temperature and relative humidity were adjusted by the climate system present in the lab (approx. 20 to 22°C and 55% relative humidity). All tests were conducted under normal standard atmosphere.

4.3.2 Temperature conditions during simulation tests

From the literature it is observed that pyrolysis experiments were performed at various temperatures in the range from 200 to 600°C. It is clear that bleed air temperature depends on air taken from high or low compression stages and type of engine. Although various laboratory studies are performed with respect to pyrolysis, it is not always well defined and supported by real measurements what the temperature profile is for typical normal bleed air temperatures. In the report of National Research Council (NRC) (2002) flight profiles were made for typical conditions from bleed air of an aircraft engine which do not exceed temperatures higher than 350°C. The information in the NRC report on the temperatures are referred to as 'typical conditions of bleed air from engine'. The provided source is 'Responses from Boeing to committee questions, July 13, 2001'. No further information on the type of engine or aircraft is provided.

ADSE Consulting and Engineering, who acts as an independent expert, to assist in case of operational questions for the consortium, was asked to work out bleed air characteristics for pressure and temperature. A flight profile and aircraft (100 to150 seats) was chosen for a typical European flight of 500 nautical mile (NM). A typical current generation engine was taken. The results of the bleed air pressure and temperature are listed in Table 4.1.

Table 4.1. Bleed air pressure and temperature for a typical European flight

Movements	Bleed pressure kPa absolute	Bleed temperature (°C)
Taxi at Ground idle	150	110
Max take off at 0 ft	940	350
Max climb rating at 1,500 ft	880	350
Max climb rating at 37,000 ft	340	300
Cruise	310	270
Flight idle at 37,000 ft	90	130
Flight idle at 1,500 ft	160	120
Holding at 18,000 ft	350	240

Ref: Vliegtuigomstandigheden ten behoeve van EASA test, 16-ME-013 V1.0, 11 February 2016, (ADSE)

The outcome of the ADSE investigation was discussed during the interim meeting in February 2016 at EASA. It was decided to take 350°C as a valid maximum temperature for the AVOIL study. Furthermore these numbers were checked at the KLM-Engineering department and were confirmed to be representative for typical worst-case temperature/pressure circumstances.

For the chemical characterization of the simulation tests the following conditions and settings profile were applied as described in Table 4.2:

Table 4.2. Conditions and settings for the chemical characterization of the simulation and blank tests.

Simulation test	Samples taken during simulation test	Sampling time (min)	Oxygen content (%)	Temperature (˚C)	Oil supply during simulation test
Ground level to top of climb	sample 1	30	20.9	20-350	no
Cruise	sample 2	60	20.9	350	yes

All tests are conducted under normal standard atmosphere.

During the first part of the simulation tests volatile compounds will be released from the oil during the warming-up of the oil from 20 to 350°C resulting in exhaustion of the oil before the second part (Cruise) of the sampling starts. Therefore oil is only continuously dropwise introduced in the reaction vessel during the cruise sampling period of 60 minutes at 375 (± 25)°C.

4.3.3 Experimental settings of the simulation tests

For all simulation tests air flows for the different samplers, temperature range and time were set. Tables 4.3 and 4.4 show the settings of air flows and temperature during the simulation tests. In order to avoid under- or overloading of analytes, air flows were chosen based on the applicability of the individual sampling methods.

Table 4.3. Air flow settings of the simulation system

Flow settings simulation tests	**Controlled values**
Flow purified air through reaction vessel (=oil vapour entering the cabin)	380 ml/min
Sampling flow mineral oil	2000 ml/min
Sampling flow OPEs	2000 ml/min
Sampling flow PAHs	2000 ml/min
Sampling flow VOCs	42 ml/min
Sampling flow aldehydes	400 ml/min
Sampling flow Condensed Particle counter	300 ml/min
Sampling flow CO-monitor	1100 ml/min
Sampling flow Bypass	3000 ml/min
Total flow out	10842 ml/min
Ventilation rate	6.5 h^{-1}

Table 4.4. Temperature settings during the simulation tests

Temperature settings simulation tests	**Controlled values**
Temperature laboratory	(20 ± 2)°C
Relative humidity laboratory	(60 ± 5)%
Temperature Emission chamber during simulation test	(21 ± 1)°C
Temperature oil during simulation test 0-30 min	(21 to 350)°C
Temperature oil during simulation test 30-90 min	(375 ± 25)°C

4.3.4 Selected oils for the simulation tests

For the simulation tests the following engine oils were tested (see also Table 4.5):

- **Oil A_n**: new oil of brand A
- **Oil A_u**: used oil of brand A. History: was taken from a certain Boeing 747 Freighter. Hours since last shop visit 13653, Cycles since last shop visit 2615.
- **Oil B_n**: new oil of brand B

Table 4.5. The properties of selected oils

Property[3]	*Oil A_n*	*Oil B_n*
Date Safety Data Sheet	*March 30, 2015*	*march 17, 2015*
Pour Point (˚C), ASTM D 97	-54	-59
Flash Point (˚C), ASTM D 92	246	> 246
Fire Point (˚C)	n.a.	n.a.
Autogenious Ignition Temp (˚C)	n.a.	n.a.
Density @15˚C, kg/l, ASTM D 4052	0.997	1
TCP (percent by weight)	1- < 2.5	1- < 3

n.a: data not available

4.3.5 Applied sampling and analytical methods

Organophosphate esters
Organophosphate esters (OPEs) were sampled using Teflon/glass fiber filters in combination with Chromosorb® 106 adsorption tubes. Extraction of the samples was carried out using accelerant solvent extraction (ASE). The extraction solvent is a mixture of dichloromethane (DCM) and n-hexane (50:50 v/v). Before extraction an internal standard TPh-d_{15} was added to the sample. After extraction the extract was concentrated followed by a clean-up using 3% deactivated florisil. Prior to analysis an injection standard 1,2,3,4-tetrachloro naphthalene (TCN) was added to the extract. The analyses were performed with GC-MS. The method is developed and validated by TNO according to NEN 7777 (NEN-EN 7777:2011) and described in an internal TNO report (TNO, 2013a).

Mineral oils
Mineral oils were sampled using glass fibre filters in combination with XAD-2 adsorption tubes. Extraction of the samples was carried out using hexane. After the extraction, the extract was pre-concentrated followed by a clean-up with florisil. The final extract was analysed with Gas chromatography-Flame Ionisation Detector (GC-FID) . Both the identification and quantification of the compounds are based on an external standard. The sampling method is based on NIOSH 5026 (NIOSH, 1996), the analysis method is based on NEN 6978 (NEN 6978:2016).

Polycyclic Aromatic Hydrocarbons (PAHs)
PAHs were sampled with a glass fibre filter in combination with XAD-2 adsorption tubes. Extraction of the samples was carried out using toluene. After extraction with Accelerent Solvent Extraction (ASE) the extract was pre-concentrated followed by clean up with silica gel. The final extract was analysed with GC-MS isotope dilution based on ISO 12884 (ISO 12884:2000).

Aldehydes
Aldehydes were sampled with 2,4-dinitrophenylhydrazone (DNPH) Sep-Pak Xposure cartridges. The aldehydes in the air reacts with DNPH and H^+ to the corresponding hydrazone derivatives. Extraction of the samples is carried out with acetonitrile.
Using liquid chromatography coupled with mass spectroscopy the determination of the compounds, quantitatively and qualitatively, was established. Identification of the individual carbonyls in the extracts is carried out on basis of the retention times and quantification is based on an external calibrated standard. The method is based on ISO 16000-3 (ISO 16000-3:2011).

Volatile organic compounds (VOCs)
Tenax^{TM} GR adsorption tubes were applied for sampling VOCs in the range of C_6-C_{12}. Subsequently the determination of organic volatiles was carried out according to internal procedure ORG-141 'Determination of Volatile Organic Compounds Using Thermal Desorption and Gas Chromatographic Analysis'. The desorption of the adsorption tubes is carried out my means of an automatic thermo sorption unit. The desorbed compounds are

[3] The Oils are used under code. The Safety Data Sheets are present at the authors office.

trapped my means of a cold-trap and are being analysed on-line using a gas chromatograph equipped with a capillary column which is coupled with a mass spectrometer. The identification of the compounds is based on the retention time and mass spectrum. Quantification is performed with the use of an external standard. The method is based on ISO 16000-6 (ISO 16000-6:2011).

Condensed particle counter (CPC) and carbon monoxide
Particle numbers are measured with the use of a butanol driven CPC 3775 (TSI) with a cut-off of 4 nm to 1000 nm particle diameter. Data points were measured each second. Concentrations of particles were calculated in particle numbers per cm^3.
Carbon monoxide is measured with a Thermo monitor type 48i. measurements are based on the principle that CO absorbs infrared radiation at a wavelength of 4.6 microns. Because infrared absorption is a nonlinear measurement technique, it is necessary for the instrument electronics to transform the basic analyser signal into a linear output. The 48i uses an exact calibration curve to accurately linearize the instrument output over any range up to a concentration of 10,000 ppm.

4.4 Results and discussion

In total, six simulation runs were performed: three were carried out for blank system measurements and three were carried out using the selected oils.

With the exception of carbon monoxide and particle number measurements, all results of the oil simulation tests were corrected for the blank concentration levels found for the blank system measurements. The results of the oil simulation tests are presented in this section.

4.4.1 Temperature and loss of weight during the simulation tests

It is obviously that due to the increase of temperature and the flow of purified air, losses in the total mass of the oil, present in the reaction vessel, take place. This is happening even though fresh oil was supplied to the vessel. In order to calculate the loss of weight during the simulation test, the mass of oil was gravimetrically determined before and after each simulation test. As the change of mass is dependent of the temperature of the reaction chamber, and thus the temperature of the oil, the temperature gradient was observed throughout each experiment. The temperature of the simulation tests for oil A_n and oil A_u are shown in Figure 4.8..

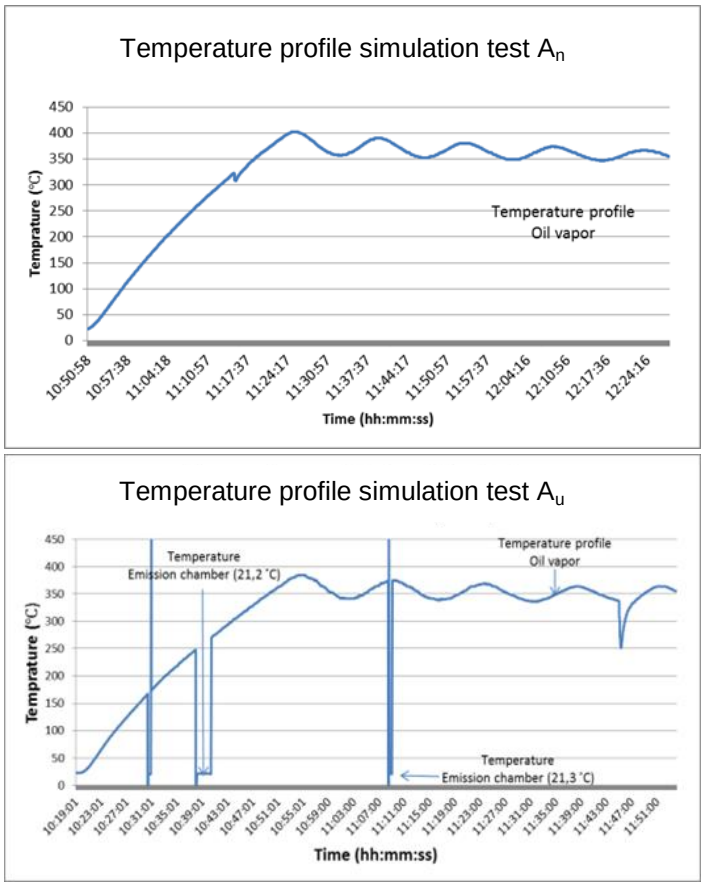

Figure 4.8. Temperature profile simulation test for new oil A_n (upper graph) and used oil A_u (lower graph). At the start of the simulation test the temperature of the oils amounts 21˚C. Within 30 minutes the temperature of the oils reached a temperature of 350˚C. During the next 60 minutes of the simulation test the temperature of the oils amounts (375 ± 25)˚C.

The temperature of the simulation test for oil B_n is shown in Figure 4.9. It has to be stated, that this temperature profile is equal to the profiles applied for the other oils. Table 4.6 shows the loss of weight during the test for oil A_n, A_u and B_n.

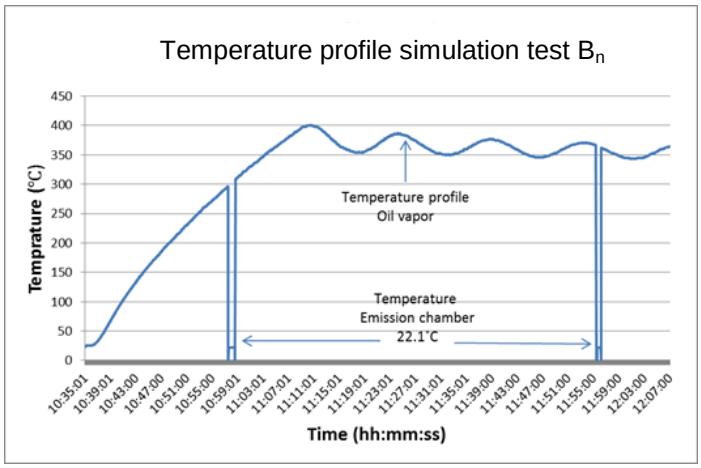

Figure 4.9 Temperature profile simulation test for oil B_n.

Table 4.6. Loss of weight of the applied oils after completion of the simulation test.

Oil type	% loss of weight after simulation test
A_n	68
A_u	11
B_n	82

From the results it is clear that the % loss of weight for the used oil A_u is significant lower than for the new oil A_n and B_n at equal settings of time, temperature and oil mass applied per simulation test.
Based on this fact, we suggest that the used oil contains less volatile- and semi-volatile fractions compared to the new oils.

4.4.2 OPEs in oil and oil vapour

The organophosphate ester group forms an important fraction of the jet oil and will also release from the applied oils. Therefore, it is important to know the original OPE concentration in the applied oils prior to the simulation tests. The results of the OPE analysis in the original oils and subsequently determined in the vapour per simulation test are presented in Table 4.7. and 4.8..

Table 4.7. OPEs found in the original oils.

Oil type Components	A_n g/kg	A_u g/kg	B_n g/kg
diphenyl (2-ethylhexyl)phosphate	0.038	0.043	0.030
tris(2-ethylhexyl)phosphate	0.048	0.020	0.025
tri(m, m ,m)- cresyl phosphate	2.5	2.2	4.1
tri(m, m, p)- cresyl phosphate	6.1	5.6	11
tri(m, p, p)- cresyl phosphate	5.4	5.2	9.5
tri(p, p, p)- cresyl phosphate	1.7	1.7	2.9
Σ TCP's (g/kg)	15.7	14.7	27.5
Σ TCP's (%)	1.6	1.5	2.8
Specification supplier (%)	1< 2.5	1< 2.5	1-3

Table 4.8. OPEs found in the vapour off the different oils.

Oil type	A_n	A_n	A_U	A_U	B_n	B_n
Temperature range (˚C)	20-350	350	20-350	350	20-350	350
Test duration (min)	30	60	30	60	30	60
Component	mg/m^3	mg/m^3	mg/m^3	mg/m^3	mg/m^3	mg/m^3
Triethyl phosphate	0.0003 <	<	<	<	<	<
Tris(2-butoxyethyl)phosphate	0.028 <	<	<	<	<	<
Triphenyl phosphate	0.0002 <	<	<	<	<	<
Diphenyl (2-ethylhexyl)phosphate	0.0003 <	<	<	<	<	<
Cresyl diphenyl phosphate	0.002 <	<	<	<	<	<
Cresyl diphenyl phosphate	0.003 <	<	<	<	<	<
Di-cresyl phenyl phosphate	0.042	0.448	0.001	0.203	0.100	0.452
Tri (o, o, o)- cresyl phosphate	<	<	<	<	<	<
Di-cresyl phenyl phosphate	0.057	0.624	0.001	0.239	0.075	0.568
Di-cresyl phenyl phosphate	0.027	0.291	0.0005	0.105 <		0.259
Tri(m, m ,m)- cresyl phosphate	0.944	3.43	0.049	1.73	1.03	3.81
Tri(m, m, p)- cresyl phosphate	2.051	7.71	0.119	3.92	2.12	8.89
Tri(m, p, p)- cresyl phosphate	1.709	7.01	0.100	3.50	1.59	7.58
Tri(p, p, p)- cresyl phosphate	0.505	2.31	0.025	1.09	0.425	2.21
Σ OPE's	5.37	21.8	0.30	10.8	5.34	23.8
Σ TCP's	5.21	20.5	0.29	10.2	5.16	22.5
Σ TCP/ΣOPE's (%)	97	94	99	95	97	95

<: below detection limit

One of the first important result coming from the TCP analysis was the fact, that there was no presence of Tri(o,o,o)-cresyl phosphate in the applied oils. Secondly it was found that the original oils contain the following isomers: tri(m,m,m,)-, tri(m,m,p)-, tri(m,p,p) and tri(p,p,p)-cresyl phosphate.

The mass fraction of TCP, calculated from the analytical results of the oils, corresponds with the oil specifications described in the MSDS sheets from the suppliers.

Looking at the mass fraction of the TCP isomers it was found that the mass fraction ratio of the TCP isomers in the oil vapour was similar to the original oils[4].
No tri(o,o,o)-cresyl phosphate was present in the oil vapours.

From the simulation test it was found that the oils heated at a steady state (350°C) emits more TCPs than compared to the simulation whereby the temperature is heated up from 20 to 370°C.
Used oil A_u showed low concentrations of TCPs in the emission chamber compared to new oil A_n. Oil A_n and oil B_n gave comparable results.

The simulation test also shows a good correlation between the composition of TCP isomers found in the original oil and in the oil vapours at different temperatures. Comparison of A_n and A_u shows no significant differences in composition of the four isomers. Figures 4.11 and 4.12 show the correlation of the TCP isomer composition in the original oils and in the corresponding oil vapours.

[4] The mass fraction per isomer is defined as the mass of isomer(a) present in the air, divided by the total mass of counting isomers.

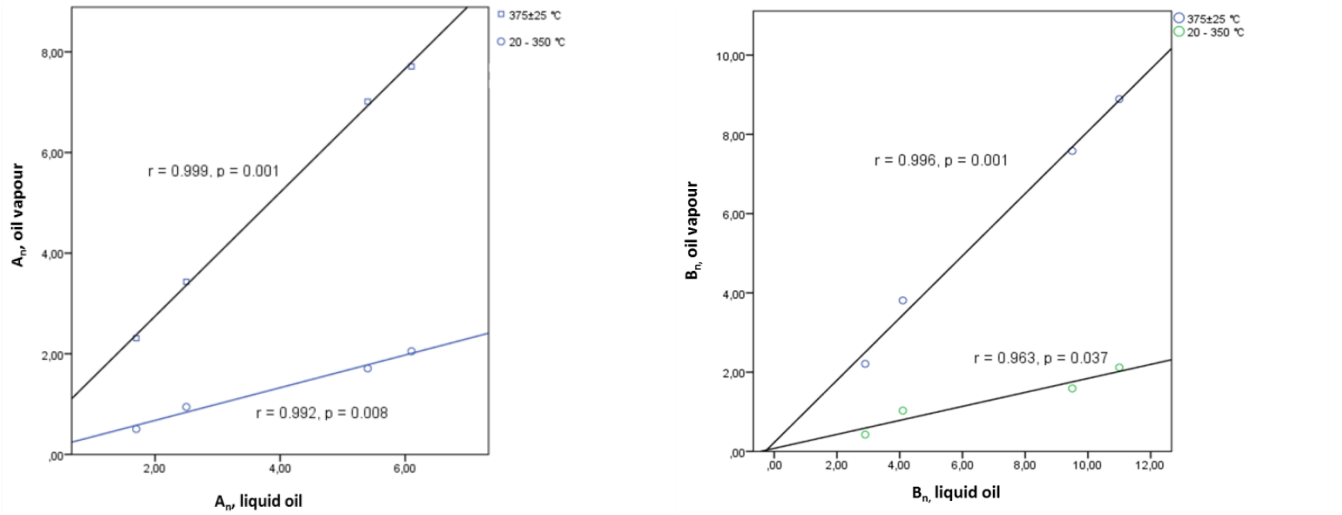

Figure 4.11. Correlation of the TCP composition for the new oils. Left the correlation of the TCP composition in the oil vapour versus the original oil from the new oil (A_n) is presented. On the right the correlation of the TCP composition in the oil vapour versus the original oil from oil B_n is presented.

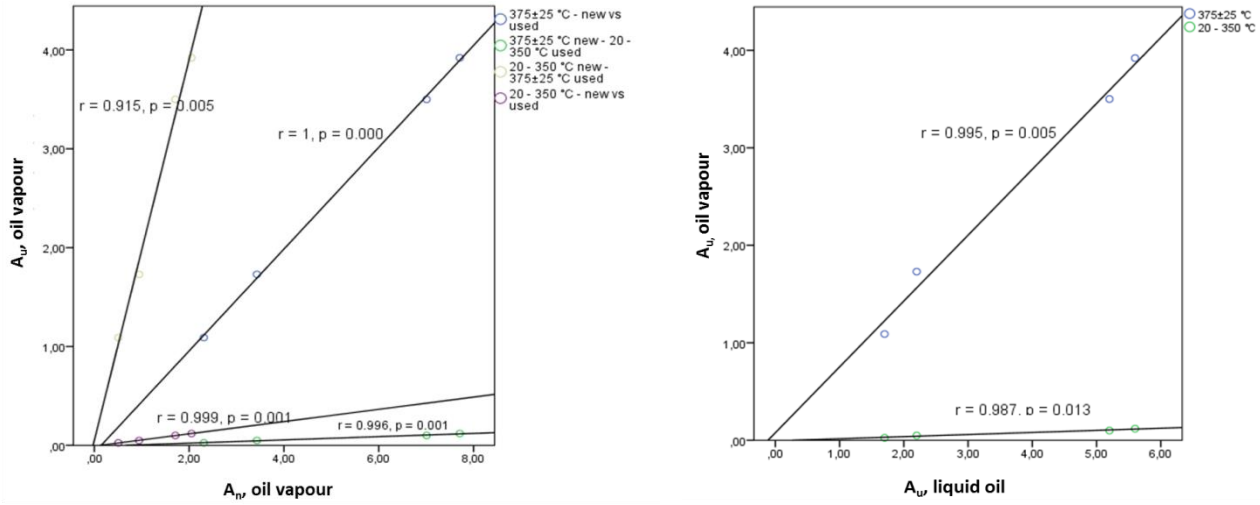

Figure 4.12. Correlation TCP composition in oil vapours for the new and used oil A. Left the correlation of the vapours of the new and used are presented. On the right the correlation TCP composition of used oil A versus used oil A vapour is presented.

Based on these findings we found that the composition of the TCPs, presented e.g. as mass TCP fraction, in turbine oils (new or used) is similar to the composition of the TCPs found in the air during the simulation tests. Based on this fact, we conclude that the four TCP isomers do not deteriorate due to the applied temperature ranges of 20 to 350°C and at 375°C. All tests in our work were carried out in purified air, thus containing oxygen. In previous work of Havermans and Houtzager comparable results of simulation tests were found under an oxygen depleted atmosphere (Havermans et al., 2015).

4.4.3 PAHs in oil and oil vapour

Another novel approach for this work is dedicated to the presence of PAHs in both the applied oils and their vapours. The main reason to include these analysis is based on the formation of PAHs during combustion of fuels, however PAHs may be present in the original oils as well. An example of a pathway causing PAHs due to combustion is given in Figure 4.13.

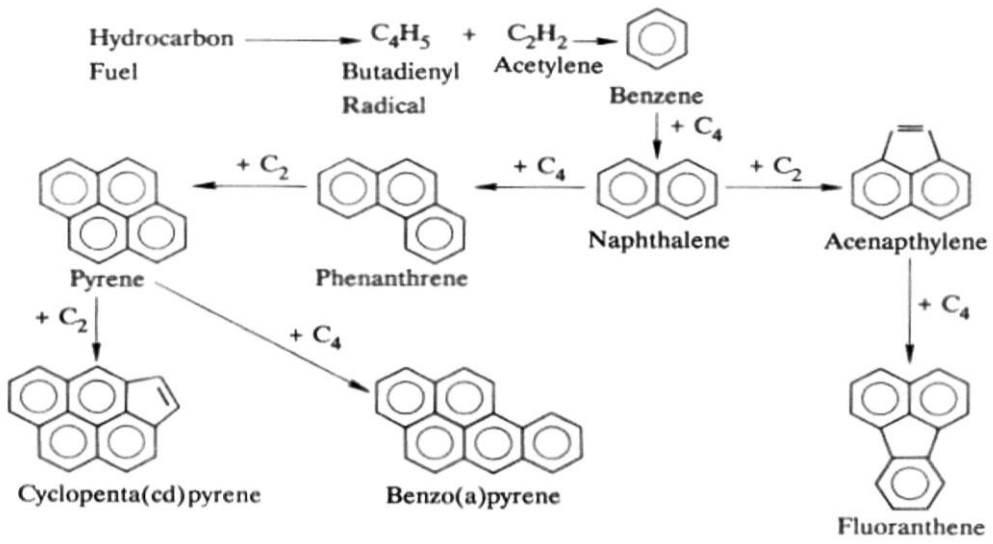

Figure 4.13. A possible pathway of the formation of PAHs during fuel combustion, where radicals from oxidation may play a serious role in the formation of PAHs according to Dandy (Dandy, 2007).

The results of the PAH analysis in the original oils and their vapours are presented in Table 4.9 and 4.10.

Table 4.9. PAHs present in the original oils.

Oil type Components	A_n mg/kg	A_u mg/kg	B_n mg/kg
Naphthalene	< 0.58	< 0.58	1.9
Anthracene	1.4	1.3	1.4
Fluoranthene	1.2	< 0.62	0.59
Total PAH's	2.6	1.3	3.8

<: below detection limit

Table 4.10. PAHs found in the oil vapours.

oil type	A_n	A_n	A_u	A_u	B_n	B_n
Temp range (˚C)	20-350	375±25	20-350	375±25	20-350	375±25
Simulation time (min)	30	60	30	60	30	60
	µg/m^3	µg/m^3	µg/m^3	µg/m^3	µg/m^3	µg/m^3
Naphthalene	0.78	4.1	0.36	8.3	8.9	93
Acenaphthylene	< 0.02	< 0.01	< 0.02	< 0.02	< 0.02	0.14
Acenaphthene	0.02	0.03	0.02	0.18	0.03	0.82
Fluorene	0.04	0.07	0.03	0.26	0.04	0.68
Phenanthrene	0.10	0.08	0.10	0.54	0.15	1.9
Fluoranthene	0.03	0.03	0.02	0.05	0.03	0.17
Pyrene	0.03	0.02	0.03	0.13	0.04	0.21
Benzo[a]anthracene	< 0.02	< 0.01	< 0.02	0.01	< 0.02	0.11
Chrysene	< 0.02	< 0.01	< 0.02	0.02	< 0.02	0.08
Σ PAH's ((semi)-volatile)	0.99	4.3	0.57	9.5	9.2	97
Σ PAH's particle bound	<	<	<	<	<	<

<: below detection limit

Based on the results, it was found that naphthalene was present in the original oil B_n in a concentration of 1.9 mg/kg. At the other hand, the original oil A_n did not contain naphthalene as it could not detected above the detection limit. However, both anthracene and fluoranthene were found in the applied oils.

By comparison the analytical results of the PAH presence in the vapour and in the original oils, it was found that the vapour does contain more different types of PAH. Therefore, we suggest the following hypotheses:

- PAHs found in the vapour may originate from small concentrations in the original oil of which we found concentrations below the detection limit.
- During heating of the oil, a partial oxidation takes place (partial combustion) that results in the formation of new PAHs take took place during heating of the oil due to incomplete combustion and according to Figure 4.13.

For the used oil A_u, we observed more different PAHs in its vapour than for the (liquid) oil. This implies that possible formation of certain PAHs occurred during (incomplete) combustion and that these PAHs do not remain in the oil but are released by its vapour.

Oil B_n contained the highest amount of PAHs, compared to the other two applied oils. The sum of PAHs found in oil B_n were about 10 to 20 times higher compared to oil A, for both new and used one.

4.4.4 Mineral oil in oil and oil vapour

A mineral oil is defined as colourless, almost tasteless, water-insoluble liquid consisting of a mixture of hydrocarbons obtained from petroleum by distillation. A mineral oil may contain mainly alkanes and cycloalkanes. Alkanes are acyclic saturated hydrocarbons and cycloalkanes are cyclic structured. An alkane consists of hydrogen and carbon atoms (C_n n=number of carbon atoms) arranged in a structure in which all carbon-carbon bonds are single. For each simulation test the mineral oil content in the oil vapour was measured and these results are given in Table 4.11.

Table 4.11. Concentration of mineral oil in the oil vapours during each simulation test.

oil type	A_n	A_n	A_u	A_u	B_n	B_n
Temp range (˚C)	20-350	375±25	20-350	375±25	20-350	375±25
experimental duration (min)	30	60	30	60	30	60
Component	mg/m^3	mg/m^3	mg/m^3	mg/m^3	mg/m^3	mg/m^3
Mineral oil	18	420	2,0	380	31	230
Mineral oil expresssed as a percentage (%) of the total amount of mineral oil measured during the simulation test	4	96	1	99	12	88

From the results it was found that during cruise a minimum of 88% and a maximum of 99% of the total mineral oil vapour measured is emitted at 375 ± 25°C.

Based on the gas chromatographic analysis, the mass distribution was obtained for each simulation test. Figure 4.13 shows from top to down the mineral oil composition of the liquid oil A_n, its composition of the vapour for simulation 1 (20 to 350°C) and its composition of the vapour for simulation 2 (new, 375°C).

Based on the results it was found that the mass distribution at room temperature of the original oil A_n (Figure 4.14, top) consists of alkane chains in the range of C_{24}-C_{50}. After 30 minutes of heating from 20 up to 350°C the composition of mineral oil in the vapour remains unchanged and is comparable with the mineral oil chains found in the original oil at room temperature (Figure 4.14, middle). After heating at a temperature of 375˚C, small changes in composition of the oil are observed in the chromatogram (Figure 4.14, bottom). There is an increase of relative low boiling point compounds during the simulation test at 375˚C. Additionally unidentified complex mixtures (UME) are formed beneath the C_{24}-C_{50} peaks.

The results of the mineral oil analysis for used oil A (A_u) gave at room temperature similar results as were found for new oil A ($A_{n)}$.

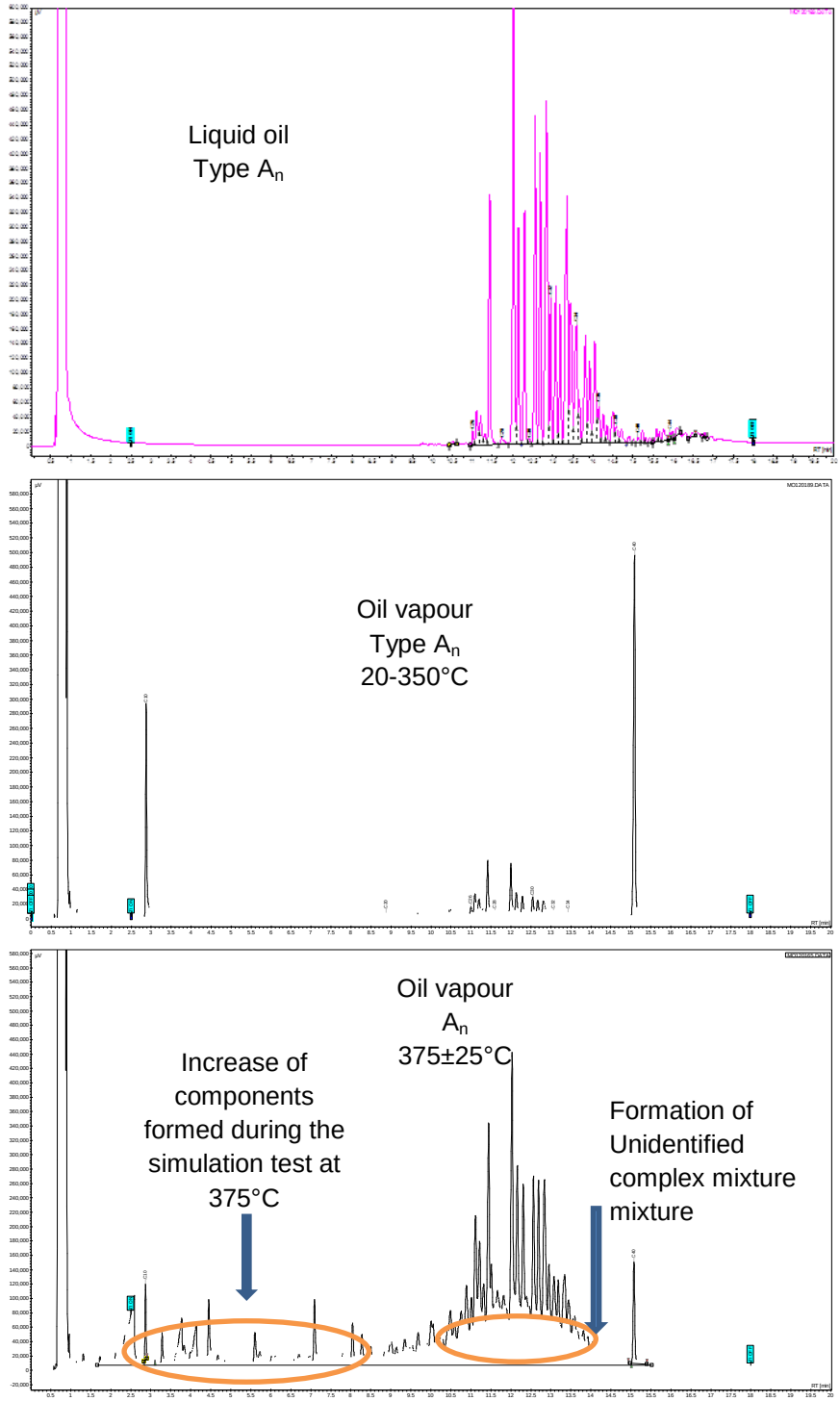

Figure 4.14. Gas chromatograms of three experimental stages of the new oil A (A_n) showing the composition of the mineral oils found per stage. Top: at room temperature. Middle for simulation 1 (20 to 350°C). And at the bottom for simulation 2 at (375 ± 25)°C.

Based on the results, it was found that the mineral oil composition for oil B_n is comparable with the composition found for oil A_n. Also for oil B_n the alkane chains have a range of C_{24} – $C_{50,}$ with the exception of one peak found around C_{21}. Additionally the ratio of the alkane chains differs between the two oils. Figure 4.15 shows the chromatograms of oil A_n and oil B_n.

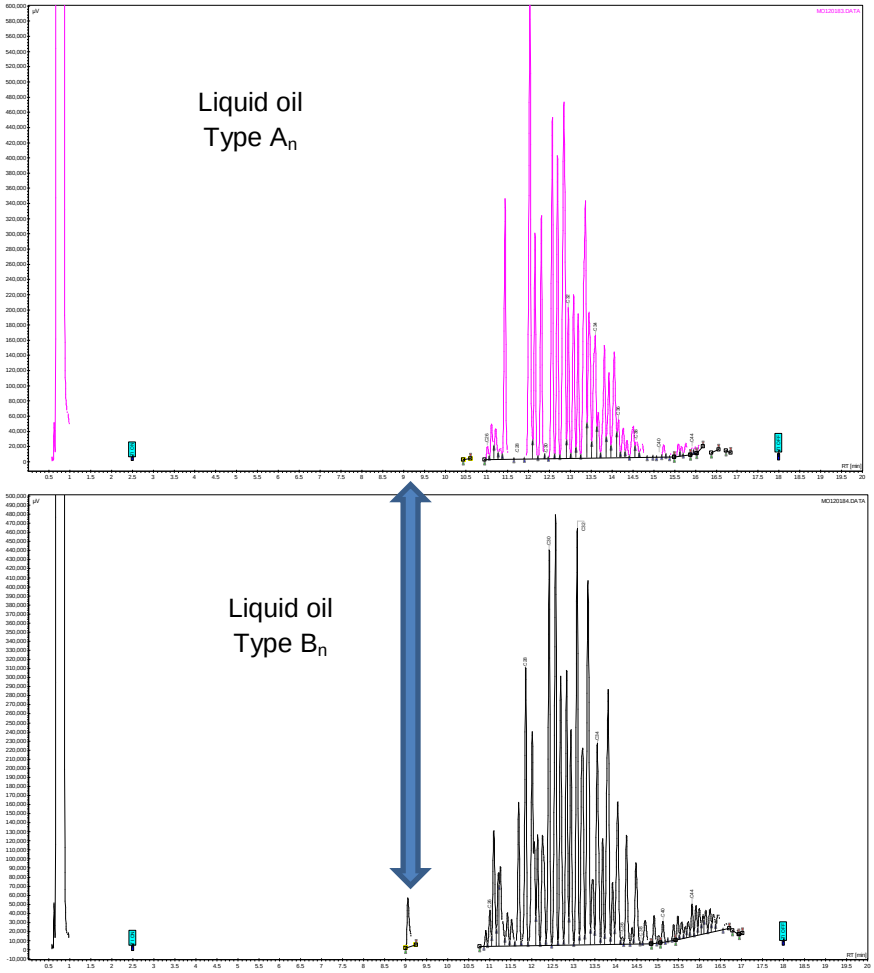

Figure 4.15. Gas chromatograms showing the composition of both new oils. Top: oil A_n. Bottom: oil B_n.

4.4.5 VOCs in oil vapour

The results of the VOC analysis in the oil vapours are summarized in Table 4.12.. The complete set of results are presented in Appendix 4 of this report.

Table 4.12. Summarized results of VOCs found in the simulation tests. Here the sum per group of compounds is presented. Additionally some various compounds are given, that do not belong to the given groups

Oil type	A_n	A_n	A_u	A_u	B_n	B_n
Temperature range (˚C)	20-350	375±25	20-350	375±25	20-350	375±25
Test duration (min)	30	60	30	60	30	60
Component	µg/m^3	µg/m^3	µg/m^3	µg/m^3	µg/m^3	µg/m^3
Σ Aromates	36	1279	6.0	1120	48	3098
Σ Alkanes	318	107	8.8	42	106	88
Various components						
2-hexanone	257	**> 10000**	47	**> 8500**	298	**> 19000**
cyclohexanone	<	**> 1000**	<	508	<	**> 2400**
phenol	62	136	18	164	239	883
3-methylphenol	289	856	38	1395	503	2467
octanal	<	321	10	461	66	1318
MIBK	<	<	<	117	<	<
dimethylphthalate	6.0	<	6.9	<	6.7	<
Σ components	968	> 2700	134	> 3806	1266	> 7855

<: below detection limit

>: minimum concentration found, chromatographic peaks out of scale and above linearity. (semi-quantitative analysis)

Based on a selection of the obtained results (aromatics, ketones, and esters) it was found that the emission of both oils differ at both simulations 'ground level to top of climb (20 to 350°C) and subsequently cruise speed at (375 ± 25)°C. For example the total VOCs (aromatics, including benzene, toluene and xylene isomers) was for the oil A_n and oil B_n at simulation 1 (i.e., 20 to 350°C), 36 µg/m^3 and 48 µg/m^3 respectively. These concentrations increase drastically for these oils at cruise simulation (375 ± 25°C), 1.279 and 3.098 µg/m^3 respectively. Similar results were observed for other volatiles as alkanes and various compounds. These findings of the presence of high concentrations of compounds at cruise simulation may indicate emissions do take place at higher temperatures.

Besides the quantitative analysis of VOCs, identification of remaining peaks were carried out for those peaks with sufficient intensity. The peaks were identified based on peak deconvolution with AMDIS followed by a target library search. The target library contains over a 1000 compounds with spectra and retention indices. Identification was based on the NET match factor, a combination of the match factor and the retention index, with a minimum NET match factor of 80. Compounds that were not identified with the target library search were tentatively identified by a search in the NIST library with a minimum match factor of 80.

Table 4.13 shows a summary of identified compound groups and the amount of different compounds per group detected in the oil vapours. The complete set of individual identified compounds are presented in Appendix 4.

Table 4.13 Number of individual compounds identified in oil vapour.

Compound groups	Number of individual compounds identified in oil vapour
Aldehydes	13
Ketones	23
Alkenes	15
Organic acids	13
Esters	11
Alcohols	2
Furanes	8
Various components	8
Total compounds observed	93

4.4.6 Aldehydes in oil vapour

The results of the aldehyde analysis in the oil vapours for the different simulations are presented in Table 4.14.

Table 4.14. The concentration of aldehydes found in the oil vapour during the different simulations.

Oil type	A_n	A_n	A_u	A_u	B_n	B_n
Temperature range (˚C)	20-350	375±25	20-350	375±25	20-350	375±25
Test duration (min)	30	60	30	60	30	60
Component	µg/m^3	µg/m^3	µg/m^3	µg/m^3	µg/m^3	µg/m^3
Formaldehyde	2953	465	670	2900	5000	3300
Acetaldehyde	2555	72	1900	920	6100	162
Propionaldehyde	190	10	<	490	1300	420
Crotonaldehyde	51	93	32	450	260	330
n-Butyraldehyde	129	14	470	1300	900	30
Benzaldehyde	<	<	<	42	25	70
iso-Valeraldehyde	11	28	39	100	<	<
n-Valeraldehyde	98	99	68	480	300	62
m-Tolualdehyde	140	100	87	220	170	110
p-Tolualdehyde	<	<	46	44	<	<
Hexanal	260	76	30	<	180	300
Σ aldehydes	6387	957	3342	6946	14235	4784

<: below detection limit

Relative high concentrations were found in the oil vapours for formaldehyde and acetaldehyde. The highest concentrations of total aldehydes were found for the new oils in the beginning of the simulation test (20 to 350°C). However, the used oil A (A_u) showed another pattern, whereby the highest concentration of aldehydes was measured at 375±25°C.
It has to be stated, that the sampling of aldehydes was affected by the matrix of oil vapours, resulting in breakthrough of aldehydes. DNPH cartridges turned from yellow to brown colour (see Figure 4.16), and no DNPH was left on the desorbed cartridge. Based on this fact, it can be concluded that due to matrix effects, the results of the aldehydes sampled are probably underestimated concentrations, results must be considered as indicative.

Figure 4.16. Left: DNPH cartridges after sampling oil vapours at 375°C. Right: DNPH cartridges after sampling the blank system at 375°C.

4.4.7 Particle number and carbon monoxide in oil vapours

Particle number concentrations (PNCs)
Particle number concentrations (PNCs) were measured during each simulation test. Measurements were performed with a cut-off of 4 nm to 1000 nm particle diameter and data points were measured each second. Concentrations of particles were calculated in particle numbers per cm^3. For each simulation test mean, minimum and maximum PNC were calculated over the following four time intervals:

1. Start simulation from 0 - 13 minutes; temperature range 20 to 180°C
2. Simulation from 13 - 31 minutes; temperature range 180 to 350°C
3. Simulation from 0 - 30 minutes; temperature range (375 ± 25)°C
4. Simulation from 30 - 60 minutes; temperature range (375 ± 25)°C

Tables 4.15 - 4.17 shows the results of the PNCs in the oil vapours during the simulation tests.

Table 4.15. PNCs in the oil vapour of new oil A (A_n) measured in the simulation test and presented for the four intervals

Oil type	A_n	A_n	A_n	A_n
Temperature range (˚C)	20-350	20-350	375±25	375±25
Test interval (min)	0-13	13-31	0-30	30-60
	#/cm^3	#/cm^3	#/cm^3	#/cm^3
Mean PNC	86	725200	83278	2156
Minimum PNC	37	237	6656	1088
Maximum PNC	760	1584000	357520	6656

Table 4.16. PNs in the oil vapour of used oil A (A_u) measured in the simulation test and presented for the four intervals.

Oil type	A_u	A_u	A_u	A_u
Temperature range (˚C)	20-350	20-350	375±25	375±25
Test interval (min)	0-13	13-31	0-30	30-60
	#/cm^3	#/cm^3	#/cm^3	#/cm^3
Mean PNC	646	47512	87815	290696
Minimum PNC	191	4934	14600	97978
Maximum PNC	4130	72665	305470	416230

Table 4.17. PNCs in the oil vapour of new oil B (B_n) measured in the simulation test and presented for the four intervals.

Oil type	B_n	B_n	B_n	B_n
Temperature range (˚C)	20-350	20-350	375±25	375±25
Test interval (min)	0-13	13-32	0-30	30-60
	#/cm3	#/cm3	#/cm3	#/cm3
Mean PNC	1610	421031	75385	13215
Minimum PNC	253	45061	14940	8235
Maximum PNC	45061	773820	286330	23677

A visual presentation on the PNCs is another way to demonstrate what is really happening during the total simulation and thus on what is happening during the four intervals where the temperature is raising from room temperature to 350°C and the steady state at (375 ± 25)°C. These results are presented in Figure 4.17.

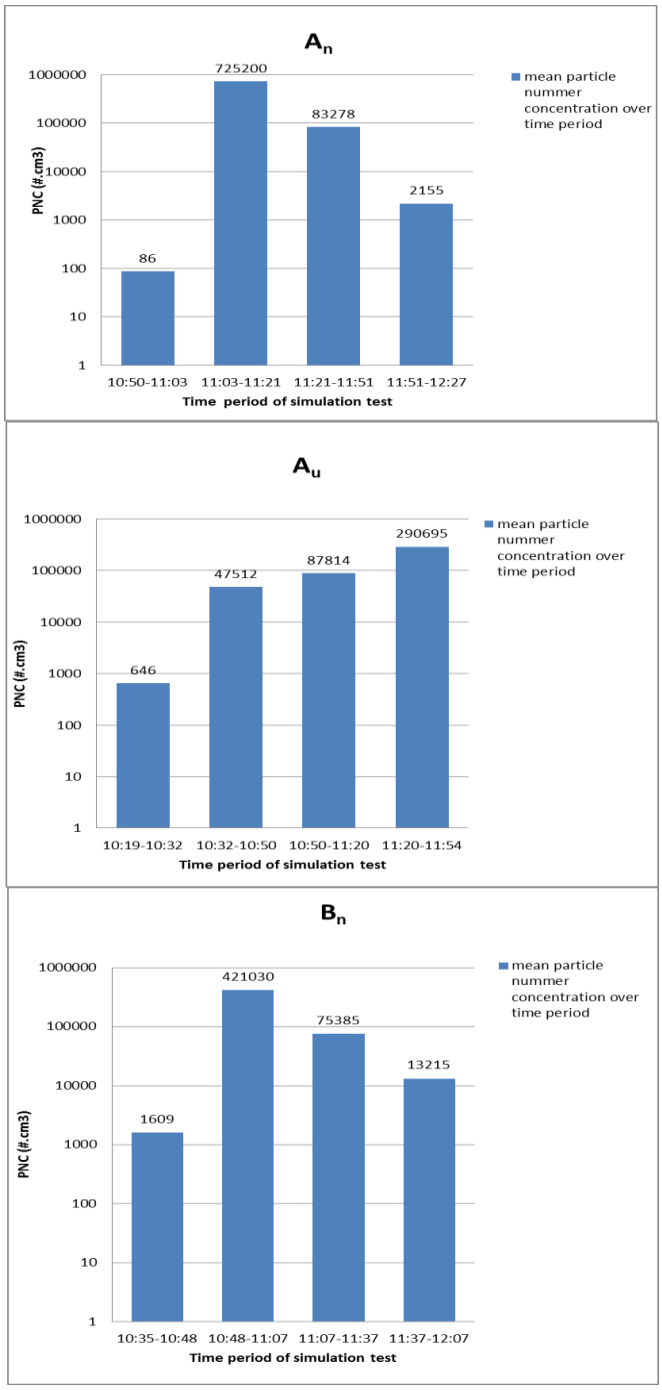

Figure 4.17. The PNCs as measured for the oils during the simulation tests and given for the four defined time intervals. Two intervals are within the heating from room temperature to 350°C and two intervals are within the steady state at 375 ± 25°C.

Based on the graphs, it is evident that PNCs are rising as the emissions of oil increases. It is found that during interval 1, i.e., from the start of the simulation test until 13 minutes after start, the oil warms up to approximately 180°C; here PNCs are relative low. However, after 13 minutes the temperature of the oil is increasing for interval 2, i.e., within a time frame for 17 to 20 minutes to 350 and 375˚C respectively; this results in the production of relative high PNCs.
Furthermore for all the simulation tests except for used oil A (A_u) a decrease in PNCs was found after reaching 30 - 40 minutes of heating (350 to 375˚C) despite of adding dropwise fresh oil to the reaction chamber.

Based on the above, it can be concluded that heating 25 g fresh (unused) oil results in an emission of a total volatile fraction within a relative short time.

Additionally, the PNCs found for the used oil in the time interval 2 (from 13 - 32 minutes after start) differ from the results of the new oils, where high PNCs were found. In the total time interval from 0 - 30 minutes the PNCs of the used oil were rising due to the start of the dropwise adding of oil into the reaction chamber.

Carbon monoxide, CO
Carbon monoxide was continuously measured during the simulation tests.
Data points were measured each 5 seconds. Concentrations of CO were calculated in mg/m^3. For each simulation test mean, minimum and maximum
CO concentrations were calculated using intervals similar to those of the PNCs:

1. Start simulation from 0 -13 minutes; temperature range 20 to 180°C
2. Simulation from 13 - 31 minutes; temperature range 180 to 350°C
3. Simulation from 0-30 minutes; temperature range (375±25)°C
4. Simulation from 30-60 minutes; temperature range (375±25)˚C

Tables 4.17 to 4.19 presents the results of the CO concentration in the oil vapours during the simulation tests for the given four intervals.

Table 4.17. The CO concentration observed in the oil vapour of new oil A (A_n) as measured in the simulation test and presented for the four intervals.

Oil type	A_n	A_n	A_n	A_n
Temperature range (˚C)	20-350	20-350	375±25	375±25
Test duration (min)	0-13	13-31	0-30	30-60
unit	mg/m3	mg/m3	mg/m3	mg/m3
Mean CO concentration	1.1	16	1432	1816
Minimum CO concentration	1.1	1.1	150	680
Maximum CO concentration	1.2	200	1891	2006

Table 4.18. The CO concentration observed in the oil vapour of used oil A (A_u) as measured in the simulation test and presented for the four intervals.

Oil type	A_u	A_u	A_u	A_u
Temperature range (˚C)	20-350	20-350	375±25	375±25
Test duration (min)	0-13	13-31	0-30	30-60
	mg/m3	mg/m3	mg/m3	mg/m3
Mean CO concentration	1.3	17	676	749
Minimum CO concentration	1.2	1	2	1
Maximum CO concentration	1.4	155	909	909

Table 4.19. The CO concentration observed in the oil vapour of new oil B (B_n) as measured in the simulation test and presented for the four intervals.

Oil type	B_n	B_n	B_n	B_n
Temperature range (˚C)	20-350	20-350	375±25	375±25
Test duration (min)	0-13	13-31	0-30	30-60
	mg/m3	mg/m3	mg/m3	mg/m3
Mean CO concentration	1.2	19	630	840
Minimum CO concentration	1.1	1.2	2.2	11
Maximum CO concentration	1.3	263	882	931

Interval 1: at the start of the simulation tests, CO concentrations are approx. 1 mg/m^3 and they can be seen as a usual background concentration levels for indoor air.

Interval 2: after 13 minutes the temperature of the oils are further increasing within a timeframe of 17 - 20 minutes to an oil temperature of 350˚C and 375˚C respectively, resulting in a fast increase of a mean CO concentration of 19 mg/m^3 with maximum concentrations of 263 mg/m^3.

Interval 3 and 4: During the following 60 minutes, now the temperature remains at 375˚C while oil is dropwise added to the vessel, the CO concentration is increasing severe. Now high levels up to 1816 mg/m^3 were observed.

Based on these results, it may be concluded that from the start of the simulation test until the oil has reached 180 ˚C hardly any emission of CO arises. However, it appears that following the increase of temperature of the oil from 180- 375˚C, CO emissions are formed due to incomplete combustion of the oil.

The conclusion given above can be illustrated with the graphs of the CO concentration observed for the given four intervals (Figure 4.18).

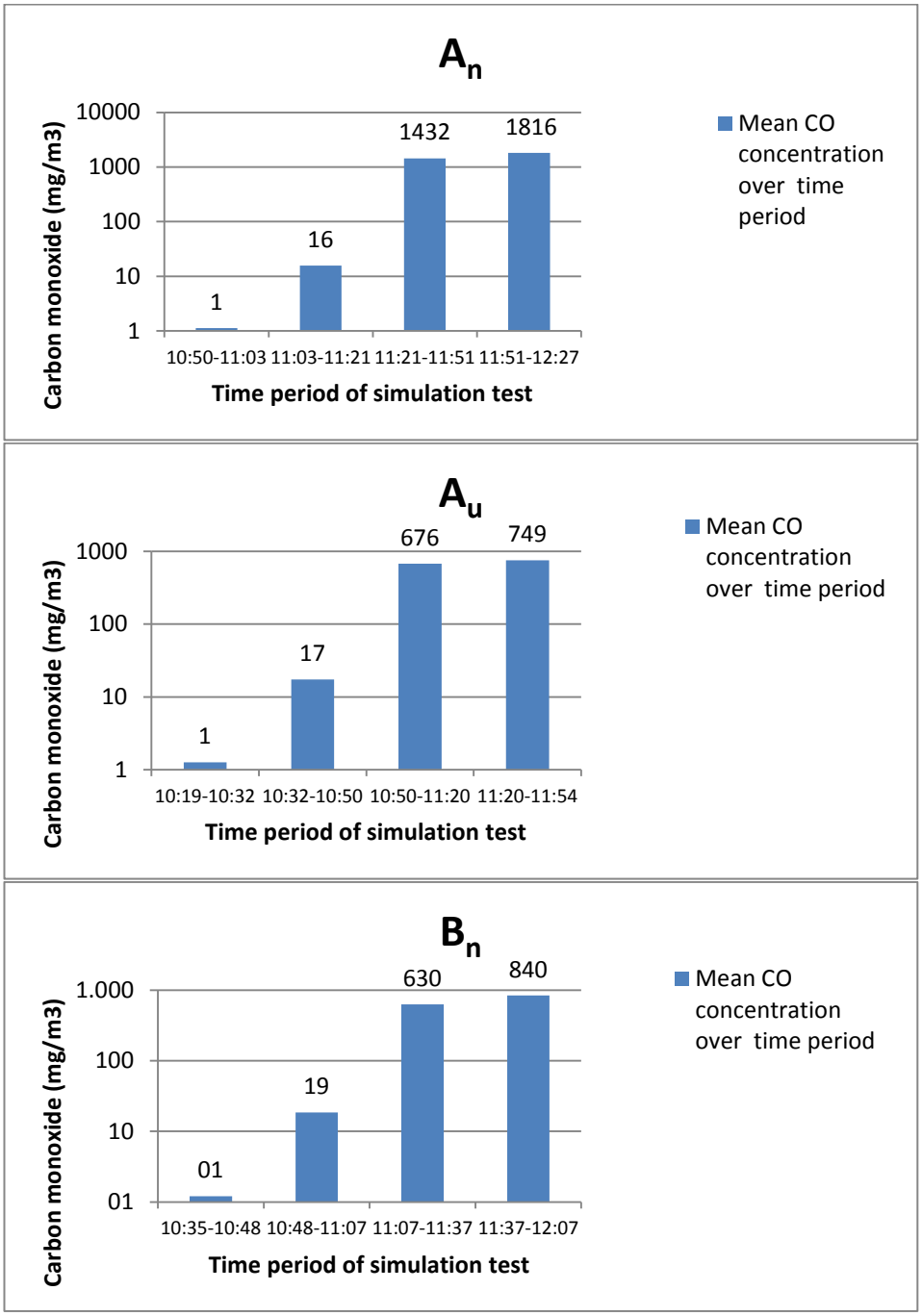

Figure 4.18. The concentration of the carbon monoxide (CO) per interval as measured in the oil vapours measured during the simulation tests. For all test counts that the CO concentration increases per interval, thus due to raising temperature. Upper: new oil A (A_n); middle: used oil A (A_u); lower: new oil B (B_n).

4.5 Limitations for upscaling of the results

The work presented in this report is based on a simulation of a worst-case scenario, i.e., under typical laboratory conditions creating contaminated bleed air from leaked oil into a cabin. Therefore, boundaries have been set, in order to assure that all experiments could be carried out within the limit of time given for this research.

The conditions were based on the following main boundaries:

- Exposure temperature of the applied oil resulting in the emission of (semi)volatiles (approx. 375°C);
- Time of exposure: only two time profiles have been applied, i.e. (1) ground level to top of climb and (2) cruise.

- Flow of the stream of fresh and contaminated air resulting in a simulated ventilation rate within the exposure chamber where the contaminants are sampled from.

For the experiments no differentiation in conditions was made related to different type of engines and engine power, It has been known, that engine conditions may influence any (semi)volatile emission.

Laboratory simulation has been performed without the use of an ECS/PACK.

For the simulation experiments carried out with the emission chamber, only one flow rate is applied causing a ventilation rate of about 6.5 h^{-1}.

Based on the fact that the reciprocal of the concentration of a certain contaminant has a linear relation with the ventilation rate and sampled in a steady state situation, extrapolation of the results is therefore not immediately possible. For extrapolation, sampling of contaminants during a steady state, and thus not during a floating state, using at least three measuring points with a different ventilation rate would be needed.

Therefore it is not meant that results of our work can be up-scaled, neither be related to typical aircraft and engines, and have to be seen as a worst case scenario performed under laboratory conditions only.

4.6 Conclusions

Based on the chemical characterization in the oils itself and in the emissions of oils performed in the simulation tests for oil A (new and used) and oil B (new), conclusions were established and are presented below.

Performance of the test
The simulation test contains two stages simulating the start of the engine to top of climb (time frame of the simulation was 30 minutes) and a steady state period for 60 minutes. During steady-state oil was added dropwise. The simulation tests were kept under controlled and comparable conditions for each oil. At the start of the simulation test the temperature of the oils were approx. 21˚C and within 30 minutes the oils reached a temperature of 350°C. During the next 60 minutes of the simulation test the temperature of the oils amounts (375 ± 25°C).

Performance of the oils
The % loss in weight for the used oil A (A_u) is significant lower than for the new oils A (A_n) and oil B_n, while the settings of time, temperature and oil mass applied for the simulation test were set equally to each other.
It may be concluded that the used oil contains less semi volatile- and volatile oil fractions compared to the new oils and thus resulting in less evaporation.

TCPs
Based on the analysis in the original oils it was found that in all oils the tri(m,m,m,)-, tri(m,m,p)-, tri(m,p,p)- and tri(p,p,p)-cresyl phosphate were detected. Tri(o,o,o)-cresyl phosphate was not detected in the oils.

The mass percentage of TCP calculated from the analysis of the oils corresponds with the oil specifications described in the MSDS sheets from the suppliers.

Based on the analysis of the oil vapours it was found that the four isomers of TCP were detected in all oil vapours in the same composition as was found for the original oils. Tri(o,o,o) cresyl phosphate was not detected in the oil vapours.

From the simulation test it was found that the oils heated at a steady state of 375 ± 25°C emits more TCPs than compared to the simulation whereby the temperature is heated up from 20 to 350°C.

The simulation test shows a good correlation between the composition of TCP isomers found in the original oil and in the oil vapours at different temperatures. Comparison of new oil A (A_n) and used oil A (A_u) shows no significant differences in composition of the 4 isomers.

PAHs

The results of the analysis of PAHs in the vapour shows more numbers of PAHs as were found in the original oils. This could imply the follow hypotheses:

- There is a possible formation of PAHs during heating of the oil and due to incomplete combustion,
- PAHs found in the vapour may originate from small concentrations in the original oil (concentrations below the detection limit).

Most of the PAHs found in the vapour of the used oil A (A_u) could not be found in the used (liquid) oil. This implies that possible formation of certain PAHs occurred during (incomplete) combustion and that they do not remain in the oil but are released by its vapour.

Oil B_n contained the highest amount of PAHs. The sum of PAHs found in oil B_n was about 10 to 20 times higher compared to oil A (for both new and used oil).

Mineral oil

Based on the results it was found that the mass distribution at room temperature of the original oil A_n (Figure 4.14, top) consists of alkane chains in the range of C_{24}-C_{50}. After 30 minutes of heating from 20 up to 350°C the composition of mineral oil in the vapour remains unchanged and is comparable with the mineral oil chains found in the original oil at room temperature (Figure 4.14, middle). After heating at a temperature of 375˚C, small changes in composition of the oil are observed in the chromatogram (Figure 4.14, bottom). There is an increase of relative low boiling point compounds during the simulation test at 375˚C. Additionally unidentified complex mixtures (UME) are formed beneath the C_{24}-C_{50} peaks.

The results of the mineral oil analysis for used oil A (A_u) gave at room temperature similar results as were found for new oil A ($A_{n)}$. Based on the results, it was found that the mineral oil composition for oil B_n is comparable with the composition found for oil A_n. Also for oil B_n the alkane chains have a range of C_{24} – $C_{50,}$ with the exception of one peak found around C_{21}. Additionally the ratio of the alkane chains differs between the two oils. Figure 4.15 shows the chromatograms of oil A_n and oil B_n.

VOCs

Based on a selection of the obtained results (aromatics, ketones, and esters) it was found that the emission of both oils differ at both simulations ‘ground level to top of climb (20 to 350°C) and subsequently cruise speed (375 ± 25°C).

The total VOCs (aromatics, including benzene, toluene and xylene isomers) for oil A and oil B at simulation one (20 to 350°C) amounts 36 µg/m^3 and 48 µg/m^3 respectively. These concentrations increased drastically at cruise simulation at 375 ± 25°C,1.279 and 3.098 µg/m^3 respectively. Similar results were observed for other (various) volatiles.

Aldehydes

High concentrations were found in the oil vapours for formaldehyde and acetaldehyde. The highest concentrations of total aldehydes were found for the new oils in the beginning of the simulation test (20 to 350°C). Used oil A (A_u) showed the highest concentrations at 375±25°C.

Due to matrix effects, the results of the aldehydes sampled at 375 ± 25°C were unreliable and results must be considered as indicative.

PNCs

PNCs are rising as the emissions of oil increases. In order to have a closer look on the emission of PNCs, four time intervals have been defined for the two simulation tests.

1. Start simulation from 0-13 minutes; temperature range 20 to 180°C
2. Simulation from 13-31 minutes; temperature range 180 to 350°C
3. Simulation from 0-30 minutes; temperature range 375 ± 25°C
4. Simulation from 30-60 minutes; temperature range 375 ± 25°C

From the start of the simulation test until 13 minutes after start, the oil still warms up at approximately 180°C: PNCs are relative low.

After 13 minutes the temperature of the oils is increasing within a time frame of 17 - 20 minutes to an oil temperature of respectively 350 and 375°C. Now relative high PNCs were observed.
For all simulation tests except for used oil A (A_u) a decrease in PNCs was found after reaching 30 - 40 minutes of heating (350 to 375°C) despite of adding dropwise fresh oil to the reaction chamber.

It can be concluded that heating 25 g fresh (unused) oil results in an emission of a total volatile fraction within a relative short time.

Carbon monoxide (CO)
It may be concluded that from the start of the simulation test until the oil has reached 180°C hardly any emission of CO arises. It appears that following the temperature of the oil from 180 to 375°C, CO emissions are formed due to incomplete combustion of the oil.

5 Task 3: Chemical characterization of the oils after pyrolysis

5.1 Background

Pyrolysis of oils during flight will result in a multi-component mixture with variable concentrations. Even the selectivity of 1-D chromatography combined with mass spectrometry is not always sufficient for full resolution and elucidation of all compounds. In that case additional selectivity is required. We therefore proposed to apply comprehensive GC-MS (GCxGC-MS) to such mixtures. This technique allows the user to fully benefit from the separating potential of the combined power of two independent gas chromatographic columns. In short, this means that it is now possible to increase selectivity to investigate and quantify even the most difficult samples, i.e., multi component mixtures like oils and pyrolysis products thereof. It is also possible to investigate low abundant compounds (i.e. present in low concentrations) in a very intense multi component mixture.

In comprehensive GC-MS, a normal length separation column is used for pre-separation of the analytes, and a very short and very thin (internal diameter) separation column is used for a second dimension separation. The addition of a mass spectrometer (e.g., a Time-of-Flight Mass Spectrometer (ToF-MS) multiplies the capabilities in terms of increased flexibility and the possibility to identify the individual peaks/compounds, yielding a higher selectivity. Furthermore, automated data analysis allows appropriate pattern recognition. ToF-MS encompasses a much higher data acquisition speed than other MS configurations, making it an excellent asset for identification of oil fume compounds. Hypothetically, a large number of presumed and suspected neurotoxic compounds are present in jet oil fumes. Extracting and analysing these compounds from such a complex matrix is demanding due to the large number of interfering compounds, some present at relatively much higher concentrations, which could jeopardize the analysis. Therefore, multi-dimensional techniques such as GCxGC-ToF-MS seem to be most promising for discriminating small amounts of toxicologically interesting compounds in the presence of high abundant compounds (Gregg et al., 2006).

A schematic overview of a 2D GC approach is shown in Figure 5.1. After a primary separation, the outlet of the first column is injected onto a secondary column. The outlet of the secondary column is attached to an appropriate detector, in this case a ToF MS, allowing on line identification of the compounds eluting from the column, which can be represented in a 2D contour plot, or a 3D plot.

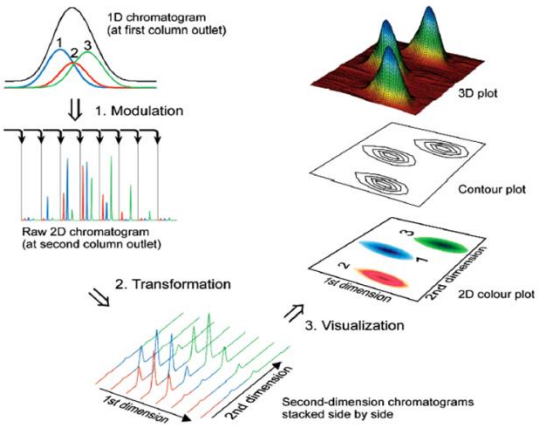

Figure 5.1. Schematic representation of the 2D and 3D viewing options of modulated chromatograms.

So, briefly, in the current set of pyrolysis experiments oil was pyrolysed in an oven in which samples can be exposed for a longer time period (hours) to a constant temperature, under various atmospheres under inert conditions, ambient air or mixtures thereof. Over time, vapours generated were sampled over time using Tenax^{TM} tubes, packed with appropriate adsorbent resin (e.g., TA) at appropriate time points.

After sampling, the Tenax^{TM} Tubes were analysed on the GCxGC-ToF-MS system for the presence, and identification of pyrolysis products in a selected set of oils.

5.2 Methods and materials

5.2.1 Experimental design

Three engine jet oils were characterized using GCxGC-ToF-MS. In the initial phase, a 2D GCxGC-ToF-MS profile of the oils was obtained. This was followed by characterization of samples obtained from fumes at preselected time points during heating the oil in an oven without oxygen (thermolytic profile) and with oxygen (pyrolytic profile) at a temperature vs time profile, resembling in-flight oil heating. Separated peaks were identified using a chemical library, and several comparisons were made to obtain similar and unique compounds in the oils.

5.2.2 Basic profiling of turbine oil with GCxGC-ToF-MS

Three turbine jet engine lubrication oils (A_n, A_u and B_n) were diluted in n-Hexane (for pesticide residue analysis, Sigma-Aldrich) to an approximate concentration of 100 µg/ml. The samples were analysed on an Agilent, Leco, GCxGC combined with a ToF-MS. First dimension separation was performed on a standard non-polar VF-5ms column (50m x 0.32mm x 0.4µm). Second dimension separation was performed on a semi polar VF-17ms column (1m x 0.1mm x 0.1µm). The dual stage modulator was set to a modulation time of 10 seconds, and liquid nitrogen was used as coolant. The GC oven was initially held at 45°C for 1 minute and then programmed to increase at 6°C/min to 360°C; after which the final temperature was held for 5 minutes. The second dimension oven as well as the hot jet had a first dimension oven offset of 10 degrees following the same ramped program. Detection was performed with a Leco Pegasus IV Time of Flight mass spectrometer with an EI source. Scan ranges were m/z 40 to 550 with an acquisition speed of 100 Hz; detector voltages were 1600 volts. The collected spectra were deconvoluted and automatically processed. A signal to noise (s/n) ratio > 150 was used as limit for peak detection. The chromatograms were corrected for background compounds. Peaks were identified (criterion: match factor >800 /999) using the National Institute of Standards and Technology (NIST) and OPCW library.

5.2.3 Simulation of the flight pattern under nitrogen and oxygen conditions

During a flight the turbine oil undergoes a series of temperature changes caused by changes in engine power. A basic temperature pattern is shown in Figure 5.2. As an aircraft climbs and descends the air pressure surrounding the aircraft changes. At cruising altitude the pressure is approximately 266 mmHg. This low pressure results in a lower oxygen concentration than at ambient pressure at sea level. The estimated oxygen concentration at 30,000 feet is 81 g/m^3. Both an oxygen-free and varying oxygen level environment were simulated in an oven (Figure 5.3).

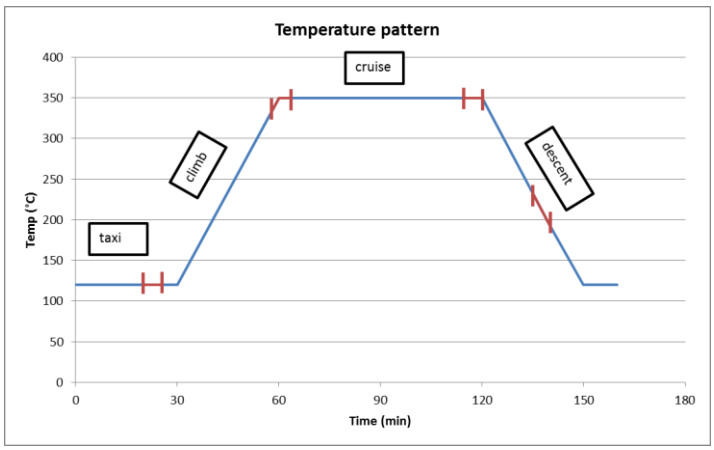

Figure 5.2. Schematic overview of a typical engine temperature profile for a flight duration of approximately 90 minutes. x-axis: time in minutes, y-axis: temperature in °C. The different flight states are labelled and TENAX sampling points are indicated in red.

The oven was programmed to follow the temperature program shown in Figure 5.2. About 4 ml lubrication oil was put in a ceramic cup and placed in the tube oven. To simulate inert conditions, a nitrogen flow of 2 l/min was used.

The oven was heated to 120°C before the ceramic cup was placed in the heated zone of the tube oven. After 30 min the oil was heated within 30 min to 350°C, i.e., the so-called cruising altitude temperature. After one hour at cruising temperature the oven was cooled within 30 min to 120°C by blowing compressed air into the oven.

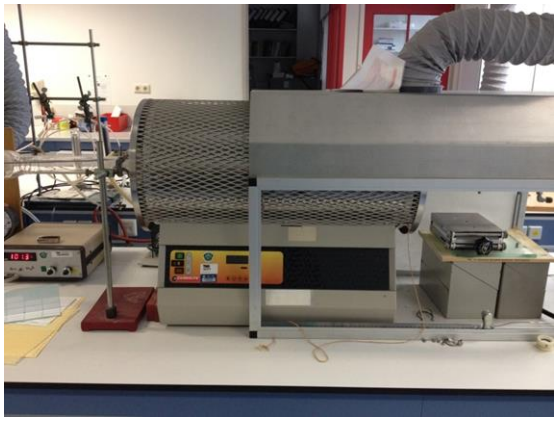

Figure 5.3. Pyrolysis oven with a temperature range from 50-1,100°C. The over can operate under air, nitrogen or other gas

To simulate pyrolysis under oxygenated conditions, a similar temperature pattern was used. During climbing and descending an average oxygen concentration of 178 g/m^3 was used. At cruising temperature, the oxygen concentration was 81 g/m^3. The oxygen concentration was changed by diluting compressed laboratory air with nitrogen retaining a constant flow of 2 l/min through the tube. In the experiments, the oxygen concentration was changed at 300°C during climbing and descending.
The exhaust of the quartz tube oven was led through a series of filters to an FTIR. The formation of CO, CO_2 and H_2O vapour was to follow the flight pattern and see if any large changes like a combustion occurred. Samples were drawn from the exhaust using a mass flow controller (5 ml/min) and a vacuum pump. The sample was led through a glass particle filter to collect high-boiling compounds and oil aerosols. The filtered air was then diluted in nitrogen 10 to 1 and thereof 50 ml/min was drawn over a SGE fritted Optic 3 liner filled with TENAX^{TM} (ta mesh 60/80) to trap organic compounds. Samples (Tenax^{TM} and glass particle filters) were taken during:

- The taxi phase (120°C, 20 - 25 min)
- At the point of reaching the cruise temperature (335 to 350°C, 58 - 63 min)
- At the end of the cruise temperature (350°C, 115 - 120 min)
- During descent (240 to 200°C, 135 - 140 min)
- Experiments were conducted in duplicate (nitrogen only).

5.2.4 Analysis of pyrolysis samples with GCxGC-ToF-MS

Volatile organic compounds were trapped on the Tenax^{TM} tubes; 300°C was the maximum desorption temperature due to Tenax^{TM} degradation. Tenax^{TM} samples were analysed on the Optic 3, Agilent, Leco TOF-MS using desorption. The Tenax^{TM} liner was placed in an air-cooled injector at 40°C and after 30 s stabilization of flows, the injector was desorbed splitless with a column flow of 2 ml/min. The injector was programmed to increase at 15°C/s to the final desorption temperature of 300°C. This temperature was held to the end of the GC run. After a total desorption time of 2 min, the split vent was opened at 250 ml/min and the column flow was 0.8 ml/min for regular chemical separation.
First dimension separation was performed on a standard non-polar VF-5ms column (50m x 0.32mm x 0.4µm). Second dimension separation was performed on a semi polar VF-17ms column (1m x 0.1mm x 0.1µm). The dual stage modulator was set to a modulation time of 4 seconds, and liquid nitrogen was used as coolant. The GC oven was initially held at 45°C for 5 minutes and then programmed to increase at 6°C/min to 200°C; then the oven was programed with 10°C/min to 300°C; after which the final temperature was held for 6.33 minutes. The second dimension oven was initially held at 55°C for 10 minutes and then programmed to increase at 6°C/min to 200°C; then the oven was programed with 10°C/min to 310°C; after which the final temperature was held for 2 minutes. The hot jets had a first dimension oven offset of 10 degrees following the same ramped program. Detection was performed with a Leco Pegasus IV Time of Flight mass spectrometer with an EI source. Scan ranges were m/z 40

to 550 with an acquisition speed of 100 Hz; detector voltages were 1600 volts. The collected spectra were deconvoluted and automatically processed. A signal to noise (s/n) ratio > 150 was used as limit for peak detection. The chromatograms were corrected for background compounds. The chromatograms were corrected for background compounds. Peaks were identified (criterion: match factor >800 /999) using the National Institute of Standards and Technology (NIST) and OPCW library.
Glass particle filters were extracted with 1 ml hexane for ~30 minutes on a rotator. The extract was analysed on Optic 3, Agilent, Leco TOF-MS. 1 µl was injected (split 50:1) at 350°C, which was kept for the entire run, while the column flow was 1 ml/min (constant flow). First dimension separation was performed on a standard non polar VF-5ms column (50m x 0.32mm x 0.4µm). Second dimension separation was performed on a semi polar VF-17ms column (1m x 0.1mm x 0.1µm). The dual stage modulator was set to a modulation time of 10 seconds, and liquid nitrogen was used as coolant. The GC oven was initially held at 45°C for 1 minute and then programmed at 6°C/min to 360°C; this final temperature was held for 5 minutes. The second dimension oven as well as the hot jet had an oven offset of 10 degrees following the same ramped program. Detection was performed as described above.

5.3 Results and discussion

5.3.1 Basic profiling

The basic oil patterns of the two brands (A and B) were obtained. This yielded the 2D total ion chromatograms (TIC) shown in Figure 5.4. The patterns of new oil (A_n) and used oil (A_u) were also compared but there were no significant differences found. Only a set of TCP isomers, 4-octyl-N-(4-octylphenyl)-benzenamine and N-phenyl-1-naphthaleneamine could be identified in the basic oil patterns. TCP isomer peaks were found in all three oils (green arrows, Figure 5.4). N-phenyl-1-naphthaleneamine was only found in oil B_n, although it did not visually appear in the TIC chromatogram, which indicates a low concentration.
Only minor differences were found between the oil A_n and oil B_n. In the red circle compounds appear to be a chain of chemically related compounds. In oil B_n more peaks are found in this red circle than in oil A_n. The peaks have an increasing retention both in the first and second dimension column, resulting in a drift upwards in the chromatogram. The peaks could not be identified with a similarity >800/999 compared to the NIST. The mass spectrum of one of the first peaks in the red circle of oil B_n is shown in Figure 5.5. The similarity is 724/999 and therefore named as “unknown”; a suggested structure is shown. Most fragments found in the peak are also present in the library spectrum, but the difference in intensities results in a low similarity. Other compounds in the found chain exhibit the same fragmentation patterns. Because of low similarity with a library spectrum they were also identified as unknown. Probably these peaks are part of a large molecule, like a polyester with a characteristic fragmentation pattern. Fragments that are characteristic to these molecules have m/z 57 and 85.

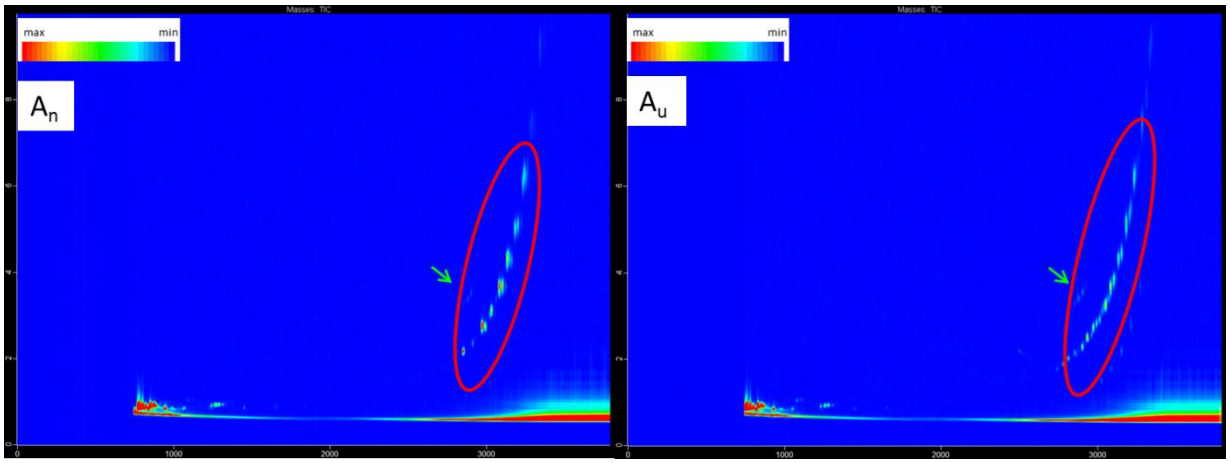

Figure 5.4. 2D Total Ion chromatogram of oil A_n and oil A_u. x-axis: 1st dimension retention time (seconds), y-axis: 2nd dimension time (seconds). The peak intensity is shown by colour gradient

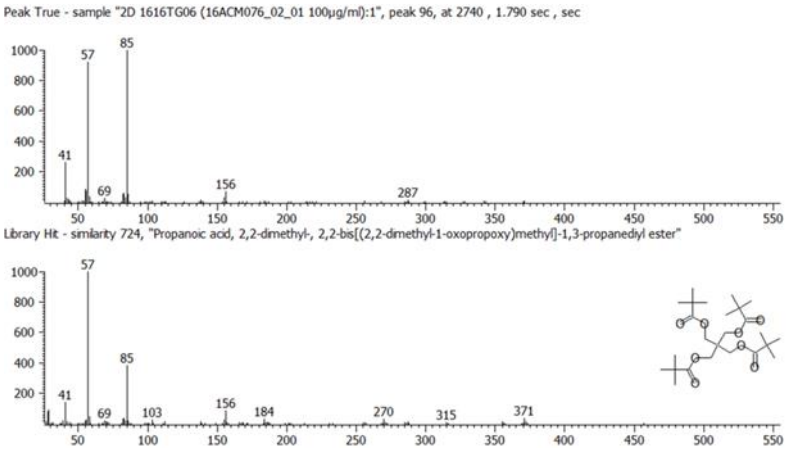

Figure 5.5. In the upper panel, the mass spectrum of peak 96 found in oil B_n at retention time 2740 sec, 1.79 sec is shown. The mass spectrum of the library hit with the best fit (similarity 724/999) is shown in the lower panel.

5.3.2 Overview of peak analysis and identification following pyrolysis

The Tenax™ samples resulting from the pyrolysis experiments were analysed using 2D GC combined with TOF-MS. Peaks were identified using the NIST library and automated data processing. After desorption of the TENAX™ samples the chromatograms were visually inspected. In Appendix 5, 2D GC Tof MS plots of samples obtained during the pyrolysis of the separate oils under both N_2 (duplicate) and O_2 conditions at the different representative flight stages are shown.
An example of a sample analysis is shown in Figure 5.6. A 3D plot of a Tenax™ sample is shown. The sample was taken from oil A at cruise temperature (350°C). The first dimension retention time is displayed (0-3800 sec) on the x-axis, the second dimension retention time is displayed on the y-axis (seconds) and the peak height is displayed on the z-axis and shown by colour gradation. Figure 5.6 shows a large variety of volatile and less volatile compounds in a broad intensity range. For example, 634 peaks were found in this sample. From these peaks, approximately 170 peaks could be matched to a NIST with a similarity >800).
It needs to be taken into account that identification was based only on library similarity, which can be prone to errors. For example, the circled intense peak was identified as 4-[2-(methylamino)ethyl]-phenol, with a similarity of 845/999. However molecular ion 151 and fragment m/z 107 were not present in the spectrum. Moreover, the retention time of the peak did not match with the expected retention time of the suggested compound, which leads to the conclusion that this is a false match. This indicates that the identification of compounds has to be considered indicative.

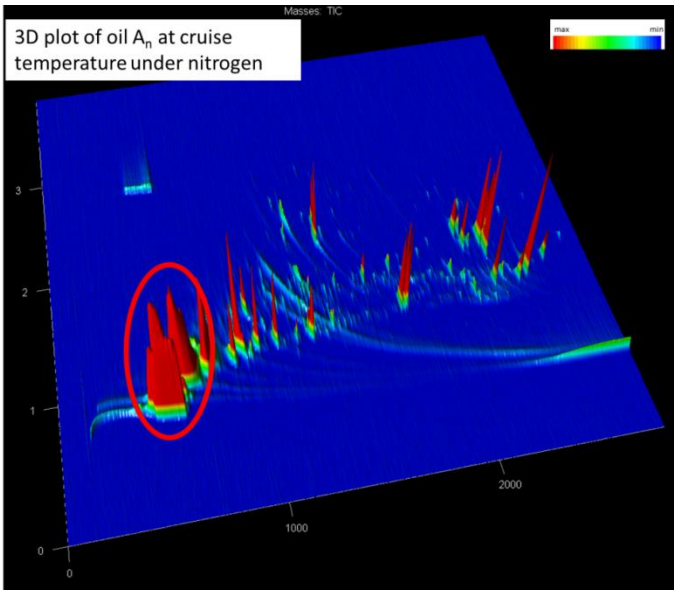

Figure 5.6. 3D plot Tenax^{TM}*, oil* A_n *at reach of cruise temperature. x-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), z-axis: peak height shown by color gradient.*

The total number of identified compounds per oil is shown in Figure 5.7. Higher numbers of compounds are present under oxygen conditions (top light parts of the bars) than under nitrogen conditions (lower dark part of the bars). In particular under O_2 conditions, more compounds were found at high temperatures (cruise, 350°C) than at lower temperatures. During the taxi stage (120°C). the highest number of compounds is generated from heating oil B_n, of which ~90% is unique compared to oil A_n. For oil B_n, the number of compounds over the flight stages remains similar, whereas higher numbers of compounds are found in oil A oil at increasing temperatures under nitrogen conditions. Remarkably, the used oil contains ~50% of unique compounds compared to new oil, indicating a substantial change of composition over the time of use. This is also reflected by the generation of higher numbers of compounds under oxygen conditions during heating of used oil A (A_u) compared to new oil A (A_n). From these observations, a comprehensive list of 127 compounds was constructed that were identified under both nitrogen and oxygen conditions, in all oils and during different flight stages (Table 5.6). Note that acetophenone and benzaldehyde might be formed due to TENAX^{TM} degradation during sampling, caused by reactive compounds in the smoke.

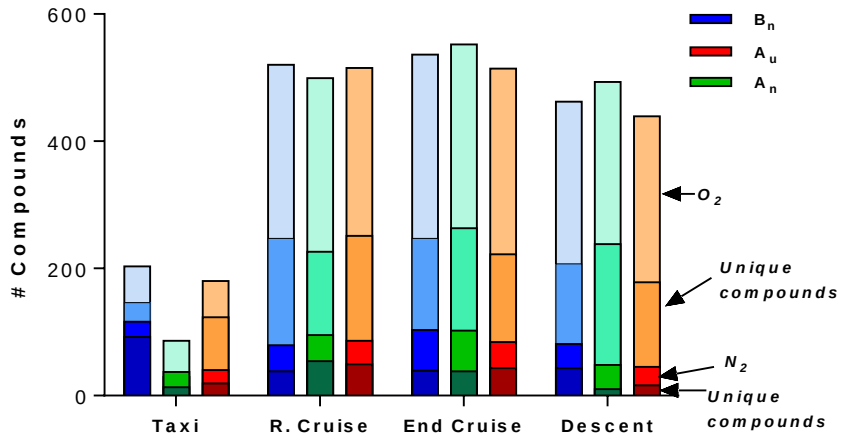

Figure 5.7. Total number of identified compounds per oil and flight stage. Samples taken during 4 flight stages: Taxi (120°C), Reach of cruise (335 to 350°C), end of cruise (350°C) and descent (240 to 200°C) under nitrogen and oxygen conditions. The shades of the bars represent from bottom to top: the number of uniquely identified compounds under N_2 *conditions, overlapping compounds under* N_2 *conditions, uniquely identified compounds under* O_2 *conditions, and overlapping compounds under* O_2 *conditions.*

5.3.3 Comparison of oil A and oil B after pyrolysis under nitrogen

Two brands of turbine oil were tested with a temperature program under nitrogen conditions as described under methods. Samples were taken at similar moments during the experiment and were visually compared. Figure 5.8 shows oil A_n (left) and oil B_n (right) during taxi state. It is clearly visible that there are some major differences in the chromatograms (marked by red circles). Peaks that were found in the orange box were both present in oil A_n as well as in oil B_n. Identification matches of compounds found in both oils are shown in Table 5.1.

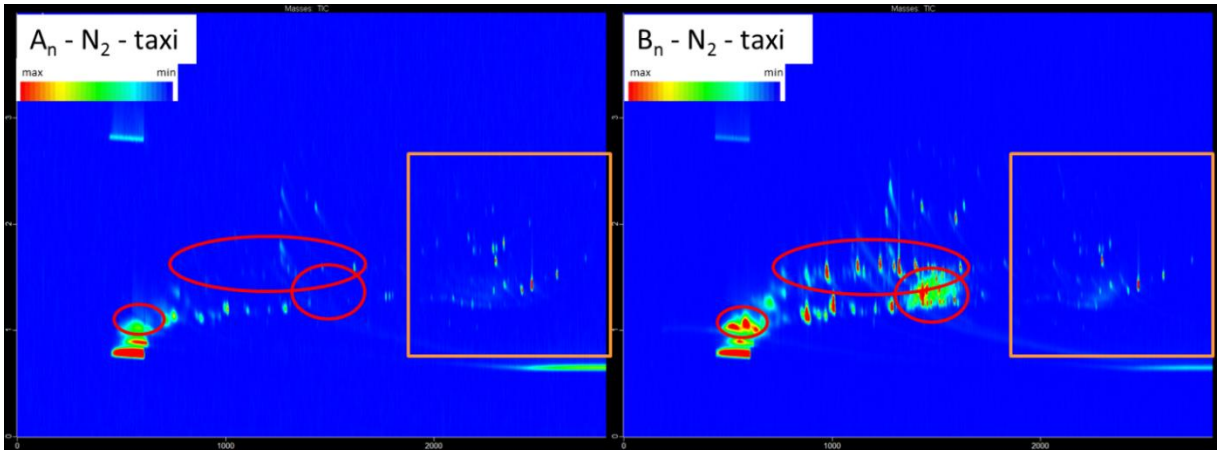

Figure 5.8. 2D TIC of a Tenax^{TM} *sample during taxi temperature. Left panel oil A_n, right panel oil B_n. X-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), peak height shown by colour gradient.*

Table 5.1. Compounds identified (match >800) in both oils, pyrolysis under nitrogen, ranked by similarity match.

Name	**CAS**
1-Tridecanol	112-70-9
2,4-Dimethyl-1-heptene	19549-87-2
2-Ethylhexyl salicylate	118-60-5
2-Propanol, 2-methyl-	75-65-0
5,9-Undecadien-2-one, 6,10-dimethyl-	689-67-8
Acetophenone	98-86-2
Benzaldehyde	100-52-7
Benzene	71-43-2
Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4
Cyclotrisiloxane, hexamethyl-	541-05-9
Decanal	112-31-2
Diethyl Phthalate	84-66-2
Dodecanoic acid, isooctyl ester	84713-06-4
Heptane, 4-methyl-	589-53-7
Hexane, 2,3,4-trimethyl-	921-47-1
Nonanal	124-19-6
Nonane	111-84-2
Octanal	124-13-0
Octane	111-65-9
Phenol, 4-[2-(methylamino)ethyl]-	370-98-9
Glycerin	56-81-5
l-Pantoyl lactone	5405-40-3
Nonane, 2,6-dimethyl-	17302-28-2
Butylated Hydroxytoluene	128-37-0

In oil A_n five compounds were found during taxi state that were not found in oil B_n during taxi state (120°C). The compounds are listed in Table 5.2 sorted on similarity. In oil B_n 70 compounds were identified in duplicate that did not occur in oil A_n. The 10 compounds with the highest similarities are listed in Table 5.3.

Table 5.2. Unique compounds that were identified in oil A_n and were not found in oil B_n during taxi state under nitrogen.

Name	CAS	Similarity
Decane	124-18-5	911
Hexane, 2-methyl-	591-76-4	855
Octane, 1-chloro-	111-85-3	826
Glycidol	556-52-5	820
Decane, 1-chloro-	1002-69-3	812

Table 5.3. Unique compounds that were identified in oil B_n and were not found in oil A_n during taxi state under nitrogen

Name	CAS	Similarity
Pentanoic acid, methyl ester	624-24-8	943
Cycloprop[a]indene, 1,1a,6,6a-tetrahydro-	15677-15-3	929
2-Hexanone	591-78-6	922
1H-Indene, 1-methylene-	2471-84-3	921
Decane, 3,7-dimethyl-	17312-54-8	917
2-Heptanone	110-43-0	915
Naphthalene, decahydro-, trans-	493-02-7	914
Dodecane	112-40-3	913
Undecanal	112-44-7	911
Undecane, 2,6-dimethyl-	17301-23-4	911

5.3.4 Comparison of new oil (A_n) and used oil (A_u) after pyrolysis under nitrogen

The used oil had a high similarity with the new oil when their basic profiles were determined. Both oils were tested under the same experimental conditions and chromatograms from Tenax^{TM} samples were visually compared. Figure 5.9 shows the 2D TIC chromatograms of a Tenax^{TM} sample taken at the moment cruise temperature was reached.
Some distinct differences between the profiles of the new oil (A_n) and the used oil (A_u) were found. It seems that in new oil more high boiling compounds were present. This is indicated by two red circles on the right side of the left chromatogram. These compounds do not appear in the used oil. On the other hand, the used oil shows a large group of peaks right in the middle of the chromatogram, which were either not present or in a much lower concentration in comparison to the new oil. It seems that in the duplicate experiments the same patterns were found in the chromatograms (Figure 5.10). In the sample taken at reach of cruise in the experiments with the new oil a 243 compounds were identified, only 95 were also identified in the duplicate experiment. In the experiment with the used oil 294 compounds were identified in the sample taken at reach of cruise, only 86 were identified in duplicate. Approximately 35% of the identified compounds were found in the duplicate experiments. This gives an indication of the bias in the experiments.

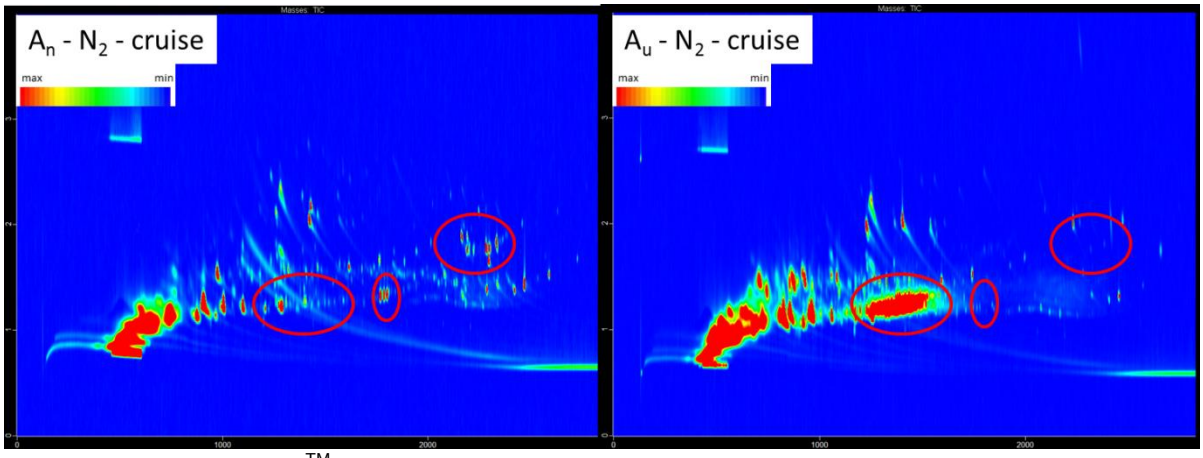

Figure 5.9. 2D TIC Tenax^{TM} *at reach of cruise temperature. Left panel new oil (A_n), right panel used oil (A_u). x-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), peak height shown by colour gradient.*

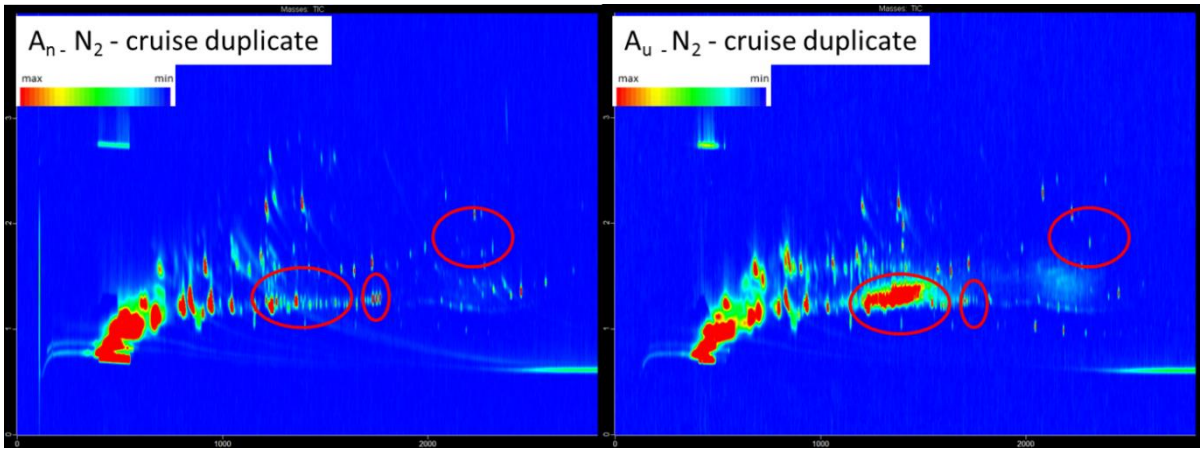

Figure 5.10. 2D TIC Tenax^{TM} *at reach of cruise temperature. Left panel duplicate new oil (A_n), right panel duplicate used oil (A_u). x-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), peak height shown by colour gradient.*

In the chromatograms above 21 compounds were identified in the new oil that were not present in the chromatograms of used oil. On the other hand, 30 compounds were found in the chromatograms of used oil that were not in the new oil. The 10 compounds with the highest similarities are shown in Table 5.4 and Table 5.5. The reported compounds were found in duplicate experiments, which makes it more plausible that usage of the oil leads to formation of these compounds during pyrolysis.

Table 5.4. Compounds found in new oil when the cruise temperature (350°C) was reached that were not found in used oil.

Name	CAS	Similarity
Cyclopropane, ethyl-	1191-96-4	917
trans-3-Decene	19150-21-1	911
Decane	124-18-5	909
3-Undecene, (Z)-	821-97-6	892
Heptane, 3-ethyl-	15869-80-4	887
Isopropyl Myristate	110-27-0	876
Decane, 3,6-dimethyl-	17312-53-7	874
Ethanone, 1,2-diphenyl-	451-40-1	874
Cyclopropane, 1,1,2,2-tetramethyl-	4127-47-3	873
Homomenthyl salicylate	52253-93-7	867

Table 5.5. Compounds found in used oil when the cruise temperature (350°C) was reached that were not found in new oil.

Name	CAS	Similarity
2-Ethylacrolein	922-63-4	914
2-Hexanone	591-78-6	911
2-Heptanone	110-43-0	910
Cycloprop[a]indene, 1,1a,6,6a-tetrahydro-	15677-15-3	896
Pentadecane	629-62-9	889
3-Tetradecene, (Z)-	41446-67-7	889
2-Butanone	78-93-3	887
Benzonitrile	100-47-0	886
Tetradecane	629-59-4	885
Pentadecane, 2,6,10-trimethyl-	3892-00-0	879

5.3.5 FTIR analysis of exhaust air, formed during pyrolysis of oil under nitrogen

The formed smoke was analysed on-line by FTIR to monitor changes in the exhaust during the experiment. The response of oil B under nitrogen is shown in Figure 5.11 (left panel). Changes in oven temperature are also indicated. The graph displays 4 lines: water vapour (green), carbon monoxide (red), ethane (black) and CO_2 (blue). These compounds could be semi-quantified using the standard calibration curves of the software. Since the smoke is a mixture of compounds and the absorption bands overlap, the concentration is only an indication. In Figure 5.11 (right) the infrared spectrum at the highest concentration of both CO and hydrocarbons is displayed. Characteristic abundance peaks are circled and named. Figure 5.12 shows two reference spectra of both ethane and CO_2. Ethane was chosen as a marker for all hydrocarbons because of the absorption band at 2600-3200cm^{-1}. As shown in Figure 5.12, the peak shapes of the sample and ethane are not completely similar to one another, which is caused by interference of other hydrocarbons like nonane and decane. These compounds were also found in the Tenax^{TM} samples.
When the oil is placed in a pre-heated oven at 120°C, the concentration of hydrocarbons in the exhaust air starts to increase. After the oven is heated to approximately 320 to 350°C the concentration rises rapidly, and at the same time CO is detected by the FTIR. After 20 minutes at cruise temperature the concentrations stabilize, followed by a progressive decline in concentration. A few minutes after starting cooling of the oven, the concentration of both hydrocarbons and CO starts to decrease rapidly.

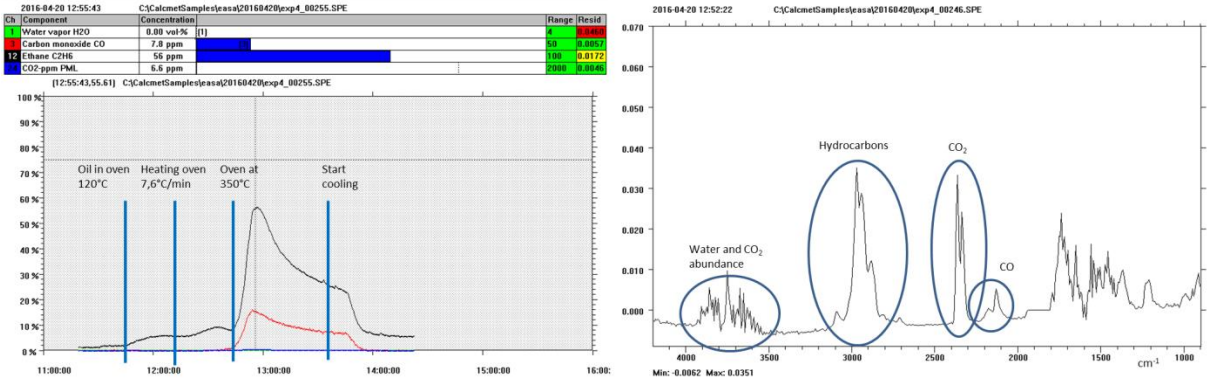

Figure 5.11. Left panel: response on the FTIR during pyrolysis of oil B under nitrogen. X-axis: time (hours), y-axis: response (%). Lines: water vapour (green), CO (red), ethane (black) and CO_2 (blue). Right panel: Infrared spectrum at highest concentration hydrocarbons.

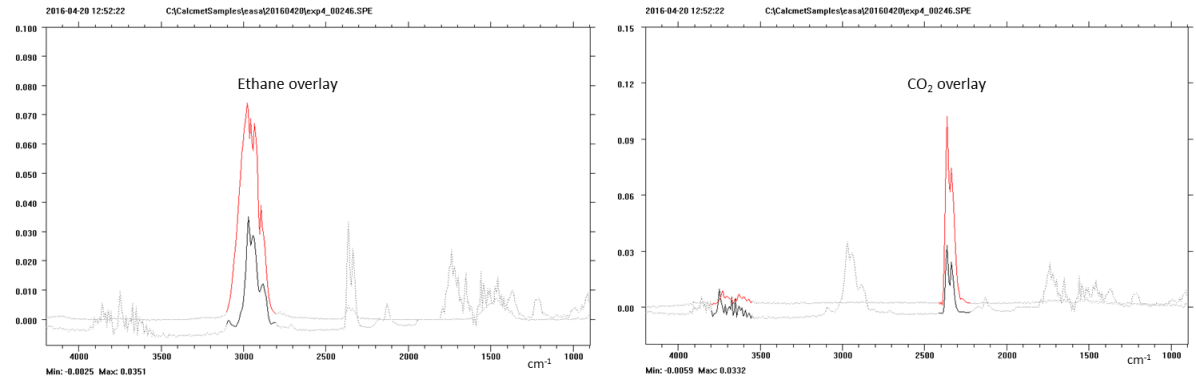

Figure 5.12. Sample (grey and black) and reference (red) overlay; on the left panel ethane reference, on the right panel CO_2 reference.

5.3.6 Pyrolysis experiments in the presence of oxygen

A series of experiments was performed with varying oxygen concentrations. Samples were taken at the same time points as in previous experiments.
After the experiment, the ceramic cup had turned completely black (Figure 5.13), which was not the case when experiments were conducted under nitrogen conditions. The quartz tube in the oven was covered with a tar like substance. The cup and tube were both cleaned by heating the oven to 900°C with an 2 l/min air flow through it. This burned off all organic compounds and cleaned the quartz tube.

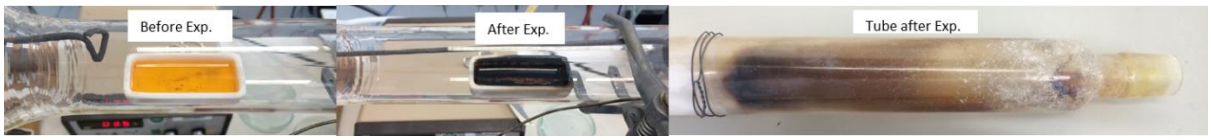

Figure 5.13. Pictures of oil B before and after the experiment

When the chromatograms of the samples taken during the taxi state (120°C) under oxygen were compared with the nitrogen equivalent they seem quite similar to one another (Figure 5.14).

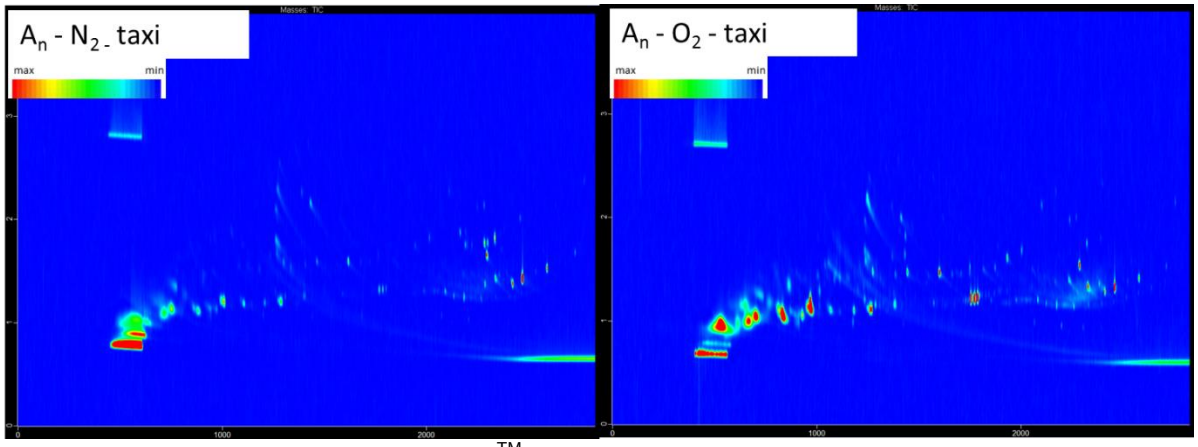

Figure 5.14. 2D TIC chromatogram Tenax^{TM} *sample during taxi temperature. Left panel Oil A under N2, right panel oil A with O_2. X-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), peak height shown by colour gradient*

Heating of the oils to cruising temperature (350°C) led to formation of a thick white/grey smoke in the tube exhaust. Chromatograms of samples taken from this are shown in Figure 5.15. Both chromatograms of oil A_n and oil B_n show a high saturation of both the number and concentration of compounds. An overload as found in these samples led to poor separation and increases the chance of false identification. To permit appropriate separation

and identification, an apparently much higher dilution was required. As this was not anticipated based on the outcomes of the nitrogen experiments, this was not performed. In the FTIR measurements, combustion of organic compounds was detected, most likely responsible for the latter findings.

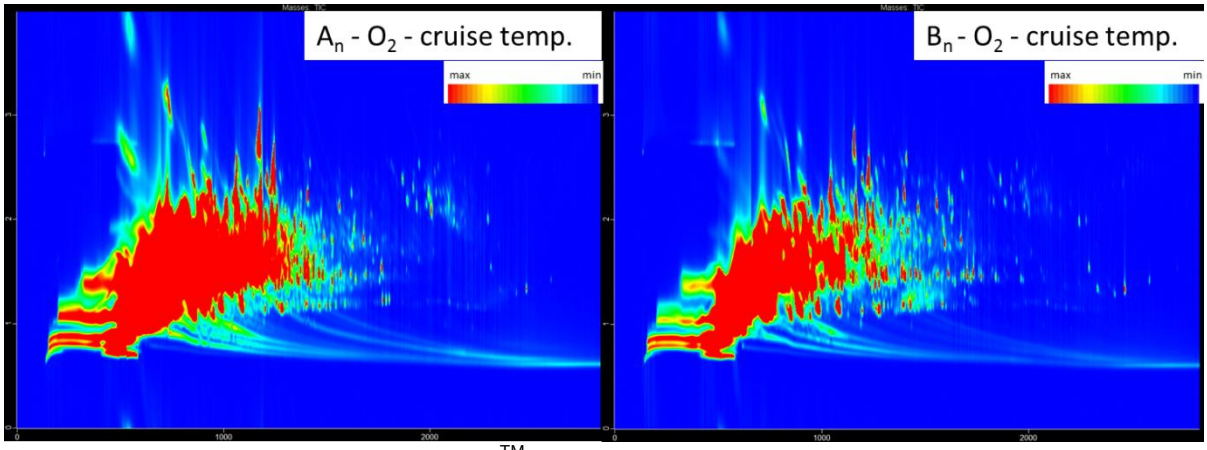

Figure 5.15. 2D TIC chromatogram Tenax^{TM} *sample reaching of cruise temperature with O_2. Left panel oil A_n, right panel oil B_n. X-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), peak height shown by colour gradient.*

The glass particle filters that were placed in line with the Tenax^{TM} sample were extracted with 1 ml hexane. The extracts were analysed with comprehensive GC. Two chromatograms of oil A_n are shown in Figure 5.16. Samples were taken when the oven reached cruise temperature under nitrogen and oxygen conditions. The pattern shown in the left panel looks similar to the chromatograms obtained from the basic profiling experiments discussed earlier. This leads to the conclusion that the bulk of the collected sample were oil aerosols. Compounds that might have been formed during the pyrolysis were either not trapped on the glass filter or were suppressed by the amount of raw oil on the filter. Due to the glass filters the Tenax^{TM} samples obtained during the nitrogen experiments did not contain the oil pattern as shown in Figure 5.16 and were therefore relevant and safe to analyse.

The right chromatogram shows oil A_n at cruise temperature obtained from the experiment with oxygen. The ambient oxygen concentration in the tube was approximately 81 g/m^3. Clearly there are some differences in the two chromatograms. The right chromatogram shows more volatile organic compounds shown in the green circle. On the other hand there are less highly boiling compounds present. One of the compounds that were not present in the nitrogen experiment was pentanoic acid. This compound was found with a library similarity 911/999.

TCP isomers could be identified on filter extracts and not on Tenax^{TM} samples because of the maximum desorption temperature. The exact chemical structure of the individual isomers of TCP could not be elucidated, however, as their mass spectra are almost identical.

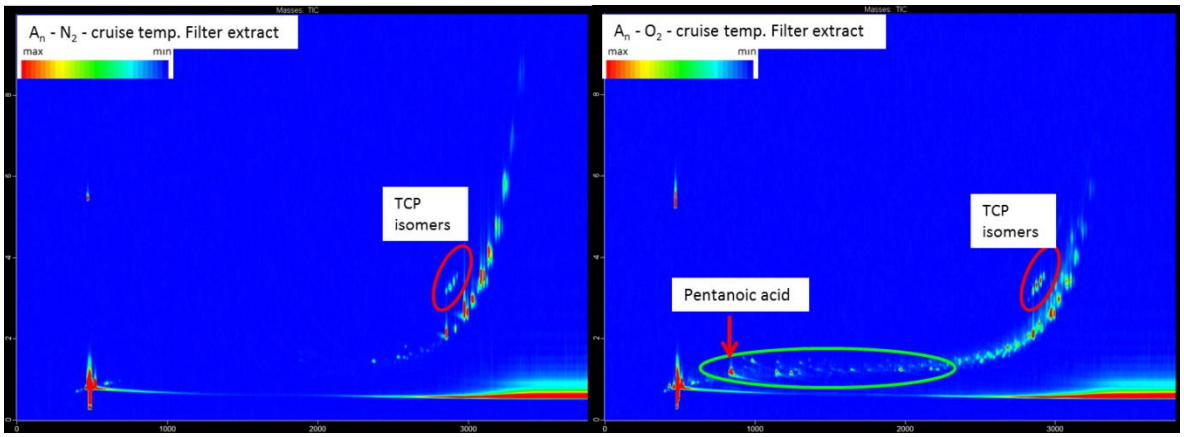

Figure 5.16. 2D TIC chromatogram filter extract at reaching of cruise temperature. Left panel oil A_n under nitrogen, right panel oil A_n under oxygen. X-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), peak height shown by colour gradient

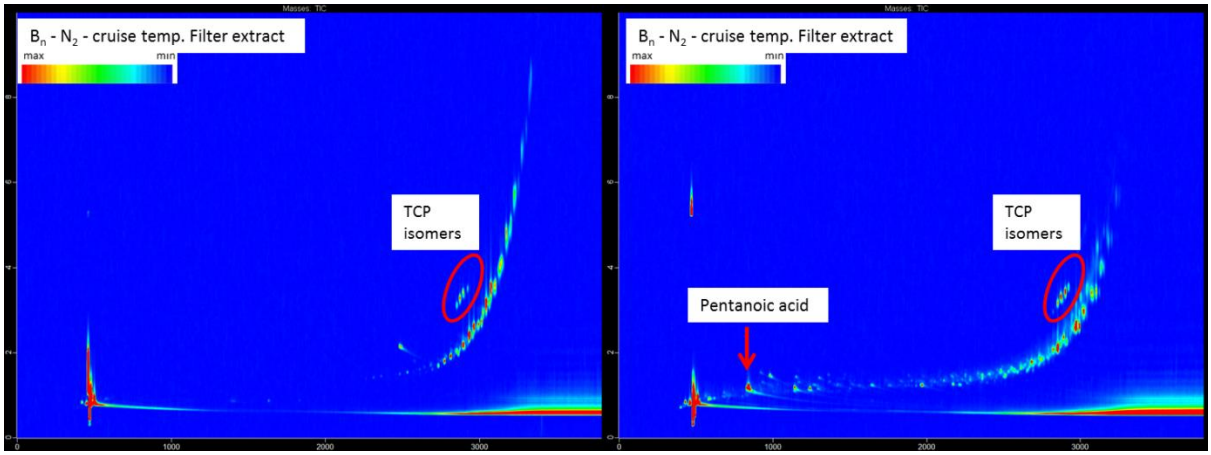

Figure 5.17. 2D TIC chromatogram filter extract at reaching of cruise temperature. Left panel oil B_n under nitrogen, right panel oil B_n under oxygen. X-axis: 1st dimension time (sec), y-axis: 2nd dimension time (sec), peak height shown by colour gradient.

5.3.7 FTIR analysis of exhaust air, formed during pyrolysis in the presence of oxygen

Before the oil is placed in the oven a concentration of CO_2 is detected by the FTIR due to compressed laboratory air that is led into the tube. This is in contradiction to the experiments under nitrogen where no CO_2 was detected before the oil was placed in the oven. Figure 5.18 shows the FTIR signal of the flight pattern of oil B_n with changing oxygen concentrations. During taxi state the signal of ethane, as representative for organic compounds, rises slightly and levels off before the heating starts. As the temperature rises the response increases; when the oven reached 300°C the concentration oxygen is reduced by mixing compressed air with nitrogen. At the same time the response highly increased. CO_2 and water vapour are formed indicating a combustion of organic compounds. CO is also formed which is probably caused by the lack of oxygen resulting in an incomplete combustion. When the oven temperature reached 350°C both ethane and CO had reached their maximum response. Before the oven had cooled down, the response of CO, CO_2 and water vapour started to decrease continuously. It might be possible that the ceramic cup was already empty at this point. The ethane response did not decrease at all. Probably the filters between the exhaust of the tube and the FTIR were completely filled with oil.

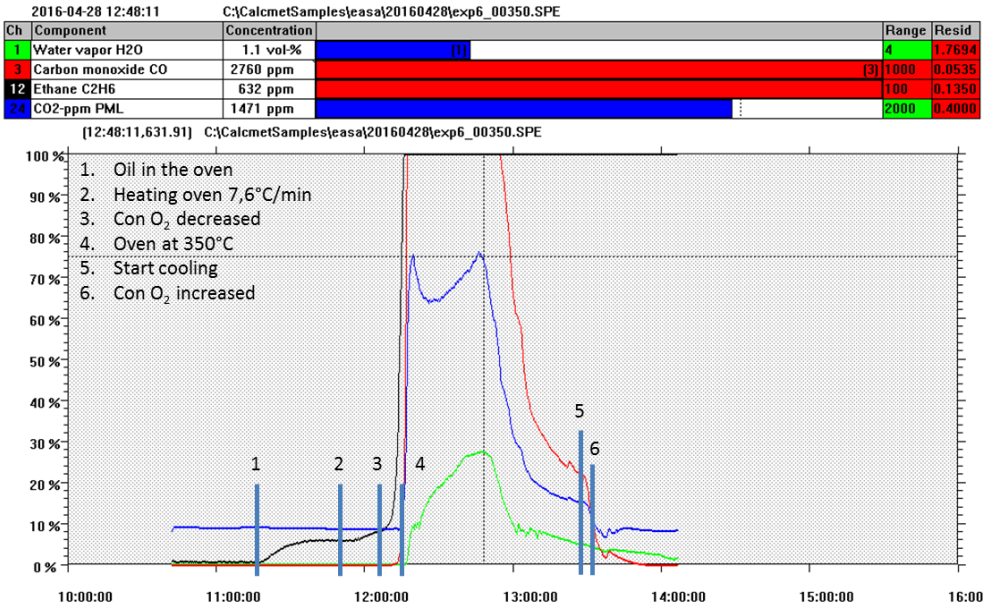

Figure 5.18. Response on the FTIR during a pyrolysis of oil B_n with changing oxygen concentrations. X-axis: time (hours), y-axis: response (%). Lines: water vapour (green), CO (red), ethane (black) and CO_2 (blue).

5.4 Conclusion

The aim was to identify compounds in three oils under a variety of conditions.
In the basic oil patterns, without heating, a set of TCP isomers and 4-octyl-N-(4-octylphenyl)-benzenamine were found in all three oils (green arrows, Figure 5.4). N-phenyl-1-naphthaleneamine was only found in oil B_n, albeit in low concentrations.

Heating under nitrogen led to an increase in the number of compounds found, and led to the identification of 24 compounds in the vapour, found in all oils (Table 5.1). A number of compounds was identified unique for either oil A_n or oil B_n. In addition, used oil (A_u) appeared to contain newly identified compounds compared to unused oil (A_n), and a number of compounds originally present appeared to have disappeared during use in an engine jet. This indicates that during the lifetime of an oil, substantial changes in composition occur.

As it cannot be excluded that oxygen is present in the jet engine, the effect of oxygen addition during pyrolysis was investigated. These experiments showed that the presence of oxygen led to combustion of the oils, resulting in a major increase of the number and amount of compounds.

To permit a safety assessment of compounds originating from jet engine oils, a list of compounds identified under both nitrogen and oxygen conditions, in all oils and during different flight stages was constructed, resulting in 127 compounds (Table 5.6). This list can be used to assess the hazard profile of these compounds using the Classification and Labelling (C&L) database of the European Chemical Agency (ECHA). As a first step, the harmonised classifications as well as the main self-classifications by manufacturers are added to the list of chemicals, as presented in Appendix 6. The classification of a substance is an indication for its toxicity.

The experiments were all performed under atmospheric pressure. In a jet engine the pressures can reach almost 10 bars. This could interfere with the formation and evaporation of organic compounds. On the basis of the physical appearance of used oil (A_u) a combustion of turbine lubrication oil is not likely to occur on a large scale. This would also result in a thick smoke and a pungent odour in the cabin during flight.

Table 5.6. Compounds found after pyrolysis in all oils, irrespective of experimental conditions

Compound #	Name	CAS
1	Diethyl Phthalate	84-66-2
2	1-Nonene, 4,6,8-trimethyl-	54410-98-9
3	2-Ethylhexyl salicylate	118-60-5
4	Acetophenone	98-86-2
5	Benzaldehyde	100-52-7
6	Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4
7	Heptane, 4-methyl-	589-53-7
8	Nonanal	124-19-6
9	2,4-Dimethyl-1-heptene	19549-87-2
10	Decanal	112-31-2
11	Dodecanoic acid, isooctyl ester	84713-06-4
12	Heptadecane, 2,6,10,14-tetramethyl-	18344-37-1
13	Octanal	124-13-0
14	Dodecane, 4,6-dimethyl-	61141-72-8

15	Heptane	142-82-5
16	5,9-Undecadien-2-one, 6,10-dimethyl-	689-67-8
17	Benzene	71-43-2
18	Glycidol	556-52-5
19	Nonane, 2,6-dimethyl-	17302-28-2
20	2-Propanol, 2-methyl-	75-65-0
21	Decane, 2,3,5,8-tetramethyl-	192823-15-7
22	Nonane	111-84-2
23	Octane	111-65-9
24	Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4
25	2,5-Hexanediol, 2,5-dimethyl-	110-03-2
26	Acetic acid, octadecyl ester	822-23-1
27	Undecane	1120-21-4
28	2-Heptanone, 4-methyl-	6137-06-0
29	Hexane, 2,3,4-trimethyl-	921-47-1
30	1,2-Benzenedicarboxylic acid, di-2-propenyl ester	131-17-9
31	1-Iodo-2-methylundecane	73105-67-6
32	Isopropyl Palmitate	142-91-6
33	n-Decanoic acid	334-48-5
34	Tridecane	629-50-5
35	TCP isomer	563-04-2
36	1-Propanol, 2-methyl-	78-83-1
37	1-Tridecanol	112-70-9
38	5-Hepten-2-one, 6-methyl-	110-93-0
39	Decane	124-18-5
40	Pentane	109-66-0
41	Pentanoic acid, methyl ester	624-24-8
42	Amylene Hydrate	75-85-4
43	Glycerin	56-81-5
44	Heptanal	111-71-7
45	Heptanoic acid, methyl ester	106-73-0
46	Octane, 3,5-dimethyl-	15869-93-9
47	Phenol	108-95-2
48	Propane, 2-ethoxy-2-methyl-	637-92-3
49	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	84-69-5
50	2-Butanone	78-93-3
51	Butylated Hydroxytoluene	128-37-0
52	Decanoic acid, 2-ethylhexyl ester	73947-30-5
53	Diazene, dimethyl-	503-28-6
54	Dodecane, 2-methyl-	1560-97-0
55	Methacrolein	78-85-3
56	Pentadecane	629-62-9
57	Benzaldehyde, 4-methyl-	104-87-0
58	Phenol, 3-methyl-	108-39-4
59	2H-Pyran-2-one, tetrahydro-	542-28-9
60	Benzene, (1,1,2-trimethylpropyl)-	26356-11-6

61	Cyclopropyl carbinol	2516-33-8
62	Hexadecen-1-ol, trans-9-	64437-47-4
63	Hexanal	66-25-1
64	Isobutane	75-28-5
65	Octanoic acid, methyl ester	111-11-5
66	(2-Aziridinylethyl)amine	4025-37-0
67	1,4-Dioxane-2,5-dione, 3,6-dimethyl-, (3S-cis)-	4511-42-6
68	1-Octene, 3,7-dimethyl-	4984-01-4
69	2-Butene	107-01-7
70	Cyclobutylamine	2516-34-9
71	n-Hexadecanoic acid	57-10-3
72	Tridecane, 3-methyl-	6418-41-3
73	1-Octene	111-66-0
74	2(5H)-Furanone, 3-methyl-	22122-36-7
75	Pentanal	110-62-3
76	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	4376-20-9
77	2,5-Furandione, dihydro-3-methyl-	4100-80-5
78	Benzeneacetaldehyde	122-78-1
79	Dodecanoic acid	143-07-7
80	Methylglyoxal	78-98-8
81	Pentadecane, 2,6,10-trimethyl-	3892-00-0
82	2-Hexanone	591-78-6
83	Hydroxyurea	127-07-1
84	(S)-2-Hydroxypropanoic acid	79-33-4
85	1,3,5,7-Cyclooctatetraene	629-20-9
86	2-Propanone, 1-hydroxy-	116-09-6
87	Butyrolactone	96-48-0
88	Cyclopentanone	120-92-3
89	Dodecane	112-40-3
90	Eicosane	112-95-8
91	Heptadecane, 2,6-dimethyl-	54105-67-8
92	l-Pantoyl lactone	5405-40-3
93	Pentanoic acid	109-52-4
94	Phthalic anhydride	85-44-9
95	trans-3-Decene	19150-21-1
96	Undecanal	112-44-7
97	1-Hexene	592-41-6
98	1H-Indene, 1-methylene-	2471-84-3
99	1-Pentene	109-67-1
100	Acetone	67-64-1
101	Decane, 3,7-dimethyl-	17312-54-8
102	Formamide, N-methyl-	123-39-7
103	Hexane	110-54-3
104	Octane, 1-chloro-	111-85-3
105	1,3,5-Cycloheptatriene	544-25-2
106	1-Hexadecanol	36653-82-4

107	3-Undecene, (Z)-	821-97-6
108	Benzonitrile	100-47-0
109	Cyclooctane, 1,4-dimethyl-, cis-	13151-99-0
110	Dodecane, 2,7,10-trimethyl-	74645-98-0
111	Heptane, 3-ethyl-	15869-80-4
112	1-Pentene, 2,4,4-trimethyl-	107-39-1
113	1-Pentene, 2-methyl-	763-29-1
114	1-Propene, 2-methyl-	115-11-7
115	2(3H)-Furanone, dihydro-5-methyl-	108-29-2
116	2-Heptanone	110-43-0
117	2-Hexenal	505-57-7
118	2-Pentanone	107-87-9
119	2-tert-Butyltoluene	1074-92-6
120	Acetic acid	64-19-7
121	cis-2-Nonene	6434-77-1
122	Decane, 1-chloro-	1002-69-3
123	Heptanoic acid	111-14-8
124	Isopropyl Myristate	110-27-0
125	Tetradecanoic acid	544-63-8
126	1-Pentene, 4-methyl-	691-37-2
127	2-Cyclopenten-1-one	930-30-3

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6 Task 3: Performance of the chemical characterization and toxic effects of the oils after pyrolysis.

6.1 Introduction

Toxicity of the oil vapour was assessed in an *in vitro* model of the human lung (human bronchio-epithelial (HBE) cell line in co-culture with human endothelial cells (HUVEC) using an air-liquid-interface system. The combination of HUVEC and HBE cells creates a realistic imitation of the human lung barrier. The lung model is exposed to the vapours via the air, which is representative for the human real-life situation.
Toxicity towards the lung model was assessed using an MTT assay. With this assay an early phase of toxicity in the lung is detectable. As no acute lung toxicity is reported in the real life situation, this endpoint will be used to determine a non-lung toxic exposure level which will be used for further experiments.
The fluid underneath the lung barrier is be used as exposure medium for the neurological model. Using this approach, the neurological model of choice (rat primary cortical neurons) is exposed to the fraction of the vapour that translocated from the air- to the fluid-compartment. Medium from underneath lung cells that have been exposed to clean air was used as negative control whereas medium that has been exposed to vapour without a lung barrier present will be used as positive control.

6.2 In vitro approach – Air Liquid Interface

6.2.1 ALI - Introduction

The pyrolysis products of the turbine engine oil can enter the human body through different routes including via inhalation and skin contact. Given the observation that aviation turbine oil is released into the aircraft environment as vapour, the consortium considers inhalation the primary or most pertinent route of exposure. The other routes can also contribute to the total body exposure; however, primary attention should be paid to the inhalation route. Considering the nature of the reported health complaints the potential effects of exposure stems from disturbance of the central nervous system function. Hence, the preferred methodology consists of an integrated in vitro approach integrating exposure of a (human) in vitro lung model with functional measurements in neuronal networks.
Most cell-based in vitro lung methods are based on exposure of submerged cell cultures. However, to execute this type of exposure, vapour and associated compounds need to be collected in a liquid medium. This procedure is likely to change the chemical identity of the mixture. Therefore, exposure to the complete volatile mixture is preferred. To achieve this, we used the Vitrocell® Air Liquid Interface (ALI; VITROCELL SYSTEMS GMBH, Waldkirch Germany) in which cells can be exposed to a stream of freshly generated vapour and its associated compounds ((Phillips et al., 2005)). Using this ALI system, an in vitro (human) lung model has been exposed to freshly generated aviation oil vapour. The lung model of choice existed of two cell types of human origin, cultured on inserts (see Figure 6.1) resembling the human lung. In this model, cells on top of the insert (human bronchio-epithelial cells) are directly exposed to the air flow, while the endothelial cells that grow on the bottom are in contact with a fluid compartment. Following exposure, the medium in the fluid compartment will contain chemical and particulate compounds from the exposure that cross the lung barrier and likely also biological signal molecules from the lung model. This medium has been used to expose the neurotoxicologically cell model. Appropriate controls have been included to be able to discriminate between effects induced by the vapour exposure and potential effects induced by signal molecules from the lung that may affect neurological signalling.

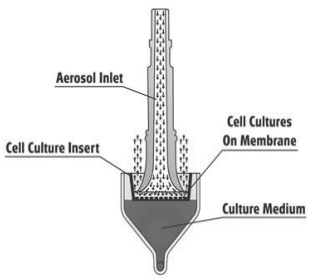

Figure 6.1. Schematic view of the setup of the exposure unit. Cells are grown on membranes with the top being exposed to the aerosol and the bottom being submerged in culture medium.

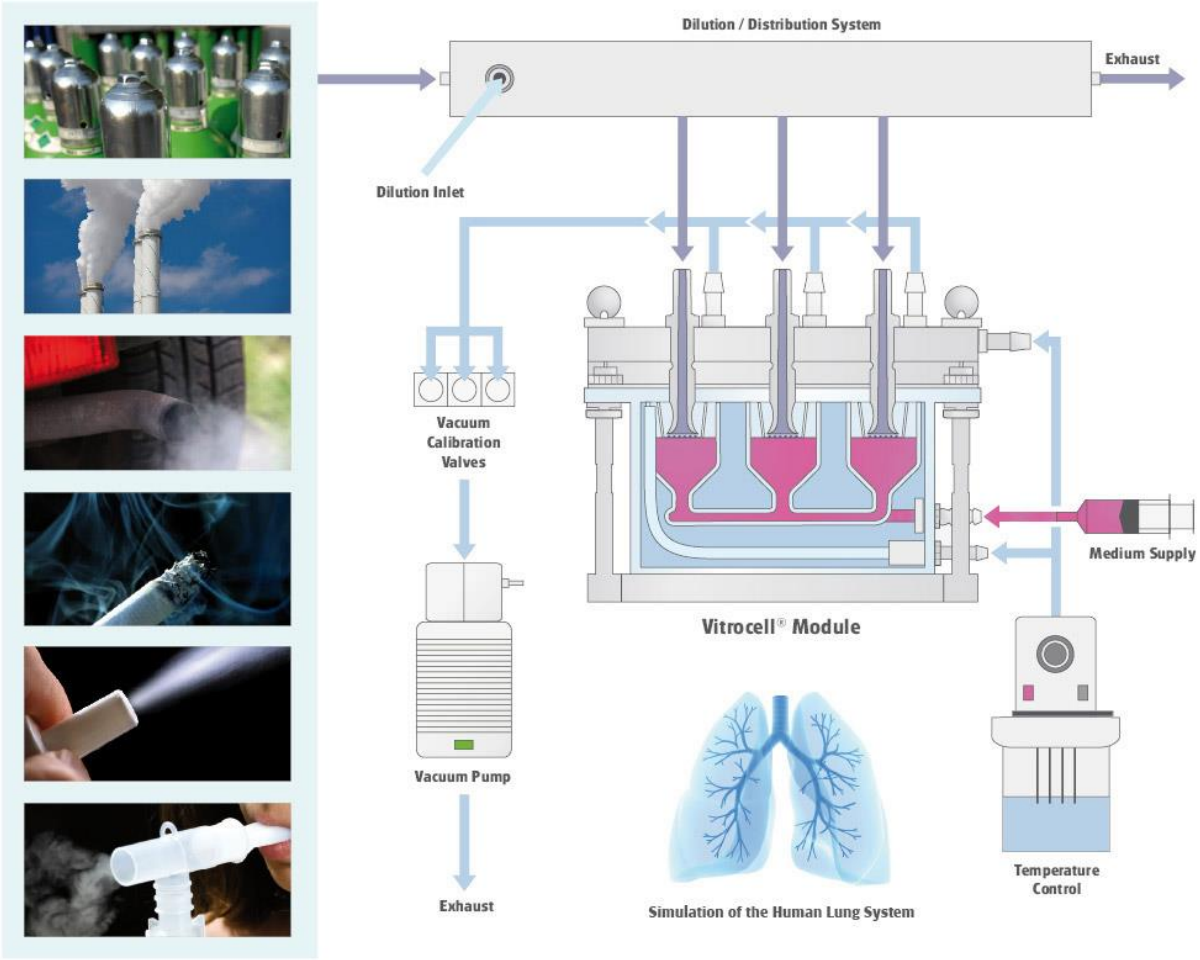

Figure 6.2 Schematic view of the ALI setup.

6.2.2 ALI - Methods

Cell Culture

Human bronchial epithelial cells 16HBE140- cells (16HBE) were kindly supplied by Dr Gruenert (University of California, San Francisco, USA) and cultured in DMEM/F12 medium supplemented with 10% FCS, 1% penstrep, 2mM L-Glutamine and 2.5 µg/ml fungizone. Human umbilical vein endothelial cells (HUVEC) cells were purchased from Life Technologies (Thermo Fisher Scientific Inc., The Netherlands), and cultured in M200 supplemented with 5% LSGS and 1% penstrep. The cells were incubated at 37 °C in a 95% humidified atmosphere containing 5% CO_2. Once confluent, the cells were trypsonized (0.05% Trypsin-EDTA) and transferred to a new culture flask. The medium was renewed once a week. To obtain a co-culture, the cells were cultured on non-coated polyester Transwell® insert membranes with 0.4 µm pores and a surface area of 4.67 cm^2 (6 well plate inserts). HUVEC cells were seeded at a density of 50.000 cells/cm^2 on the basolateral side of the Transwell® inserts. After 3 hours, the Transwell® inserts were returned to their original orientation and the 16 HBE cells were seeded at a density of 200.000 cells/cm^2 on the apical side. The bi-culture was incubated at 37°C in a 95% humidified atmosphere containing 5% CO_2 overnight.

Exposure in the Air-Liquid Interface

After 24h incubation, the inserts were exposed for 4h to the oil vapour from oil A (A_n) or oil B (B_n) in the ALI system. To maximize the probability of particle deposition, exposure was performed under high voltage conditions applying an electrical charge to the particles.

Aerosol generation

Oil was dosed using a motor driven (TSE type S40200, TSE Systems, Inc. Chesterfield USA) syringe with a Schlick compressed air spray nozzle (SCHLICK Mod.970/5 S 9, Düsen-Schlick GmbH, Untersiemau/Coburg Germany).

The oil was nebulized into a heated mixing chamber with pre-heated compressed air, controlled by a Mass Flow Controller (MFC) (Type F201, Bronkhorst Nederland B.V., Veenendaal, the Netherlands). The nebulizer, mixing chamber and compressed air were heated to improve the nebulization by decreasing the oil viscosity and surface tension. The oil aerosol then passed through a stainless steel tube heated by two tube ovens (Heraeus type ROK/A 6/30, Heraeus Holding GmbH, Hanau Germany) where the oil is vaporized/pyrolized. The air/oil mixture temperature was measured inside the tube just before the end of the second tube oven by a thermocouple (Voltcraft K201 thermometer + Type K inconel 600VV thermocouple). At the exit of the ovens, the air/oil mixture was diluted and cooled with compressed air controlled by a MFC. The dilution flow is concentric and to the outside of the air/oil mixture to minimize thermal diffusion losses by shielding it from the cold walls of the connection tube to the ALI. Prior to entering the ALI the mixture passes through a pre selective inlet, designed to remove particles larger than 2.5µm. The generator deliverers less air than what the ALI and monitoring instruments demand, so at the cut off impactor inlet an atmospheric make up air inlet with a HEPA filter allows air to balance the generator and ALI flows.

Downstream of the pre-selective inlet and before the ALI (Figure 6.3), a sample flow was taken to characterize the air/oil mixture. A flow splitter (TSI model 3708, TSI Incorporated, Shoreview, MN USA) distributes the air/oil mixture to inline instruments for measurement of time weighted Particles Mass concentration and vapour composition. Gravimetric measurement was performed with two parallel filters, one Teflon (R2PJ047; Pall corp., Ann Arbor MI, USA) and one Glass Microfibre GF/F (Whatman, Maidstone, England) plus a downstream vapour cartridge. A Sartorius MC-5 microbalance (Sartorius, Goettingen, Germany) was used in controlled relative humidity (40 – 45%) and temperature (21 – 23°C) conditions to do the mass measurements, the filters were weighed before and after each exposure. Laboratory and field blanks were used for quality assurance. The filter volume flow was measured with dry gas meters (Gallus 2000 G1.6, Actaris Meterfabriek B.V, Dordrecht the Netherlands).
To measure mass concentration online, a TEOM (Tapered Element Oscillating Microbalance, Series 1400; Rupprecht & Patashnick, Thermo Fisher Scientific, East Greenbush, NY USA) was connected.
To measure the number concentration and particle size distribution of particles present in the aerosol, a CPC (Condensation Particle Counter 3022A, TSI Incorporated, Shoreview, MN USA) and a SMPS (Scanning Mobility Particle Sizer 3080 including CPC 3788 and Advanced Aerosol Neutralizer 3088) were connected. An aerosol diluter (Main) was used upstream of the CPC and SMPS to bring the air/oil mixture within their measurement range.

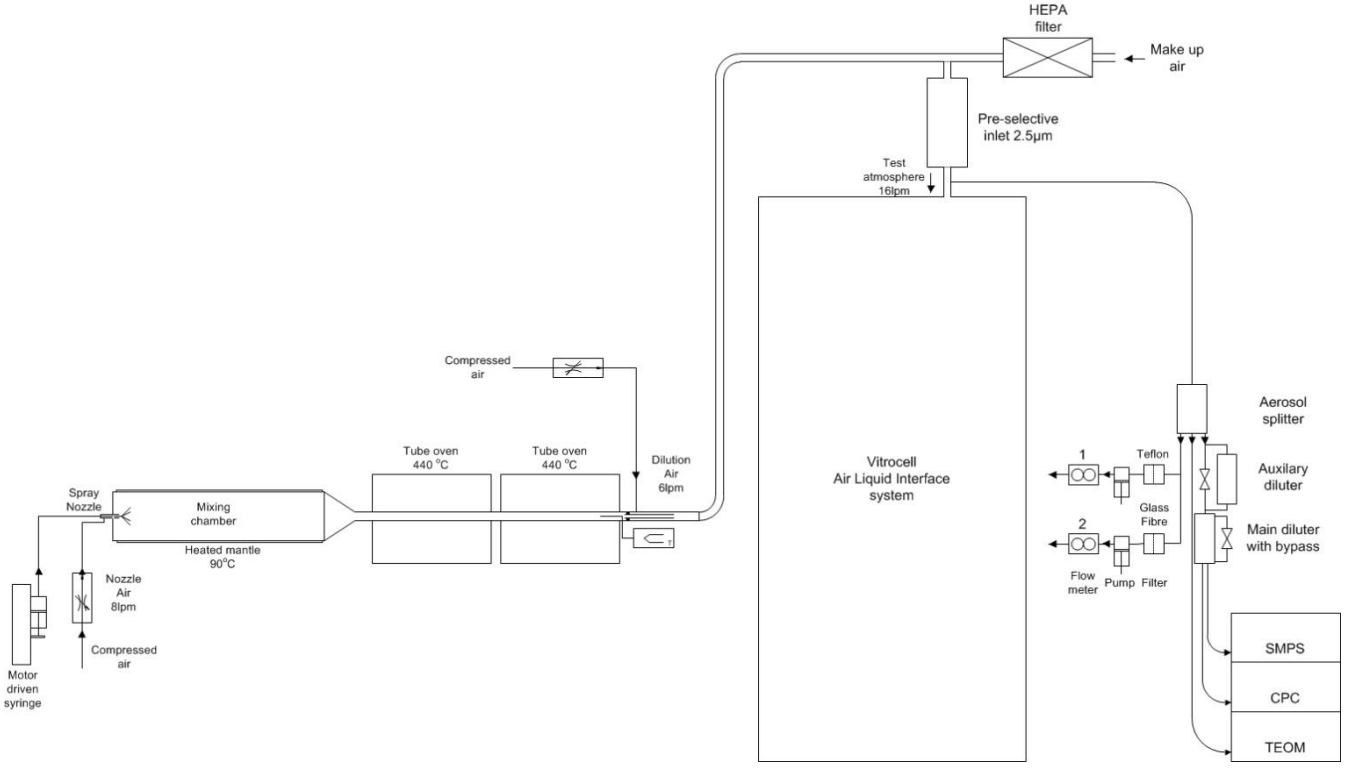

Figure 6.3. Schematic view of the aerosol generation equipment and the placement of the detection and measurement instruments.

Cytotoxicity assessment

Cytotoxicity was measured using the widely used MTT assay, which is based on the conversion of MTS tetrazolium into coloured formazan by the mitochondria of live cells. Thus, a decrease in cell viability caused by the treatment can be detected as decreased conversion of the colourless substrate into the coloured product. The effect of the treatment on cell viability was assessed 24h after exposure to be able to detect delayed toxicity as well.

6.2.3 Result ALI

Cytotoxicity in the human lung model

To determine the exposure level where exposure was maximal with minimum cytotoxicity in the lung model, range finding experiments have been performed.

Based on the outcome of these experiments, an oil dose of 50ul/h was chosen which resulted in an exposure level of around 1 mg/m^3. All medium in the bottom compartment of the wells was pooled per treatment and frozen in a clean glass vial awaiting further use in the neurotoxicological experiments.

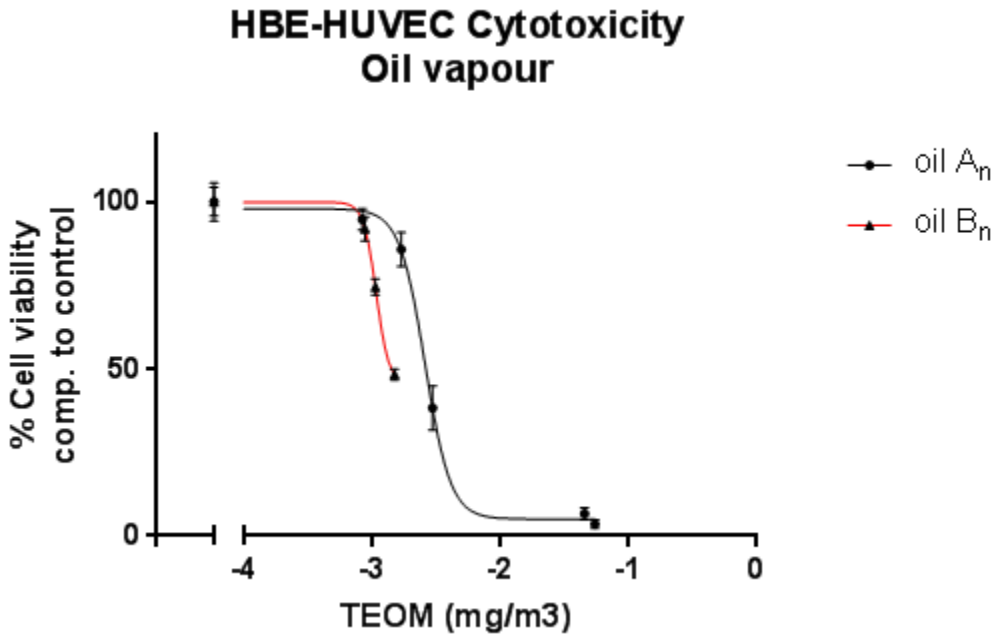

Figure 6.4. Graph displaying the cell viability data in the human lung model following exposure to the two different oils. Cell viability data is plotted against mass concentration as measured using the TEOM. Each data point represents the average cell viability from one experiment with six biological replicates.

Exposure characterization

Using the setup described above, a stable vapour generation was achieved. During the exposures various parameters were monitored including exit temperature, mass concentration, particle number and size as well as total carbon content of the gaseous phase. There were slight inter-experiment variations in the exit-temperature ranging from 330 to 340 °C.

For all exposures an injection speed of 50 µl/h was used resulting in exposure levels between 0.84 and 1.50 mg/m^3 based on TEOM data (Figure 6.5). TEOM data correlated well with the gravimetric measurements on Teflon filters that were used as control for the electronic measurements (R2=0.92). As no filter recording was obtained in one experiment, the TEOM data is used to plot the data. Particle size varied slightly with the dose but only in a very small, well-respirable range (24-46 nm; Figure 6.6). In all exposures the number of particles was comparable (*~1*108 particles/cm^3*; Figure 6.7). No useful TCA measurements were obtained, most likely because of saturation at these exposure levels.

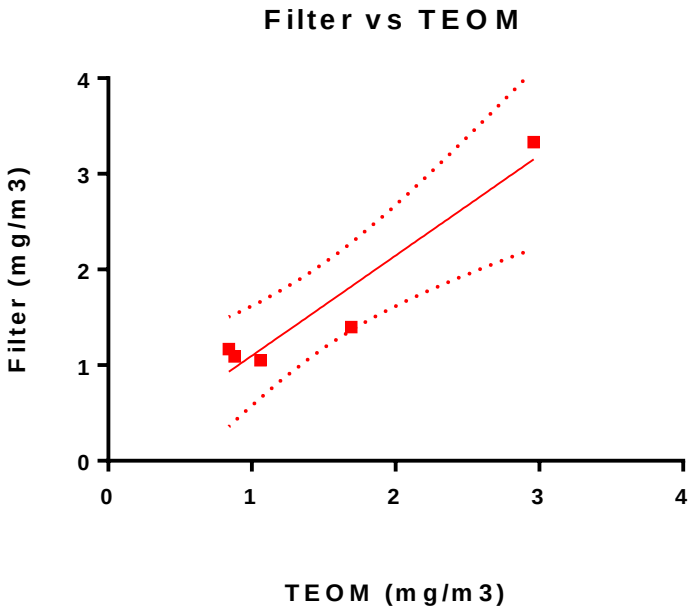

Figure 6.5. Graph displaying the correlation between the TEOM recordings and the gravimetric measurements on Teflon filters. A good correlation between the two measurements was observed (R2 = 0.92).

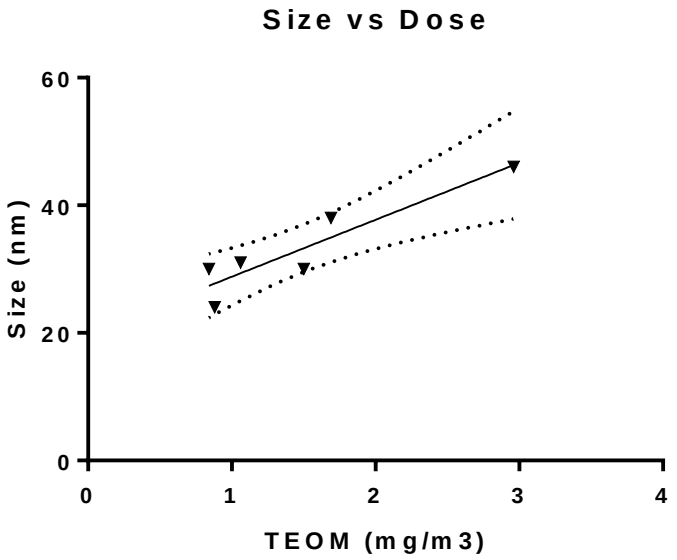

Figure 6.6. Graph displaying the size of particles formed plotted against the measured dose. The data demonstrates a correlation (R2 = 0.85) between particle size and dose with all particles well within the inhalable range (24-46 nm).

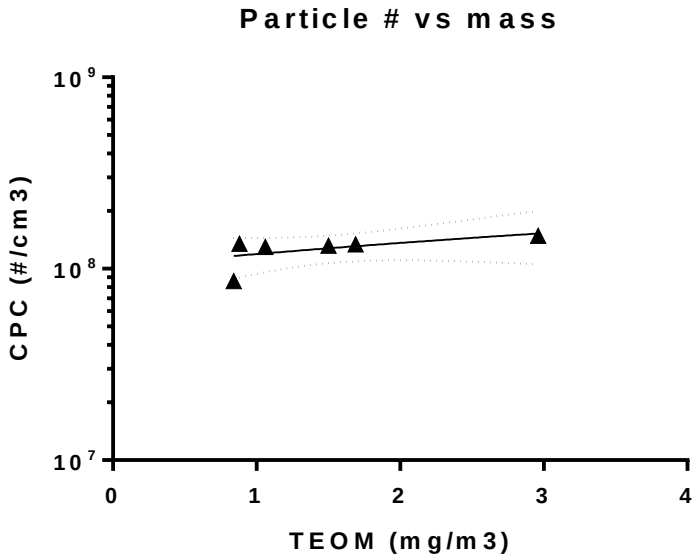

*Figure 6.7. Graph displaying the correlation between the parameters dose and particle number. No correlation (R2 = 0.40) was observed as the particle number was in all experiments ~ 1*108 particles/cm^3.*

6.2.4 In vitro approach - microelectrode arrays (MEA)

6.2.5 Introduction

Current data indicate that the nervous system is most sensitive to potential effects of pyrolysis products of turbine engine oil (Winder et al., 2002b, Ross, 2008, Furlong, 2011, Liyasova et al., 2011, Abou-Donia et al., 2013). Disturbance of neuronal function can induce detrimental short-term as well as long-term consequences, including cognitive psychological defects as well as impaired neurodevelopment and neurodegeneration.

Earlier studies have shown effects of tricresylphosphates (TCP) on several endpoints involved in neuronal function. For example, ToCP was recently shown to inhibit voltage-gated calcium channels (VGCC), glutamatergic calcium signalling and neurite microstructure in mouse embryonic neurons following 1-6 days of exposure (Hausherr et al., 2014, Hausherr et al., 2016). Yet, effects of pyrolysis products are largely unknown.

Effects of pyrolysis on neuronal function can be assessed in vitro using primary cortical neurons grown on microelectrode arrays (MEAs). MEAs consist of a cell culture surface with an integrated array of microelectrodes that allows for the simultaneous and non-invasive recordings of local field potentials at millisecond time scale as measure for neuronal (network) function (for review, see Johnstone et al.(2010), Figure 6.8).

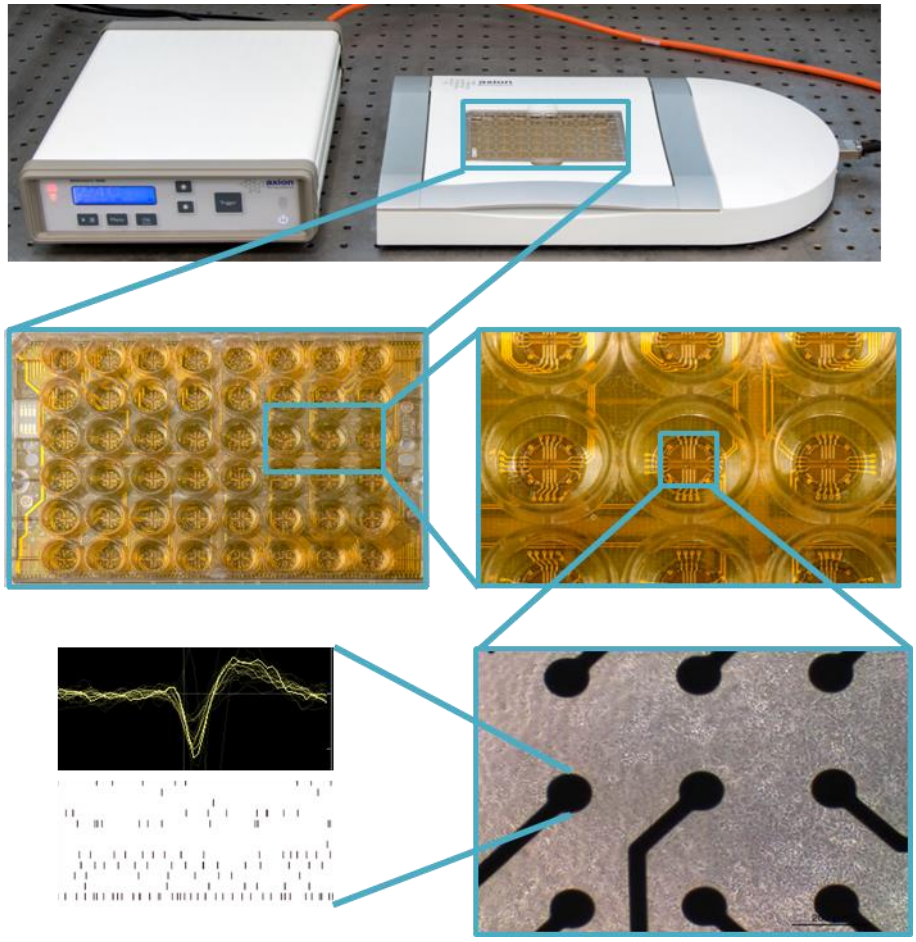

Figure 6.8. Maestro 768-channel amplifier with integrated heating system and temperature controller (top) for recording of neuronal activity. Recordings are performed using 48-well MEA plates (middle left), with each well containing 16 nano-textured gold microelectrodes (middle, right).Detail of primary rat cortical neurons cultured on top of the electrode grid (bottom, right) and the resulting signals representing spontaneous neuronal activity (bottom,left).

Neuronal networks grown on MEAs possess many characteristics of neurons in vivo, including (the development of) spontaneous activity with bursting (Robinette et al., 2011) and responsiveness to neurotransmitters and pharmacological agents (Gross et al., 1997, Johnstone et al., 2010, de Groot et al., 2013). Moreover, MEA recordings have shown consistent reproducibility and reliability across different laboratories(Novellino et al., 2011) as well as high sensitivity and specificity (McConnell et al., 2012, Nicolas et al., 2014, Valdivia et al., 2014). Recently, it was confirmed that primary cortical neurons grown on MEAs are responsive to a range of neurotransmitters and pharmacological modulation of neurotransmitter receptors (Hondebrink et al., 2016) as well as to toxin-induced modulation of ion channels (Nicolas et al., 2014), highlighting the usability of this system as an integrated screening tool that is sensitive to a wide range of compounds.

Therefore, effects of pyrolysis products were investigated in vitro using MEA recordings of primary cortical neurons to assess neuronal network function as an integrated read out, rather than testing all individual processes (modes of action) involved in neuronal function.

6.2.6 Methods

Cell culture.
Experiments were approved by the Ethical Committee for Animal Experiments of Utrecht University and were in accordance with Dutch law. Primary cultures of rat cortical neurons were prepared from postnatal day 0 to 1 Wistar rat pups as described previously (Nicolas et al., 2014, de Groot et al., 2016, Hondebrink et al., 2016), with minor modifications. Briefly, pups were decapitated and cortices were rapidly dissected on ice and minced into small pieces, which were mechanically dissociated by gentle trituration and filtered through a cell strainer (BD Falcon, 100 µm nylon). Cells were resuspended in dissection medium, i.e. Neurobasal-A supplemented with sucrose (14 g/500 mL), 200 mM L-glutamine (Life Technologies, Bleiswijk, The Netherlands), 2.5 mM glutamic acid, 10% fetal bovine serum (FBS, Life Technologies), and 1% of a solution containing 10 000 units/mL of penicillin and 10 000 µg/mL of streptomycin (Life Technologies). The cell-containing medium was centrifuged for 5 min at 800 rpm and supernatant was removed. Primary cortical cells were diluted in dissection medium and seeded on 0.1% polyethyleneimine ([PEI; diluted in borate buffer [24 mM Sodium Borate/50 mM Boric Acid in Milli-Q adjusted to pH 8.4])-coated 48-well MEA plates (Axion Biosystems Inc., Atlanta, USA) at a density of approximately 1 × 105 cells/well for measurements of neuronal activity.
Cells were cultured in a humidified incubator at 37°C and 5% CO_2. At day in vitro (DIV) 1, the dissection medium was replaced by glutamate medium, i.e. Neurobasal-A supplemented with sucrose (14 g/500 mL), 200 mM L-glutamine, 2.5 mM glutamic acid, 2% B-27 (Life Technologies), and 1% penicillin/streptomycin. On DIV4, glutamate medium was replaced by FBS culture medium, i.e. Neurobasal-A supplemented with sucrose (14 g/500 mL), 200 mM L-glutamine, 10% FBS, and 1% penicillin/streptomycin (Life Technologies).

Measurements of neuronal electrical activity using microelectrode arrays (MEAs).
Primary rat cortical neurons grow neuronal networks consisting of βIII-tubulin positive neurons and GFAP positive astrocytes (see Figure 6.9). These primary rat cortical neurons were cultured on 48-well MEA plates, with each well containing 16 nano-textured gold microelectrodes (40–50 µm diameter; 350 µm centre-to-centre spacing) with four integrated ground electrodes (see also Figure 6.8). Spontaneous electrical activity was recorded at DIV9-11 at a constant temperature of 37°C using a Maestro 768-channel amplifier with integrated heating system and temperature controller (Axion Biosystems Inc.) as described previously (de Groot et al., 2014, Nicolas et al., 2014, de Groot et al., 2016, Hondebrink et al., 2016). Axion's Integrated Studio (AxIS 1.7.8) was used to manage data acquisition. Channels were sampled simultaneously with a gain of 1200× and a sampling frequency of 12.5 kHz/channel using a band-pass filter (200-5000 Hz), resulting in raw data files.

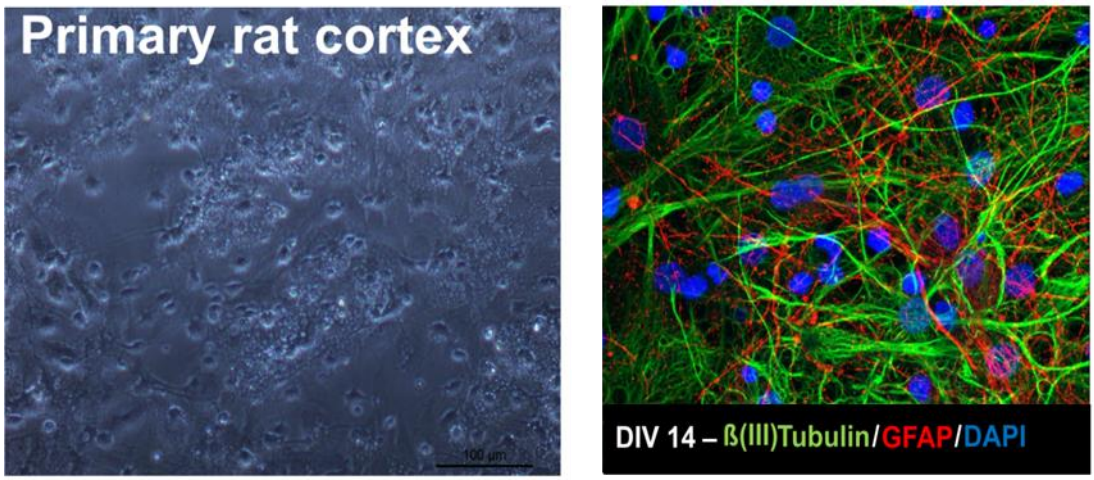

Figure 6.9. Light microscopic image of primary rat cortical cultures (left) and confocal immunofluorescent image (right) demonstrating the presence and distribution of βIII-tubulin positive neurons (green) and GFAP positive astrocytes (red) at14 days in culture (DIV14). Blue staining (DAPI) represents nuclei.

MEA plates were allowed to equilibrate in the Maestro for 5-10 min prior to recordings of electrical activity. At DIV9/10, a 30 min baseline recording of spontaneous activity was made. After this recording the cells were exposed by adding 55 µL of the test chemical (final dilution 10 and 30x) or 165 µL of the test chemical (final dilution

4x; minimum dilution possible) and a subsequent 30 min recording was performed directly following the onset of exposure to determine the acute effect of the test compounds compared to baseline spontaneous activity (paired comparison). At DIV10/11 neuronal activity was measured again to determine the effects of test compounds following 24h exposure. Next, effects of test compounds were normalized to time-matched medium controls to prevent confounding by changes in neuronal activity by ongoing development of cortical cultures over time.

Data analysis and statistics.
For MEA data, raw data files were re-recorded to generate Alpha Map files for further data analysis in NeuroExplorer® software (Nex Technologies, Madison, USA). During re-recording, spikes were detected using the AxIS Spike Detector with dynamic threshold detection (Adaptive threshold crossing, Ada BandFlt v2) set at seven times standard deviation of the internal noise level (rms) on each electrode. The spike count files (Alpha Map files) were loaded into NeuroExplorer for further analysis of the percentage of active wells (defined as ≥1 active electrode), the percentage of active electrodes (defined as ≥0.1 spikes/s) per well, and the average mean spike rate (MSR; spikes/s) per active electrode. Effects of test compounds were calculated as follows: MSRs were averaged per well (20-31 wells (n) from at least 3-4 independent isolations) and effects of test compounds were calculated as percentage change compared to baseline. Next, the effects test compounds were expressed (mean ± SEM from n wells) normalized to time- and dilution-matched medium control wells.
Wells that showed effects two times standard deviation (SD) above or below average are considered outliers (~5%) and were excluded from further analysis. Effects of test compounds on spontaneous neuronal activity were tested for significance using unpaired two-sample t-tests. Effects were considered statistically significant if p-values <0.05.

6.2.7 Results

Medium derived from lung epithelial cells exposed via the air-liquid interface (ALI) model was used to expose the in vitro brain model. Test conditions included medium containing oil A_n-derived pyrolysis products (4, 10 or 30x diluted), oil B_n-derived pyrolysis products (4, 10 or 30x diluted), medium derived from lung epithelial cells exposed via ALI to clean air (4, 10 or 30x diluted), or medium derived directly from the ALI system following exposure to pyrolysis product but in the absence of lung epithelial cells ('no insert'; 4 or 10x diluted). Effects of these media on neuronal activity were compared to time- and dilution-matched medium controls and activity was assessed during the initial phase of the exposure (acute 30 min exposure) and following 24h exposure (subchronic 24h exposure).

Acute (30 min) exposure.
Neuronal activity in cortical cultures acutely exposed to oil A_n-derived pyrolysis products showed a modest and concentration-dependent increase. However, even at the 4x dilution the increase in neuronal activity compared to medium controls did not reach statistical significance (Figure 6.10, left). Similarly, acute exposure to oil B_n-derived pyrolysis products induced a modest and concentration-dependent increase in neuronal activity that did not reach statistical significance (Figure 6.10, right).

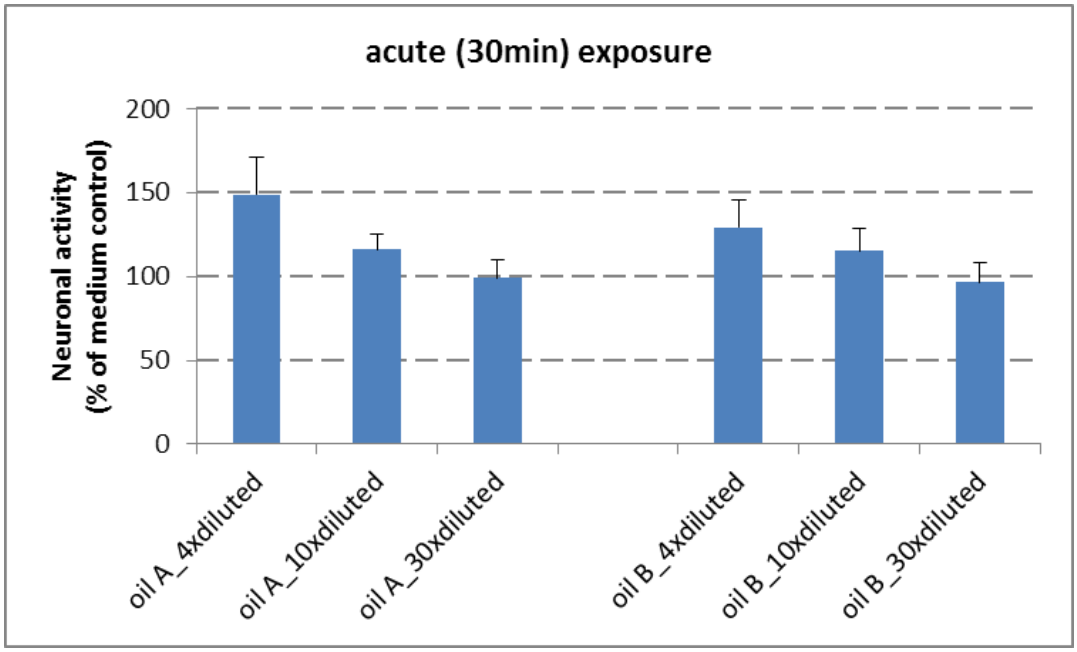

Figure 6.10. Changes in neuronal activity (expressed as percentage of medium controls (n=24-31) induced by acute (30 min) exposure to different dilutions of oil-derived pyrolysis products following transfer to the air-liquid interface. None of the effects reached statistical significance. Data are presented as mean ± SEM from 20-26 wells.

Subchronic (24 h) exposure.

Neuronal activity in cortical cultures exposed to oil A_n-derived pyrolysis products for 24h did not reveal any changes compared to medium controls (Figure 6.11, left). Similarly, exposure to oil B_n-derived pyrolysis products did not induce any changes in neuronal activity compared to medium controls (Figure 6.11, right).

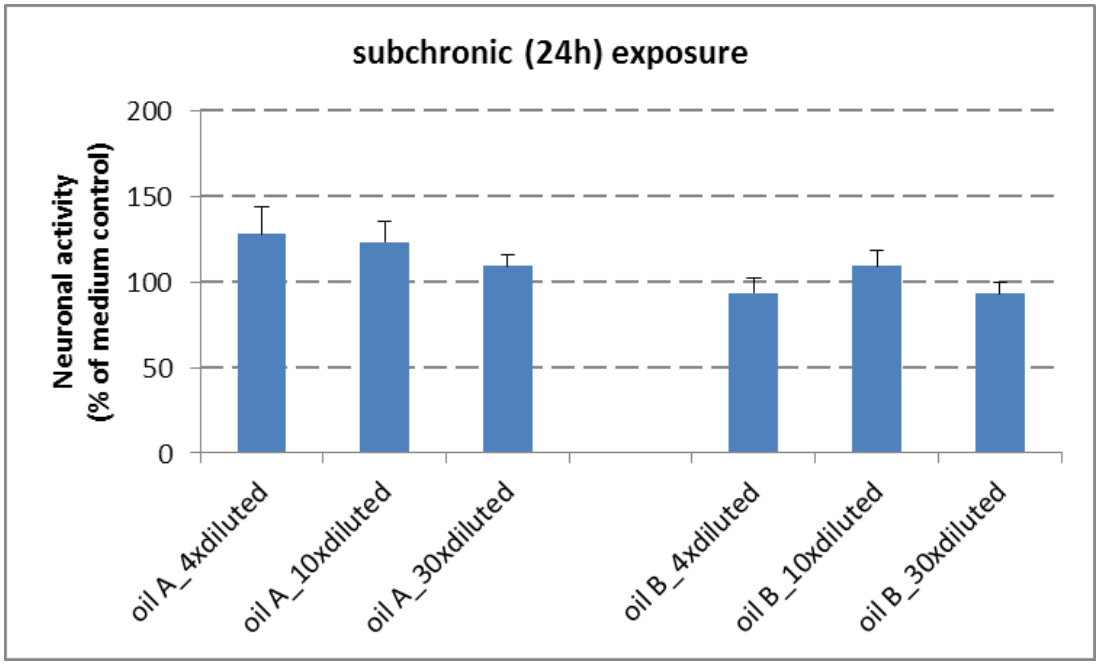

Figure 6.11. Changes in neuronal activity (expressed as percentage of medium controls (n=24-31) induced by subchronic (24 h) exposure to different dilutions of oil-derived pyrolysis products following transfer to the air-liquid interface. None of the effects reached statistical significance. Data are presented as mean ± SEM from 23-29 wells.

Direct exposure to pyrolysis products.
Notably, cortical cultures acutely exposed to medium containing pyrolysis products of oil A_n derived directly from the ALI system in the absence of long epithelial cells ('no insert') showed a significant increase in neuronal activity compared to medium control (194 ± 23, n=24, p<0.05), but only at the highest concentration (4x diluted; Figure 6.12, left). Acute exposure to medium containing pyrolysis products of oil B_n derived directly from the ALI system in the absence of long epithelial cells ('no insert'), did also induce a significant increase in neuronal activity compared to medium control (163 ± 15, n=23, p<0.05), but only at 10x dilution (Figure 6.12, right). The increase in neuronal activity in cortical cultures exposed to oil-derived pyrolysis products was no longer visible following subchronic (24h) exposure (not shown).

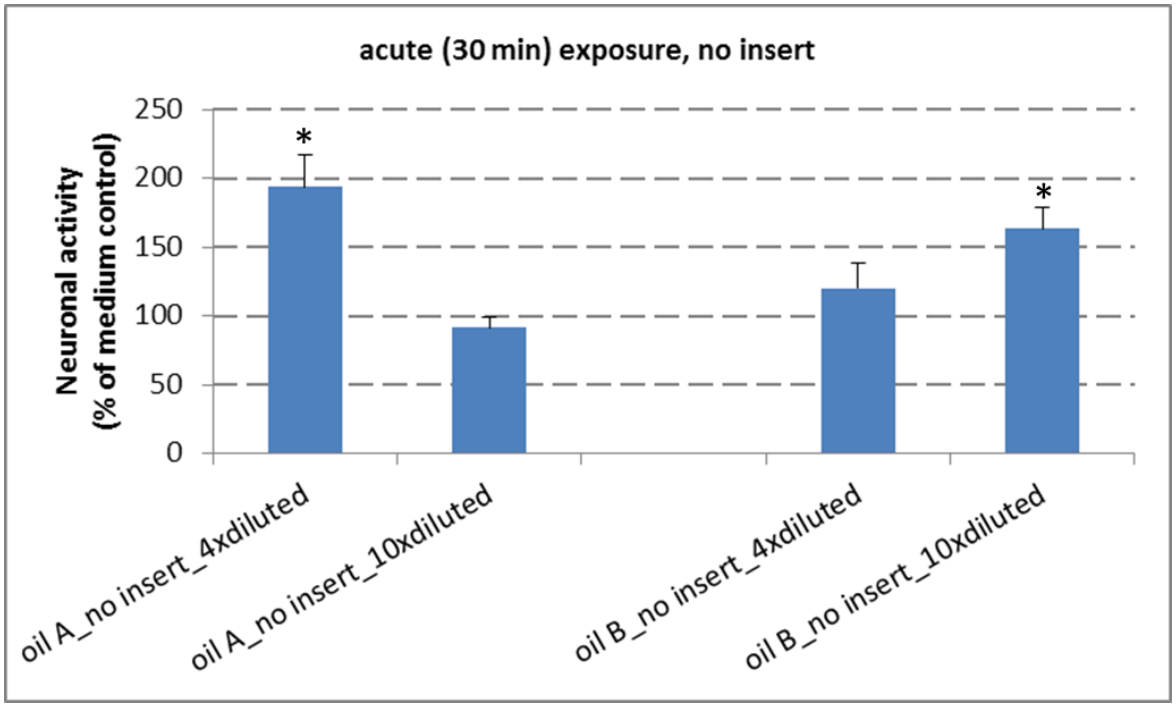

*Figure 6.12. Changes in neuronal activity (expressed as percentage of medium controls (n=24-31) induced by acute (30 min) exposure to different dilutions of oil-derived pyrolysis products following transfer to the cell-free (no insert) air-liquid interface. Data are presented as mean ± SEM from 23-29 wells; *, p <0.05.*

6.2.8 Conclusions and discussion

The current data indicate that acute exposure of primary rat cortical cultures to medium containing pyrolysis products derived from oil A_n or oil B_n following transfer to an air-liquid interface equipped with lung epithelial cells does not induce significant changes in neuronal activity (Figures 6.10 and 6.11). The lack of effect at neuronal activity is also a clear indication for the absence of cell death/cytotoxicity of neuronal cells. Nevertheless, the highest concentrations tested (4x diluted) do show a trend towards an increase in neuronal activity and it cannot currently be ruled out that higher concentrations may affect neuronal activity. However, it is practically not possible to increase the concentration of pyrolysis products in the medium (minimal dilution is 4x, and further increasing the concentration in the air-liquid interface will induce acute cytotoxicity of the lung epithelial cells).
The observation that neuronal activity is increased when cortical cultures are exposed to medium containing pyrolysis products following transfer to a cell-free air-liquid interface equipped (no insert, i.e., no lung epithelial cells present; Figure 6.12) is however suggestive for the presence of neuroactive compounds in the medium. Apparently, the lung model properly acts as a barrier and prevents that pyrolysis products permeate to a degree that is sufficient to cause effects.

Due to a lack of insufficient in-flight monitoring data for pyrolysis products it is not easy to compare the in vitro exposure levels applied in the AVOIL study to real-life exposure as may be expected for cabin air environment under normal flight conditions.

Nevertheless, based on measurements performed by TNO (2013b), it is possible to make an estimation as to how the levels in vitro relate with respect to real-life exposure levels. TNO investigated the concentrations of TCPs in the oil and in the cockpit air measured under normal flight conditions. The TCP contents and isomeric composition of the TCPs in the oils used in the aircraft investigated was comparable to the oils tested in the AVOIL experiments. Therefore, the sum of TCPs (∑TCP) can be used as tracer compound to compare the results from the in vitro measurements with levels found during the in-flight measurements.
Concentrations reported from the TNO investigation indicate average ∑TCP levels of up to 155 ng/m^3 in-flight. Chemical analysis of the vapour generated for the in vitro exposures displayed ∑TCP levels of 17 (± 3) and 33 (± 7) µg/m^3 for oil A_n and for oil B_n respectively (Appendix 7) indicating a factor ~100 higher exposure in the in vitro lung model compared to normal in-flight levels. As an extra dilution factor of 4 is applied in the neurotoxicity experiments also the exposure level should be divided by 4 when considering the neurotoxicity data. This reduces the difference between the in vitro exposure levels and everyday exposure levels to a factor ~30-50. Nevertheless, everyday exposure levels reported in literature range from 0.3 ng/m^3 to 50 µg/m^3 (Crump et al., 2011, Denola et al., 2011, Rosenberger et al., 2013) depending on the aircraft investigated, indicating that great care should be taken when interpreting these data.

The current data indicate that neuroactive pyrolysis products are present, but that their concentration in the presence of an intact lung barrier is too low to be of major concern for neuronal function. Moreover, the non-significant effects appeared to be transient as neuronal activity following 24h exposure is very comparable to medium controls. This is in line with recent MEA data showing that neuronal function of primary cultured rat cortical cultures following acute (30 min) exposure to different TCP isomers or commercial mixtures of TCPs is not or only limitedly affected (Duarte et al., 2016). Similarly, the effects of TCPs appeared to be transient and virtually absent following 24h exposure. However, when exposure continued up to 48h neuronal activity was markedly decreased by the majority of TCP isomers and mixtures. It is therefore possible that effects of pyrolysis products develop only following more prolonged (i.e. 48h and longer). In line with this notion, the effects of TCPs on (glutamatergic) calcium signalling and neurite microstructure (Hausherr et al., 2014, Hausherr et al., 2016) were also observed following more prolonged exposure (1-6 days).

So, while the present data indicates limited concern following exposure up to 24h, prolonged exposure to pyrolysis products may aggravate their potential neurotoxicity. Given the general human (occupational) exposure scenario, additional research may thus need to focus on prolonged and/or repeated exposure to pyrolysis products. Notably, such studies may need to focus also on the use of human induced pluripotent stem cell-derived neurons, which are gradually becoming more accessible for neurotoxicological research (Tukker et al., 2016).

7 Task 4: Analysis of the human sensitivity variability factor

7.1 Introduction

There are concerns among international governments, pilots, cabin crew and passenger (and other stakeholders of commercial jet aircraft) about health effects related to the presence of fumes in the air supplied to aircraft cabins. Pilots, cabin crew as well as passenger have reported a whole range of symptoms (see Appendix 2 and Task 1). Cabin air quality under routine conditions has only been partially characterized or documented within a limited number of flights hours, types of aircraft and number of chemical substances (see Appendix 3 and Task 1). There are, furthermore, no published studies, which describe and quantify air quality in aircraft under abnormal operating conditions; such as fume events.
The available information about the potential exposure to hazardous substances in the cabin suggests that environmental factors, including air contaminants, can be responsible for some of the many physical symptoms in cabin crew and passengers (such as sleeping problems, dizziness, concentration problems, headaches, respiratory complaints). However, the reported complaints tend to include a much broader range of (un)specific types of symptoms and within an equally exposed group of individuals, only some people have been found to develop symptoms, which makes it difficult to cluster the symptoms in a specific disease or syndrome.
It is therefore extremely difficult, if not impossible, to establish a causal relationship between cabin air quality and the self-reported symptoms which come forward from the different reviews. Extensive reviews (Nagda and Koontz, 2003, Griffiths and Powell, 2012) were unable to attribute any clinical outcomes to specific exposures. Moreover, in an aircraft there is potential exposure to a large number of substances. Inadequate hazard profiles, mixture toxicology and the lack of exposure levels allows only for a partial risk assessment. Key issues related to the difficulty to link the cabin air quality with reported symptoms are the broad variety in reported symptoms, the lack of systematic routine collected data about health complaints and the lack of clustered symptoms in crew as well as passengers. For the pilots and other crew members a healthy worker effect might also play a role, and on the other hand it is also possible that symptoms are underreported in the crew due to the fear of the consequence of a medical examination, potentially resulting in a “not fit to fly” status.
The variety in somatic complaints and their unspecific nature indicate, at least partly, that we might be dealing with somatically unexplained physical symptoms; a term which refers to diffuse symptoms after exposure to low doses of everyday environmental factors.

The main aim of this section is to explore the factors that can contribute to the development of health complaints caused by exposure to potentially contaminated cabin air and provide potential explanations why a broad range of (un)specific types of symptoms are reported in only a limited number of individuals exposed. Hereto the available knowledge on the following topics is summarized:

- Genetic differences in metabolism and detoxification (7.2)
- The influence of stress/coping strategies on underlying biological pathways leading to health complaints (7.3)

7.2 Genetic differences in metabolism and detoxification

It is widely accepted that differences exist between humans with respect to chemical sensitivity. Part of these differences can be explained by ‘normal’ biological variation. Several obvious factors can increase the degree interindividual differences, including sex, age, general health status and life style factors such as diet, smoking and drug or alcohol consumption. In addition, specific genetic differences (polymorphisms) can contribute significantly to the chemical sensitivity of an individual. Such genetic differences can act at different levels to alter an individual’s chemical sensitivity, for example by altering the bioavailability and/or potency of the chemical of interest.
The intensity of a toxic effect relates to the concentration and persistence of the ultimate toxicant at its target site. The concentration of the ultimate toxicant at its target site depends on the relative effectiveness of multiple processes that affect its delivery (Figure 7.1). In toxicology this is generally referred to as delivery or ADME: absorption, distribution, metabolism and excretion.

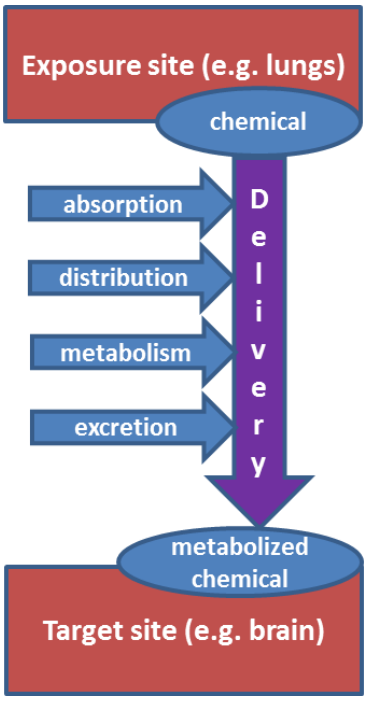

Figure 7.1. Schematic presentation of the processes that affect the delivery of a toxicant (Klaassen et al., 2013).

ADME largely determines if a particular chemical enters the body, how long it stays in the body and if it can reach its molecular target. Absorption following oral exposure for example is usually higher in children than in adults. However, for inhalation exposure which is the most obvious exposure route for CAC, differences in absorption seem less obvious. With respect to distribution in the body; lipophilic compounds tend to accumulate in fatty tissues, whereas hydrophilic compounds are usually easily excreted. A person's fat percentage can thus to some extent affect the distribution/excretion of a chemical, and consequently alter in theory a person's chemical sensitivity. However, the most notable contribution to differences in sensitivity is to be expected from differences in metabolism.

Metabolism, also known as biotransformation, usually involves transformation to a compound with increased polarity and potentially altered potency by cytochrome P450 enzymes in phase 1 and subsequent conjugation of the phase 1 product in phase 2, for example by glucuronidation or sulfation. Interindividual differences in the activity of phase 1 and/or phase 2 enzymes can thus affect which metabolites are formed and to which extent. Current knowledge about the enzyme variability with these metabolic pathways in the human population is largely based on well-studied pharmaceuticals, such as acetaminophen (paracetamol). For example, interindividual variability in glucuronidation (phase 2 metabolisms) has been indicated to contribute to differences in susceptibility to acetaminophen intoxication and associated liver injury in humans. Subsequent research demonstrated a substantial degree of interindividual variability (more than 15-fold) for the responsible phase 2 enzyme UDP-glucuronosyltransferase (UGT)(Court et al., 2001).

Current knowledge about the enzyme variability with regard to CACs is mainly restricted to ToCP. For ToCP it is known that cytochrome P450 enzymes are responsible for the formation of a toxic metabolite (bioactivation; also see (Reinen et al., 2015)), whereas paraoxonase 1 (PON1) is likely responsible for its detoxification. Individuals with an enzyme profile that favours bioactivation (cytochrome P450 enzymes) and/or hampers detoxification (PON1) of ToCP are thus likely to be more sensitive to its toxic effect. Earlier human studies with different cytochrome P450 enzymes, including 2C19, 3A4, 2D6 and 1A2, indicated a difference in individual constitutive hepatic activity of approximately 50-100-fold (Rendic and Di Carlo, 1997, Tamminga et al., 1999, Hagg et al., 2001). An additional 40-fold difference in the constitutive activity of the detoxification enzyme PON1 has previously been found in humans (Costa et al., 2005). As a result of these interindividual differences in P450 and PON1 enzyme activities, a 4000-fold difference can be expected between individuals expressing a very low and very high sensitivity (de Ree et al., 2014). Notably, this interindividual difference may be exaggerated due to the experimental

design, which relies on high substrate concentrations to determine enzyme activity, as a recent study indicated that the difference in PON1 activity is well within a factor 10 for more realistic substrate concentrations (Coombes et al., 2014). Yet, this may render a specific subpopulation that could be approximately 1000-fold more sensitive to ToCP. However, the complete metabolic pathway and the contribution of interindividual variability in the metabolic enzymes is still largely unknown for the majority of industrial chemicals, including CACs. Nevertheless, similar differences in sensitivity can be expected for other compounds that rely on cytochrome P450 enzymes for their metabolism.

In addition to (genetic) metabolic differences, other factors may be involved in the occurrence of interindividual differences in sensitivity, such as interaction of the chemical of interest with other organ systems and/or targets. While differences in immune-sensitivity have been suggested, for example in relation to 'sick-building syndrome', there is currently no evidence for interindividual differences in immune-sensitization for ToCP or the different chemical compounds in CACs. Similarly, genetic differences can alter an individual's chemical sensitivity directly at the target such as a specific receptor , enzyme or ion channel. If for example a particular isoform of a receptor has an altered affinity for the chemical of interest, this may result in altered sensitivity of the individual. Yet, there is currently no evidence for such interindividual differences directly at the target(s) for ToCP or the different chemical compounds in CACs.

While the involvement of genetic differences may (partly) explain why some individuals are more sensitive than others, the question remains whether current exposure levels are sufficiently high to evoke such complaints even in sensitive individuals.
It should be noted that the range of reported health complaints is very broad and therefore difficult to pinpoint to a particular CAC. Common acute symptoms include sensory (irritative) effects in eyes and airways as well as neurological symptoms such as headache. However, although ToCP at sufficiently high concentrations can cause (acute) respiratory failure by inhibition of acetylcholinesterase, TCPs are generally not considered airway irritants and it appears that sensory symptoms can be exacerbated by or even due to environmental and occupational conditions present in the aircraft, such as low relative humidity and low cabin pressure (reviewed in (Wolkoff et al., 2016)). Moreover, typical symptoms associated with acute inhibition of acetylcholinesterase (SLUDGE: salivation, lacrimation, urination, diaphoresis, gastrointestinal upset, emesis) are hardly reported.
Next to acute symptoms, there is a wide range of reported chronic health complaints, including fatigue, dizziness, muscle weakness, nerve pain, tremors and cognitive impairment and even neurodegeneration (reviewed in (Wolkoff et al., 2016)). While chronic exposure to ToCP can cause organophosphate-induced delayed neuropathy mediated by inhibition of neuropathy target esterase, these symptoms at best partially match with those reported following cabin air exposure. It can therefore be questioned if the reported health complaints are related to ToCP exposure.
Flying is associated with unique exposure conditions including exposure other CACs, radiation, hypoxia and changes in temperature, gravitational forces and pressure. Whether or not these occupational conditions are responsible for the reported complaints remains unknown until the complete set of potential chemical exposures is known, including their exposure levels, resulting internal dose levels, full spectrum of molecular targets (i.e., all different modes of action) and the related no-effect concentrations.

7.3 The influence of stress/coping strategies on underlying biological pathways leading to health complaints

A strong body of experimental studies has demonstrated that somatically unexplained physical symptoms occur when people perceive they are being exposed to environmental stressors irrespective of actual exposure. Perceived exposure can thus trigger or contribute to the occurrence of symptoms. More specific triggers such as smell, temperature, stuffy air are hereby relevant and well documented (Bailer et al., 2005, Das-Munshi et al., 2006). A whole range of explanatory models has been described in the literature for the development and maintenance of somatically unexplained physical symptoms. Currently a cognitive psychological approach is the most common (see e.g. Brown (2004)). In this approach symptom-focused attention is considered as central in both symptoms development and maintenance. Misinterpretation of symptoms, illness beliefs, worry and rumination, negative affect and personality features are assumed to play an important role in this process.

One characteristic element often associated with the occurrence of somatically unexplained physical symptoms is the lack of personal control or learned helplessness, well-known factors to enhance stress reactions, which in their own right can lead to acute health complaints and long-term health effects (Seligman, 1975, Lazarus and Folkman, 1984). Also in the case of symptoms attributed to environmental factors the lack of control over the exposure is characteristic (Campbell, 1983). There is a shared notion within the field of environmental stressor that we are dealing with a generic mechanism. A large body of stress research in the past 40 years shows that environmental factors are assessed by the individual in terms of threat and control referred to in the stress approach by Lazarus and Folkman as primary and secondary appraisal. The actual health effects in this process are highly dependent on how people cope and whether coping options are available. These coping strategies can roughly be subdivided in active coping and avoidance (Lazarus, 1966, Folkman et al., 1986, Roth and Cohen, 1986, Baliatsas, 2015).

Characteristic for the context of an enclosed environment, such as in an airplane, is that the range of coping options is very limited and that the work situation is characterized by a range of stressors (McNeely et al., 2014). Also it is important to emphasize that this mechanism applies to situations where there is actual exposure as well as perceived exposure. An immediate pathway between exposure and health can co-exists with an indirect pathway (via beliefs/expectations, personality traits, context and for example media coverage and early experiences). For several environmental exposures these pathways have been studied in detail such as environmental noise (van Kamp, 1990), electromagnetic fields (Baliatsas, 2015) and odour (Cavalini et al., 1991, Winneke et al., 1996).
Two basic approaches can roughly be discerned in the field of stress research: 1) a psychological approach with emphasis on the intervening mechanisms between a stressor and its long-term effect and 2) a biological approach with emphasis on the immediate physiological reaction between stressor and effect. These approaches are complementary and several models integrated the two approaches.
Representative for these approaches are the models are the Lazarus cognitive approach to stress and Ursins psychobiological approach to stress.
The cognitive stress model of Lazarus distinguished several phases in the stress process: via primary and secondary appraisal people make a judgement whether there is a threat and whether they can control it. Based on this appraisal process they choose a suitable coping strategy. These strategies can roughly be subdivided into active and passive coping. The outcome of this is dependent on the type of stressor, but in general it shows that avoidance enhances symptoms while problem oriented coping in many cases results in a perceived reduction of stress and symptoms. The model is transactional in the sense that the outcome leads to reappraisal of the exposure.

In the diagram below (Figure 7.2) this process in schematized, taking environmental noise as example.

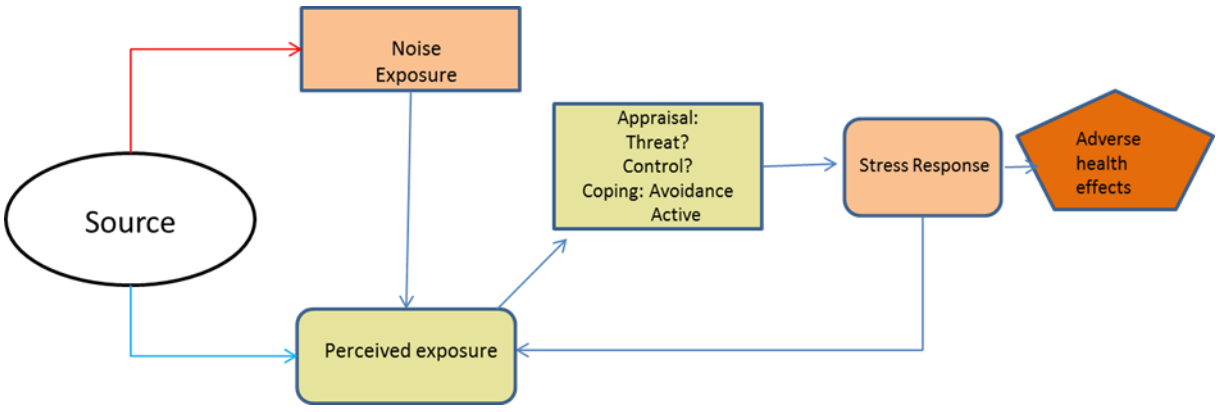

Figure 7.2. Example of cognitive stress model of Lazarus.
In the model developed by Ursin and his team, stimulus and response expectancy play a key role. Ursin based this model on earlier work among pilots and parachute jumpers. In this model the person environment interaction and the concept of homeostasis are central. Stimulus and response expectancy (comparable with primary and secondary appraisal in the Lazarus and Folkman approach) predict the level of arousal (autonomic and endocrine) and either lead to restoration of homeostasis or to sustained activation. Sustained activation is associated with subjective health co plaints and unexplained symptoms.

7.3.1 Stress as healthy alarm.

Originally the Ursin model was referred to as psychobiological but in more recent work the model is described as the Cognitive Activation Theory of Stress (CATS) (Ursin and Eriksen, 2004, Ursin and Eriksen, 2010). CATS (see Figure 7.3) has been developed and tested with regard to stressful work environments (Eriksen HR, 2002, Kristenson et al., 2004, Ree et al., 2014), but has been also applied to specific research field such as environmental noise (van Kamp, 1990, Klæboe, 2011) (using a predecessor of CATS combined with Lazarus' stress model). According to CATS, the stress response results from a perceived "discrepancy between what is expected to be 'normal' and what is happening in reality (actual value)" (Ursin et al., 2004). This perceived discrepancy (i.e. the stressor) is assumed to induce neurophysiological activation. Neurophysiological activation due to this discrepancy remains elevated until the individual has succeeded in reducing the disparity between expected (feared or desired) and actual values (of personal noise exposure, for example). If the difference between expected and actual values persists, arousal sustains and induces pathophysiological processes. In CATS, "the real concern is sustained arousal occurring when there is no solution" (Ursin et al., 2010), as the organism is vainly struggling for homeostasis by trying to exert control on expected and/or actual values. The higher the individual rates her or his chances to reduce or remove the difference between expected and actual values, the more likely it is for the organism to return to the arousal level observed prior to the activation. As mentioned before two concept are key: stimulus expectancy and outcome expectancy. The stimulus expectancy arises from the perceived probability of an event associated with a specific stimulus while outcome expectancy refers to the perceived relationship between actions (behaviours) and their results (outcome): positive effects, no control, and negative effects. A positive outcome expectancy is comparable with active coping while a negative outcome expectancy is related to avoidance or defence. According to the authors the model has explanatory power in epidemiology, prevention and treatment of "subjective health complaints". Since the model is primarily a model on work stress it is fit to be applied to the situation of flight attendants and pilots.

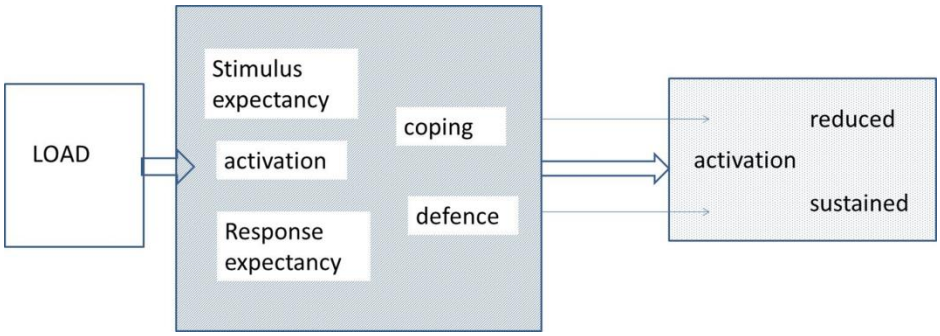

Figure 7.3. Schematic presentation of the CATS model (van Kamp, 1990)

7.3.2 Definition of somatically unexplained physical symptoms

The diagnosis of somatically (partly) unexplained physical symptoms is the most prevailing diagnosis in medical practice. We speak of somatically unexplained physical symptoms when the symptoms exist over several weeks and adequate tests have not resulted in a sufficient explanation for the symptoms. The diagnosis is however often made at a later stage or patients are often not properly informed about the diagnosis. Symptoms such as fatigue, headache, sleep difficulties, gastrointestinal disturbances and musculoskeletal pain are often referred to as non-specific because they are very common in the general population, occur in multiple organ systems and can be caused by a variety of factors, sometimes unknown (Yzermans et al., 2016). In general practice e.g. at least 50% of the presented complaints are not explained by organ pathology. A whole range of terms has been suggested to refer to these symptoms (for review see Creed et al. (2009). Symptoms attributed to environmental factors form a subcategory within the domain of somatically unexplained physical symptoms. Although there might be a causal foundation for the attributions, as in the case of contaminated cabin air in aircraft, it is often concluded that there is no scientific base for a causal mechanism, given the actual low exposure levels. Within the broader domain of symptoms attributed to environmental factors again several subgroups are discerned, related to different types or sources of exposures such as multi chemicals, indoor climate, (low frequency) noise, electromagnetic fields and food additives. In some cases not the symptoms obtain a key role, but the assumed sensitivity behind them such

as noise sensitivity, multi-chemical sensitivity, electro sensitivity and more broadly: environmental sensitivity. This genetic and/or acquired sensitivity might then explain why some people suffer from complaints after exposure to low doses of some toxic agent while others do not.
Recent research (Baliatsas, 2015) comparing symptom patterns between different (self-declared) groups of sensitives versus non sensitives showed considerable overlap between self-reported as well as GP registered symptoms, but also specific patterns related to organ systems. For example multi chemical sensitives scored higher on respiratory and gastrointestinal symptoms, while electro-hypersensitive people scored higher on cardiovascular symptoms and symptoms related to the psychological and neuro-vegetative function system.

7.3.3 Prevalence

Here we briefly discuss what is known about the prevalence of health problems and symptoms in cabin crew and in the general population and subgroups. In the specific literature on symptoms in cabin crew we make a distinction between relatively large and small epidemiological studies and case reports. This overview has by no means the pretention of completeness and rather serves as an indication for the type of health complaints reported by cabin crew while using prevalence figures in the general population (and its subgroups) as reference.

Derived from a relatively large epidemiological study
McNeely et al (2014) made a comparison between the self-reported health of US flight attendants (pilots were not included) and the general population. The association of symptoms with exposure to the broad aircraft environmental was also explored. Job tenure was used as proxy of exposure. Data from a survey among flight attendants of two large domestic US airlines gathered in 2007 were compared with data obtained from surveys in a cohort in the framework of the National Health and Nutrition study (NHANES) over the period between 2005 and 2008 adjusting for age, gender, education, smoking and body mass index (BMI). Moreover, only participants from the general population were selected who matched with the flight attendants in terms of income, level of education and current employment (note: as indicators of social economic status). The response rate was 48% bringing the number of participants to 2.613, completed by an additional 1.398 surveys in attendants of the same airlines. Health status was inventoried in a broad way. Our focus is on somatically unexplained physical symptoms only. Also at the exposure side a broad range of conditions is considered which are characteristic for current work circumstances of flight attendants. These include disrupted circadian rhythm, physical demands in restricted cabin quarters, cosmic radiation, air contaminants, noise, vibration, low pressure and humidity and gravitational forces. Longer tenure implies longer exposure to these potential hazards. Frequent acute symptoms in the flight attendants include symptoms related to different organ systems such as sinus congestion, bloating, anxiety, and musculoskeletal symptoms prevailing in 23 – 29% of flight attendants. From the longer term symptoms, the respiratory symptoms (sinusitis, allergies and reactive airways) score by far the highest (54%), followed by general fatigue (37%), joint aches and pains (33%) and severe headaches (23%).
The inventoried symptoms among flight attendants only partly overlap with those from the NHANES study. Prevalence was compared by means of the standardized prevalence rate (SPR), weighted by age and analyzed separate per gender. With the approach expected prevalences are derived from the data in the general population and compared with the observed prevalences in the flight attendant group. This comparison revealed an increase in chronic bronchitis, with prevalences of 3.6 versus 13.5% in males and 5.1 and 16.1% in females (SPR: 3.5 and 2.7 respectively). This is of value considering that the number of smokers is considerable lower in the flight attendants. Sleep disorders are quite prevalent in both males as females with prevalences of 32 NS 34% versus 8 and 6% in the general population (SPR's of 3.5 and 5.5). Fatigue was twice as high in male attendants than in the general population and symptoms of depression 5 times higher in males and 2 times higher in females (prevalence of <1 ad 1.5 in the general population and 3.7 in male and 3.8 in female flight attendants. Chronic bronchitis was shown to be associated with longer job tenure after adjusting for smoking, age, education, and overweight. This is according to the authors possibly linked to passive smoking(SHTS exposure), with 41% working longer than 20 years. Another explanation could be the cabin air quality, but as stated before, the broad range of symptoms which could theoretically be attributed to the cabin air quality have not been systematically studied in cabin crew. Other associations with job tenure found were skin cancer, hearing loss, depression and anxiety, while this was not the case for sleep disorders and migraines.

In conclusion: the McNeely study showed that a whole range of health conditions pertaining to different organ systems (respiratory, neurological, musculoskeletal, gastrointestinal) was higher in flight attendants as compared to the general population and some of these show an association with job tenure.

Derived from smaller epidemiological studies and case reports

Reviews of studies on flight attendants health commonly conclude that most studies are not based on random samples, outdated while the industry is changing rapidly, based solely on self- reported data and suffer from low response rates (Nagda et al., 2003, Griffiths et al., 2012, McNeely et al., 2014).

In the framework of this project again a systematic literature review was performed (see section 3) including a set of smaller epidemiological studies and case reports among pilots and flight attendants.

In appendix 1 and 2,the findings regarding symptom report among aircraft crew are summarized. In the following paragraph these findings are compared with what was reported by McNeely and symptom patterns in the general population.

The 19 included studies consisted of 3 case control studies,11 case studies and 5 small epidemiological studies. Although most studies are small, often not representative and primarily based on self-reported symptoms, they give good insight into the symptoms people attribute to cabin air quality and how these relate to those found a the larger epidemiological study discussed above and in the general population. The most frequently reported symptom is headache which shows up in 11 out of 19 studies with prevalence estimates ranging between 15-86%, followed by cognitive functioning in 8 studies with rates between 25-78% and dizziness and disorientation also in 8 studies (8-72%). Nausea, fatigue/exhaustion and irritated eye show up in 7 studies with again a broad range of estimated prevalences 29-58%, 48-78% and 14-76% respectively. Other symptoms including respiratory complaints were only mentioned in 2-4 studies, while depression only showed up in 1 study.

In most studies people attributed their symptoms to cabin air quality (fumes and smoke) and in two studies people 26-53% of the participants described themselves as sensitive to multiple-chemicals. As stated before, the quality of the studies was moderate and suffered from selection bias, low response rates, small sample sizes. The prevalences reported are highly dependent on the specific participants included and the way they were selected or self-selected. This makes it hard to allow for any comparison with other data, both from the same professional groups as in the study of McNeely et al. (2014) and the general population. Even though there is some parallel in the most prevalent symptoms the patterns is not consistent and the estimated prevalences are in most cases not comparable and in general higher than in the well-designed epidemiological study among flight attendants. This confirms again that we are dealing with selection bias. The next paragraph summarizes the main differences.

Background prevalence estimates from different studies

In the Netherlands, the percentage somatically unexplained physical symptoms in outpatient services of somatic specialists is estimated to be 41 to 66% (Tak and Bax-aan de Stegge, 2014) and 35% in a neurological clinic (Snijders et al., 2004). It is estimated that in 10-30% of these patients the symptoms become chronic. Haller et al. (2015) estimates the prevalence among outpatients to be 40-49%. In the general population headaches, belly aches and fatigue are the most common symptoms with prevalences between 30-35% (Kroenke et al., 1990, Kroenke, 2003) and 52% (Nimnuan et al., 2001). In an overview of Creed (2009) a summary is given of the prevalence of symptoms per type of clinic: 30% concerned neurological symptoms, 40% gastrointestinal and 53% general medicine, which is highly comparable to what Nimnuan found. In flight personnel general fatigue, headaches, and muscular skeletal complaints come forward as the most common symptoms in general and in smaller studies with specific groups of participants and case studies after an event, with prevalence estimates between 48-78%, 15-86%, 23-29%. While respiratory symptoms come forward as the most prevailing in cabin crew, these symptoms do not show up in the specific case studies. Nausea, and neuropsychological symptoms such as cognitive impairment, disorientation/dizziness are most often mentioned and attributed to the exposure to contaminated air in the cabin as well as irritated eyes.

Estimates on the prevalence of psychiatric comorbidity are inconclusive, but in general it is found that the number of symptoms is a good predictor of psychiatric problems (see also thesis Van Bellis van den Berg (2007)). It is well known that psychiatric problems are often missed in the group of people who tend to attribute their symptoms to environmental factors and moreover they are also taboo especially in this patient group. These associations have not been studied thus far in the specific occupational group of flight personnel.

7.4 Concluding observations

There is for quite some time concern about the association between the air quality in aircraft (and its broad variety of compounds) and health complaints typical among pilots and cabin crew concerning respiratory system, mood, concentration, dizziness etc. These complaints have not been systematically mapped; no causal relation has jet been established between these prevailing symptoms and exposure. A broad range of symptoms related with different organ systems has been found among flight personnel, but these have not been systematically studied.

However, overall statements about the prevalence of somatically unexplained physical symptoms are hard to make because symptoms overlap, estimates are dependent on the definitions used and the participants in the study. This is true for specific studies in flight attendants and pilots, but also for the international literature on the prevalence of somatically unexplained physical symptoms. When trying to compare the estimated prevalence of somatically unexplained physical symptoms, problems concerning comparability in terms of study population, composition of the sample and symptoms included are encountered.

The main aim of this section was to explore the factors that can contribute to the development of health complaints caused by exposure to potentially contaminated cabin air and provide potential explanations why a broad range of (un)specific types of symptoms are reported in only a limited number of individuals exposed.

The complete metabolic pathway and the contribution of interindividual variability in the metabolic enzymes is still largely unknown for the majority of industrial chemicals, including CACs. Nevertheless, differences in sensitivity can be expected for compounds that rely on cytochrome P450 enzymes for their metabolism. This may render a specific subpopulation that could be approximately 1000-fold more sensitive to specific chemicals. The broad range of compounds in the air in combination with other stressors, typical for working in an aircraft at irregular times has not been systematically mapped. In view of this great variety in symptoms and the lack of specificity, it cannot be ruled out that part of the symptoms cannot be explained by actual exposure levels. Whether or not occupational conditions are responsible for the reported complaints remains unknown until the complete set of potential chemical exposures is known, including their exposure levels, resulting internal dose levels, full spectrum of molecular targets (i.e., all different modes of action) and the related no-effect concentrations.

The literature shows that we are dealing with symptoms which are also quite common in the general population and in primary and secondary health care patients. It has been found that these symptoms do not always profit from treatment and often become chronic. Key feature is that the symptoms are often attributed to external causes, This does not necessarily imply a priori knowledge about plausibility of a relation between the symptoms and reported symptoms.

Preliminary points of attention in case of further epidemiologic research in cabin crew:

- Define relevant symptoms and clear case characterization, possibly distinguish hereby between specific and non-specific symptoms.
- Systematic study among pilots and flight attendants and control group of these groups of symptoms
- Include context, personality situational factors and pay attention to triggering and maintaining factors as well.
- Include aspects as perceived threat and control and coping strategies.
- Ideal design would be a combination of survey among specific groups and control group + diary and provocation study. However, this might be quite problematic because it cannot be done at random moments during the flight.

8 Conclusions

The objective of the AVOIL study was to characterize the toxic effects of chemical compounds that are released into the cabin or cockpits of transport aircraft. The characterisation was aimed at the toxic effects of aviation turbine engine oil as a mixture of compounds, including potential pyrolysis breakdown products.

From the experimental work on detecting chemicals it is concluded that the commercial oils included in this study do contain TCP, however no tri-ortho cresyl phosphate isomers could be detected. The overall emission of both oils differs at both simulations. Due to increase of temperature, more TCPs will emit, however, the mass fraction of the emitted TCP isomers was comparable to the TCP oil composition. PAHs such as naphthalene are formed probably due to partial oxidation. VOCs (e.g., 2-hexanone), aldehydes (e.g. formaldehyde and acetaldehyde) were clearly present at relative high concentrations. The CO and mineral oil concentration increased drastically at cruise simulation temperature, i.e., 375°C (± 25 °C).

Dedicated work on pyrolysis of the oils with comprehensive GC-MS (GCxGC-MS) conformed the above formulated conclusions. A list of 127 compounds was identified under both nitrogen and oxygen conditions in all oils and during different simulated flight stages. The basic oil patterns of the two tested oils demonstrated no significant differences. A set of TCP isomers, 4-octyl-N-(4-octylphenyl)-benzenamine and N-phenyl-1-naphthaleneamine could be identified in the basic oil patterns. TCP isomer peaks were found in all three oils. N-phenyl-1-naphthaleneamine was only found in oil B_n, although it did not visually appear in the TIC chromatogram, which indicates a low concentration. Heating under nitrogen led to an increase in the number of compounds found, and led to the identification of 24 compounds in the vapour, found in all applied oils. A number of compounds was identified unique for either oil A_n or oil B_n. In addition, used oil (A_u) appeared to contain newly identified compounds compared to unused oil (A_n), and a number of compounds originally present appeared to have disappeared during use in an engine jet. This indicates that during the lifetime of an oil, substantial changes in composition occur.

Based on the study on toxic effects of the oils after pyrolysis it was concluded that the current data indicate that neuroactive pyrolysis products are present, but that their concentration in the presence of an intact lung barrier is that low that it could not be appointed as a major concern for neuronal function. Moreover, the non-significant effects appeared to be transient as neuronal activity following 24h exposure is very comparable to medium controls. However, prolonged exposure to pyrolysis products may aggravate their potential neurotoxicity. Additional research may thus need to focus on prolonged and/or repeated exposure to pyrolysis products.

Analysis of the human sensitivity variability factor showed that the complete metabolic pathway and the contribution of inter individual variability in the metabolic enzymes is still largely unknown for the majority of industrial chemicals, including CACs. Differences in sensitivity between humans can be expected for compounds that rely on cytochrome P450 enzymes for their metabolism. This may explain the symptoms observed in a specific subpopulation of the people with health problems that may be related to cabin air. However, the broad range of compounds in the air in combination with other stressors, typical for working in an aircraft at irregular times has not been systematically mapped. Further, in view of the great variety in symptoms and the lack of specificity, it cannot be ruled out that part of the symptoms cannot be explained by actual exposure to chemicals. Whether or not occupational conditions are responsible for the reported complaints remains unknown until the complete set of potential chemical exposures is known, including their exposure levels, resulting internal dose levels, full spectrum of molecular targets (i.e., all different modes of action) and the related no-effect concentrations. A suggestion for follow-up research is to define the specific symptoms as reported by flight and cabin crew, in order to investigate if a syndrome can be defined.

For future work on risk assessment and maximum exposure levels, it needs to be taken into account that the conditions in cabin air may differ from the standard conditions on which exposure limits are normally based, for example the air pressure, humidity and longer working hours. These aspects need further consideration. In addition, also possible effects relating to mixture toxicology need further investigation.

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Appendix 1 Selection of additional literature

Reference	Included	Justification
https://www.faa.gov/data_research/research/med_humanfacs/oamtechreports/2010s/media/201520.pdf	Yes	Very recent and potentially relevant, include in analysis category 4
http://www.ohrca.org/wp-content/uploads/2014/05/Medicalprotocol031909.pdf	Yes	OHRCA full study report was already selected for analysis category 1 & 2 as it focusses on health effects and exposure. On second inspection it also contains some new engine oil measurements (page 39) and will therefore include as well in the analysis category 4
http://www.ohrca.org/wp-content/uploads/2014/05/quickreference.pdf		
http://www.ohrca.org/wp-content/uploads/2014/08/finalreport.pdf		
http://www.cdc.gov/niosh/docket/archive/pdfs/niosh-220/95-117.pdf	No	This report contains good practice guidelines for air sampling in general and is not within the scope of this literature review. Therefore it will be excluded from the analysis.
http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=52702&published=on&includesc=true	No	This report contains good practice guidelines for air sampling in general and is not within the scope of this literature review. Therefore it will be excluded from the analysis.
http://www.iom-world.org/pubs/IOM_TM1106.pdf	Yes	This report was already selected for analysis of category 2 as it focusses on exposure assessment.
http://ec.europa.eu/environment/air/quality/standards.htm	No	This report contains air quality limit values in general and is not within the scope of this literature review. Therefore it will be excluded from the analysis.
http://www.airspacemag.com/flight-today/clearing-the-cabin-air-1-19794696/	No	This is an online article about a research consortium yet to be started and does not contain any relevant data.
https://dspace.lib.cranfield.ac.uk/bitstream/1826/5305/1/AircraftCabinAirSamplingStudyPart1FinalReport%2020110420.pdf	Yes	The Cranfield study was already selected for analysis category 2 as it focusses on exposure assessment
http://asrs.arc.nasa.gov/docs/rpsts/cabin_fumes.pdf	No	Narrative description of fire, smoke, fumes or odor incidences. Not within the scope of the focused literature review.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4444275/	No	The literature review focusses on effects related to engine oil, fumes and pyrolysis products. Therefore, effects of ozone are not taken into account.
http://www.ncbi.nlm.nih.gov/gquery/?term=cabin+air+quality	No	See methodology for details on used search term
http://www.ncbi.nlm.nih.gov/books/NBK207470/	Yes	Potentially relevant for all four categories, include in analysis
http://www.ncbi.nlm.nih.gov/books/NBK207485/pdf/Bookshelf_NBK207485.pdf		
CAQ sensing technologies & studies – state of play	Yes	This note relates to the overall cabin air quality and exposure measurements. It gives a summary of some exposure

		measurement campaigns focused on VOCs. It can be included for category 2 as it focusses on exposure assessment; depending on the focus of the analysis of the exposure measurement data.
Source apportionment of volatile organic compounds (VOCs) in aircraft cabins	No	This article focusses on exposure to VOCs in the cabin and is already discussed in the summary note above.
In-Flight/Onboard Monitoring: ACER's Component for ASHRAE 1262, Part 2	No	This article focusses on exposure to VOCs in the cabin and is already discussed in the summary note above.
Net in-cabin emission rates of VOCs and contributions from outside and inside the aircraft cabin	No	This article focusses on exposure to VOCs in the cabin and is already discussed in the summary note above.
Measurements of volatile organic compounds in aircraft cabins. Part II: Target list, concentration levels and possible influencing factors	No	This article focusses on exposure to VOCs in the cabin and is already discussed in the summary note above.
Measurements of volatile organic compounds in aircraft cabins. Part I: Methodology and detected VOC species in 107 commercial flights	No	This article focusses on exposure to VOCs in the cabin and is already discussed in the summary note above.
Professor Michael Bagshaw. (2014). Health Effects of Contaminants in Aircraft Cabin Air. Summary Report v2.7	Yes	This report was already selected for analysis of category 1 as it focusses on health effects. On second inspection it also contains some theoretical maximum oil concentrations in the cabin and will therefore include as well in the analysis category 2.
Evaluation of shipboard formation of a neurotoxicant (trimethylolpropane phosphate) from thermal decomposition of synthetic aircraft engine lubricant	No	This article also retrieved with the literature search but was excluded because it focusses on shipboard fires
Sensors and Prognostics to Mitigate Bleed Air Contamination Events 2012 Progress Report	Yes	This article contains an overview of previous studies on thermal degradation of aviation engine oil and some new data. Will be included in analysis.

Appendix 2 Summarizing the health symptoms reported by cabin crew after exposure to contaminated bleed air.

Table A.1. Frequency of symptoms recorded for 36 aircrew exposed to contaminated cabin air (Somers 2005) .

Symptom	Total number with symptom	Case number 1 2 3 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 25 26 27 28 29 30 31 32 33 34 35 36 37 38
Nausea	32	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Lethargy	32	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Sore throat	30	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Cognitive dysfunction	29	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Sore eyes	28	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Headaches	27	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Improve away from fumes	23	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Breathing difficulties/ chest tightness	22	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Prolonged recovery	18	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Dizziness	17	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Chemical sensitivity	17	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Paresthesia/tingling	13	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Balance disturbance	12	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Anxiety	11	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Depression/stress	10	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Palpitations	9	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Altered smell/taste	8	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Abdominal discomfort/ diarrhoea	8	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Epistaxis	5	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Blurred vision	3	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Reflux	3	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Hair loss	2	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Rash	2	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Swollen glands	1	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x
Bladder dysfunction	1	x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x x

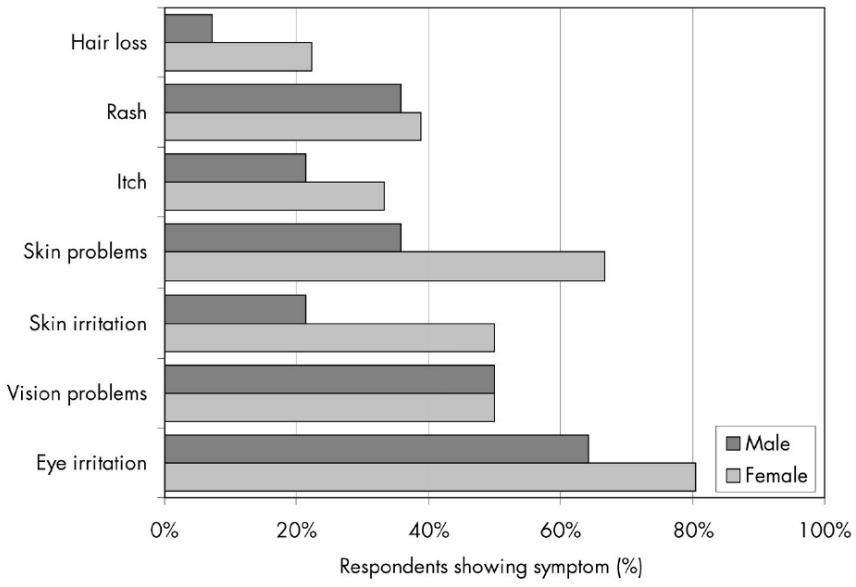


Figure A.1. Data on eye and skin irritation signs and symptoms from 50 exposed aircrew members (BAe 146 and A320) (Winder et al., 2002).

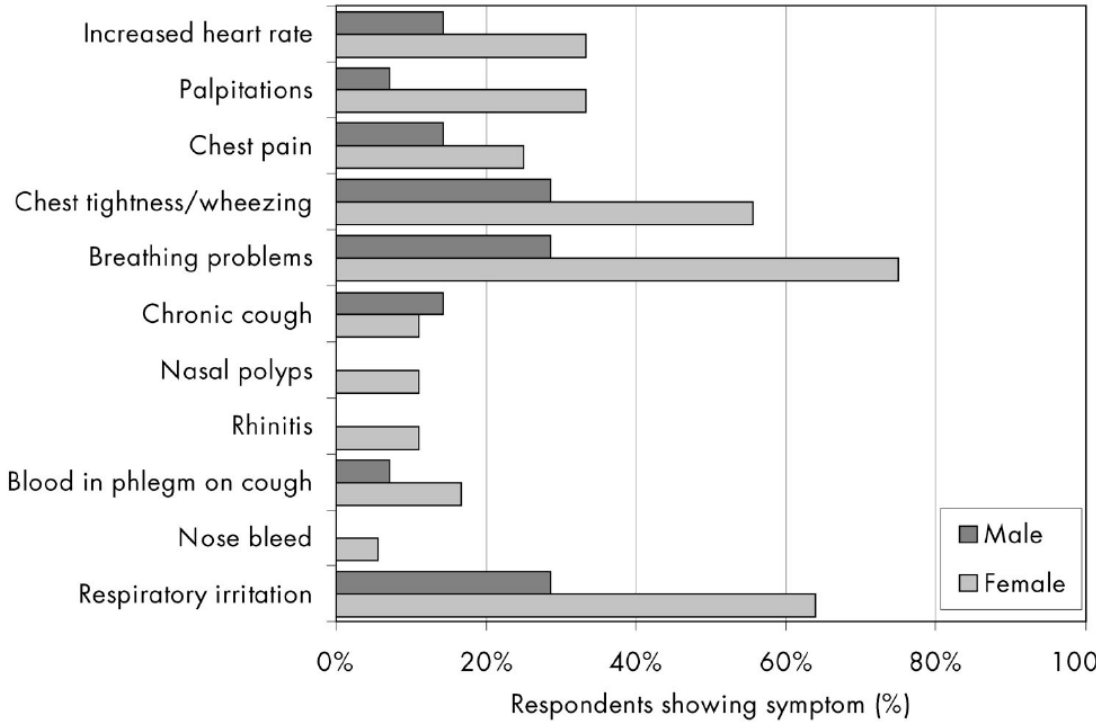


Figure A.2. Data on respiratory and cardiovascular symptoms from 50 exposed aircrew members (BAe 146 and A320) (Winder et al., 2002).

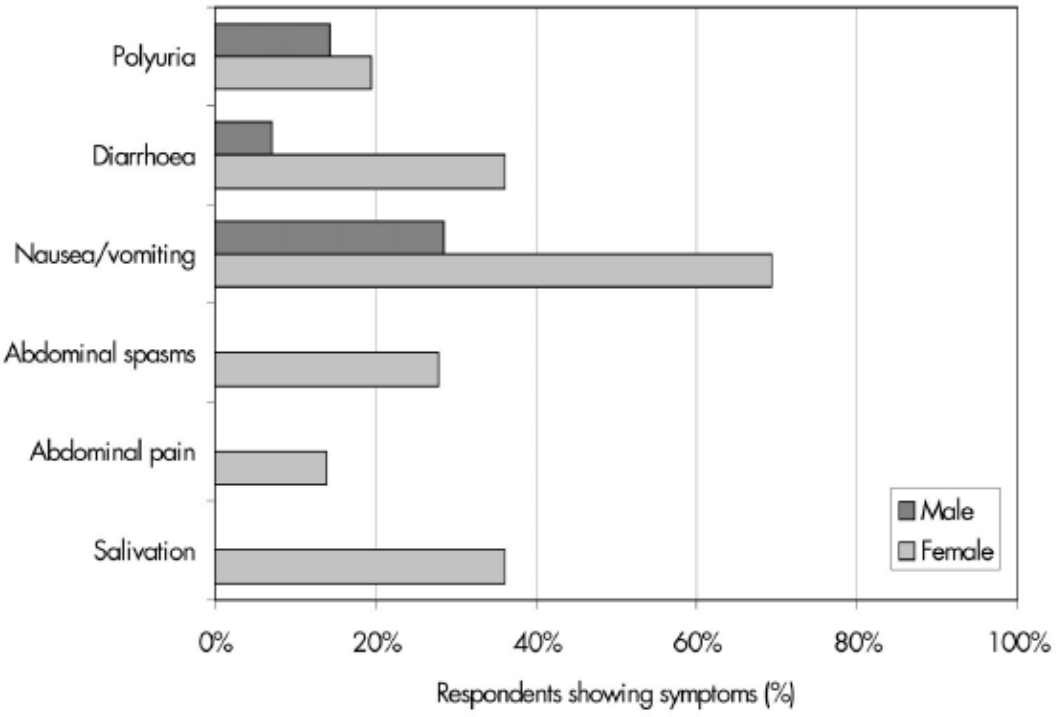


Figure A.3. Data on gastrointestinal/renal signs and symptoms from 50 exposed aircrew members (BAe 146 and A320) (Winder et al., 2002).

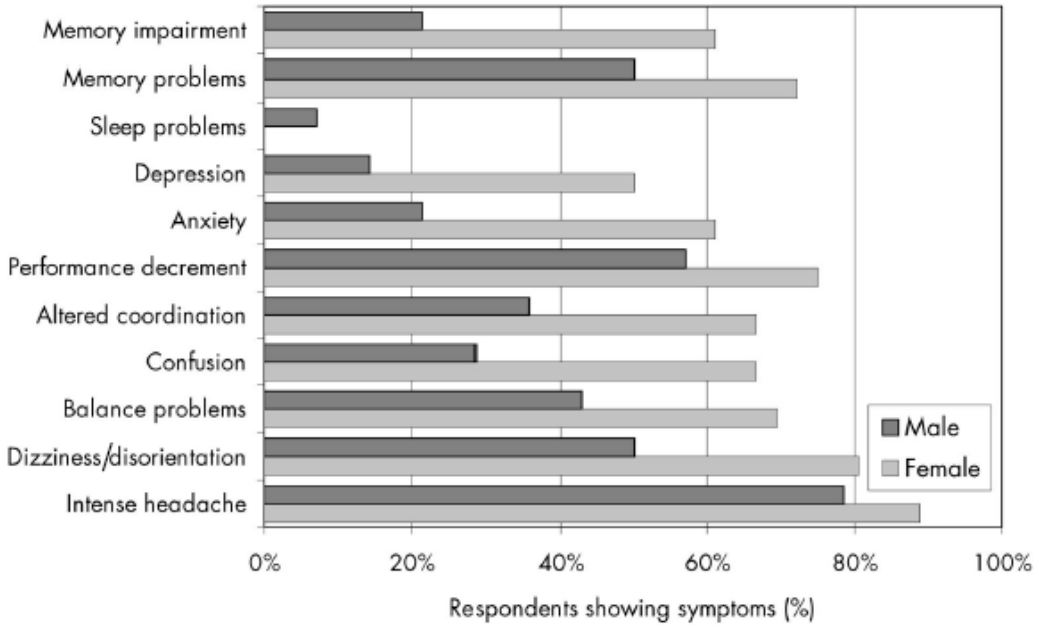


Figure A.4. Data on neuropsychological signs and symptoms from 50 exposed aircrew members (BAe 146 and A320) (Winder et al., 2002).

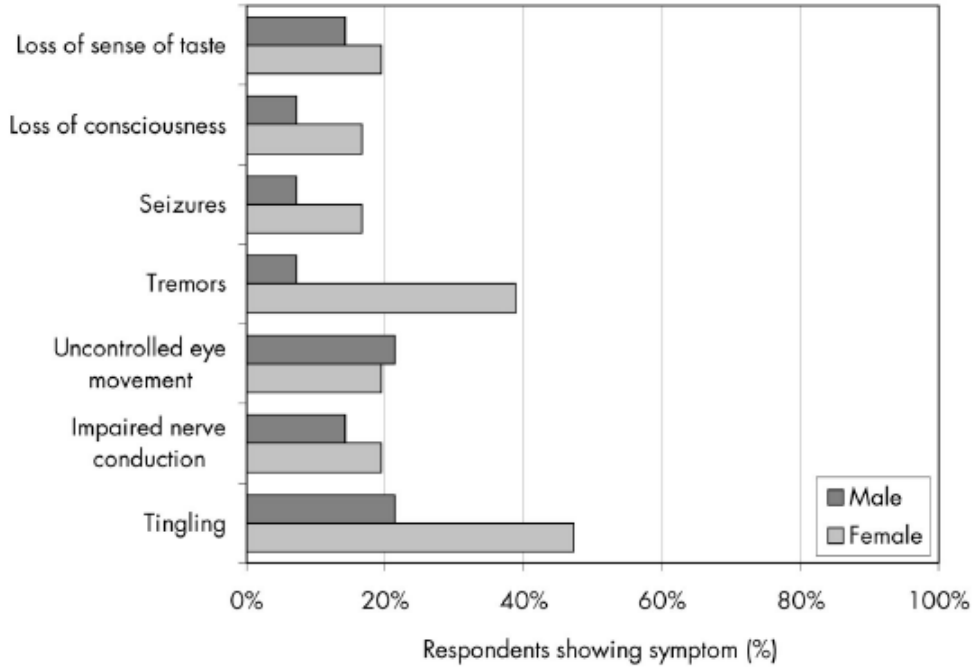


Figure A.5. Data on neurological signs and symptoms from 50 exposed aircrew members (BAe 146 and A320) (Winder et al., 2002).

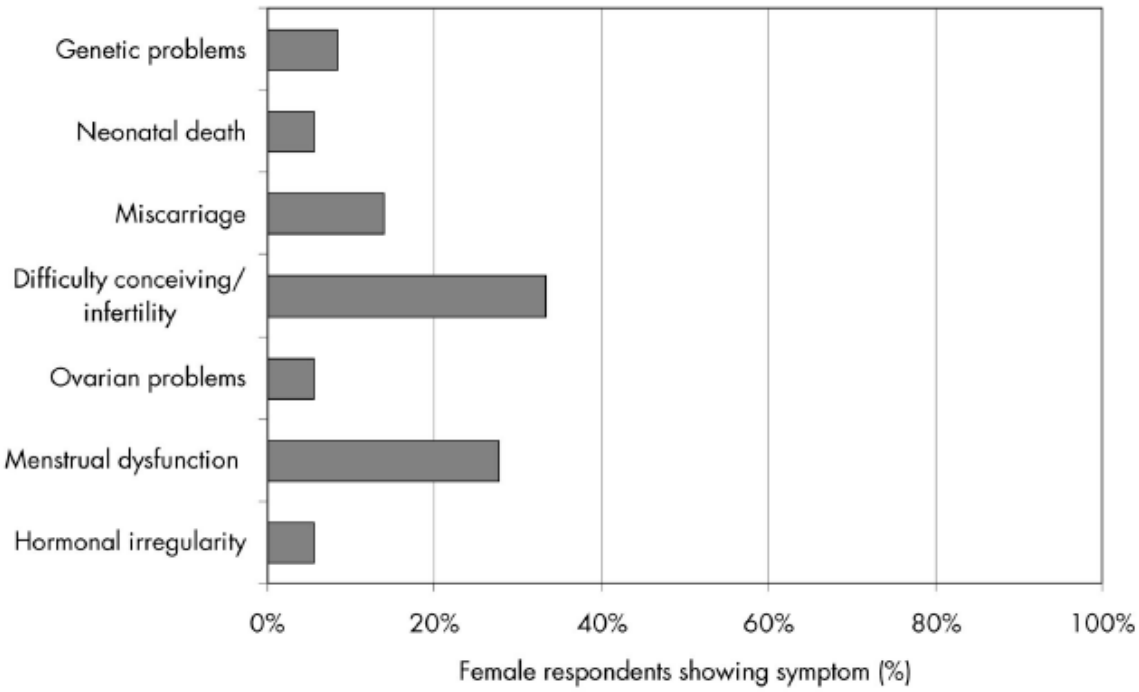


Figure A.6. Data on reproductive signs and symptoms from 50 exposed aircrew members (BAe 146 and A320) (Winder et al., 2002).

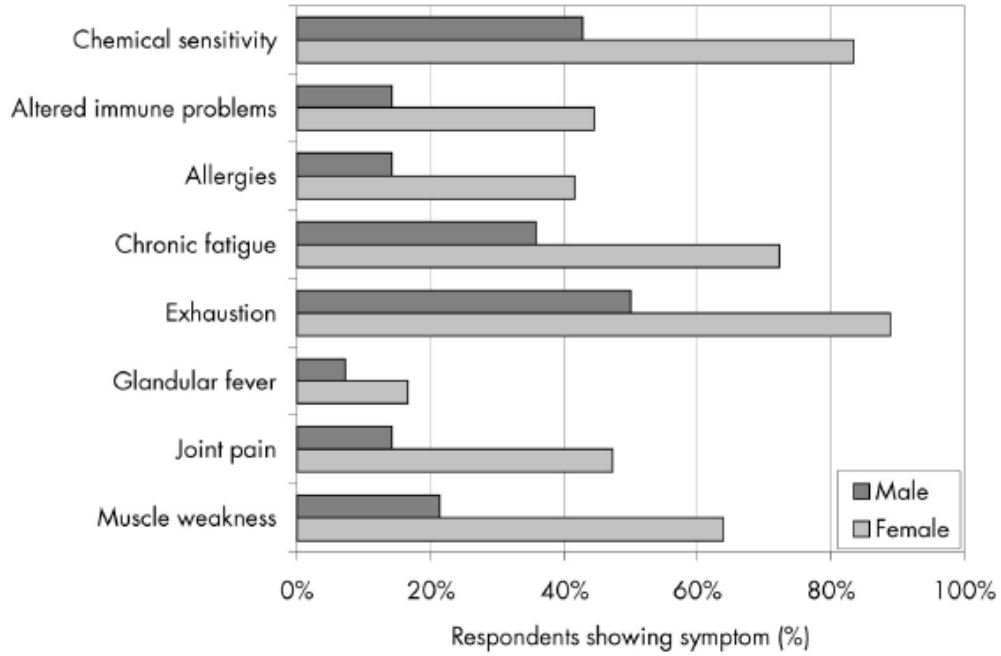


Figure A.7. Data on general signs and symptoms from 50 exposed aircrew members (BAe 146 and A320) (Winder et al., 2002).

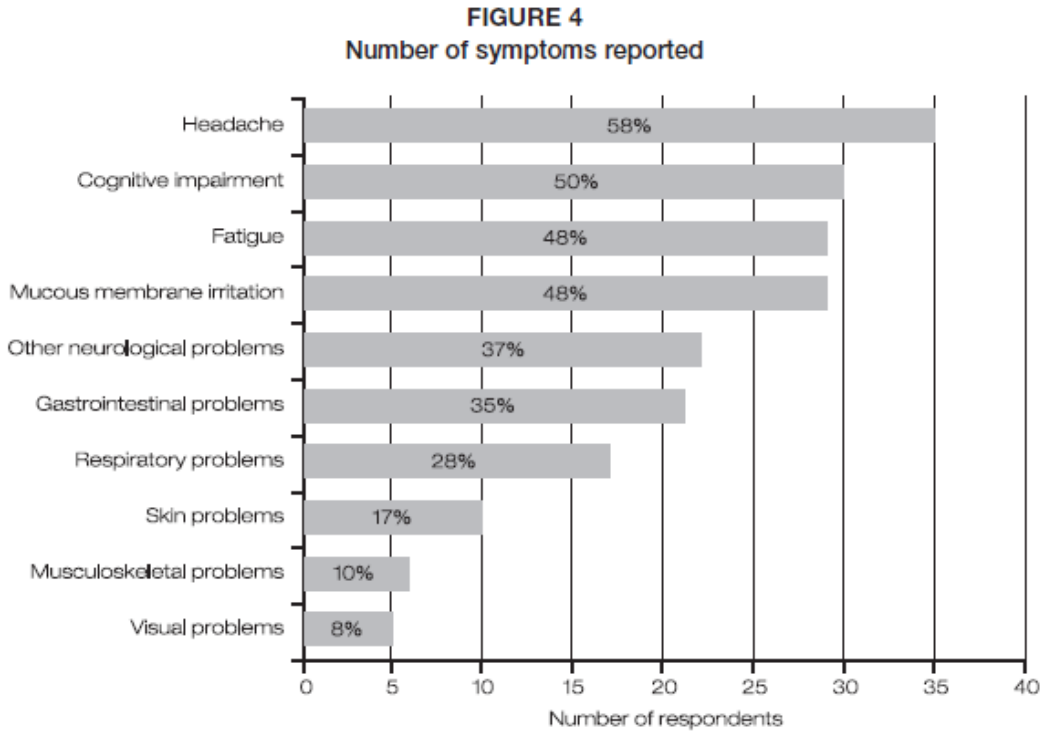


Figure A.8. Numbers of symptoms reported by 39 pilots exposed to contaminated aircraft cabin air (Harper, 2005).

Table A.2. Numbers of symptoms reported by 19 pilots and 2 flight attendants flying on a BAe 146 aircraft (Cox and Michealis. 2002).

Symptom severity	*No answer*	*Occasional*	*Sometimes*	*Often*	*Long term*
Headaches, light-headedness,dizziness	32%	21%	21%	10%	16%
Irritation of eyes, nose and throat	16%	16%	32%	16%	21%
Disorientation	74%	5%	5%	0%	16%
Memory impairment (short-term)	53%	10%	5%	5%	26%
Concentration difficulties, confusion	53%	16%	10%	0%	21%
Blurred vision, tunnel vision	90%	5%	0%	5%	0%
Nausea, vomiting, gastrointestinal problems	90%	0%	0%	5%	5%
Fatigue, weakness, decreased performance	32%	21%	26%	0%	21%
Respiratory distress/difficulties	63%	0%	26%	0%	10%
Numbness (head, limbs, lips, fingers)	74%	5%	0%	10%	10%
Balance/coordination difficulties	74%	0%	16%	0%	10%
Joint pain, muscle weakness	84%	5%	5%	0%	5%
Intolerance to chemicals/odours	53%	5%	16%	0%	26%
Intolerance to foods/alcohol	84%	0%	0%	5%	10%
Skin irritations	79%	5%	16%	0%	0%
Immune system disorders	79%	0%	0%	0%	21%
General increase in feeling unwell	53%	5%	16%	5%	21%
Diarrhoea	90%	0%	5%	0%	5%
Cancer	100%				

Table A.3. Numbers of symptoms reported by 106 pilots flying on a B737, B757 and A320 aircraft (Michealis, 2003).

Health problems in pilots

Symptom	*No answer*	*Occasionally*	*Some-times*	*Often*	*Long term*	*Never*
Irritation of eyes, nose and throat	3	39	19	4	1	40
Blurred vision, tunnel vision	9	4	1	0	0	92
Respiratory distress difficulties	10	4	2	0	1	89
Headaches, light-headedness, dizziness	4	35	15	3	2	47
Balance/coordination difficulties	10	3	2	0	0	91
Disorientation	12	9	3	0	0	82
Memory impairment (short-term)	8	12	4	1	2	79
Numbness (head, limbs, lips, fingers)	5	13	3	1	0	84
Fatigue, weakness, decreased performance	7	32	18	5	1	43
Concentration difficulties, confusion	7	22	7	2	1	67
Skin irritations	10	8	7	6	0	75
Nausea, vomiting, gastrointestinal problems	9	16	5	0	1	75
Diarrhoea	12	17	11	2	1	63
Joint pain, muscle weakness	9	10	5	1	0	81
General increase in feeling unwell	6	29	7	2	3	59
Immune system disorders	10	3	2	0	0	91
Intolerance to foods/alcohol	10	4	4	1	2	85
Intolerance to chemicals/odours	10	4	11	1	0	80
Cancer (please state type)		2 (1 basal cell carcinoma and 1 prostate)				

Table A.4. Description of subject and results of examination of the 26 airline attendants (Heuser et al., 2006).

Case No	Gender	PET	Neuropsychological evaluation	Neurological/ Physical Examination
1	F		TE, LDM	Abnormal
2	F	Abnormal	TE, LD, RD	Abnormal
3	F	Abnormal	TE	Abnormal
4	F		TE. LDM	Mildly abnormal
5	F		TE, LDM	Within normal limits
6	F		TE, LD, RD, DST	Mildly abnormal
7	M	Abnormal	TE	Abnormal
8	F		TE, LD, DST	Within normal limits
9	F	Abnormal	TE	Abnormal
10	F		TE, LDM	Mildly abnormal
11	F	Abnormal	TE, LD Reading	Within normal limits
12	F		TE, DST	Within normal limits
13	F	Abnormal	TE, LD	Abnormal
14	F		TE, LD, Depression	Within normal limits
15	F	Abnormal	TE, LDM, DST	Abnormal
16	F	Abnormal	TE. LDM	Mildly abnormal
17	F		TE, LDM	Within normal limits
18	M		TE, LD, Depression	Within normal limits
19	F		TE, LD, DST	Within normal limits
20	F	Abnormal	TE	Abnormal
21	F	Abnormal	TE, LDM	Within normal limits
22	F		TE, Depression	Within normal limits
23	F		TE, DST, Depression	Mildly abnormal
24	M	Abnormal	TE, LDM	Abnormal
25	F		TE, DST, LDM	Within normal limits
26	F	Abnormal	TE, DST, Depression	Mildly abnormal

Abbreviations
TE: Toxic encephalopathy
LD: Learning disabilities
RD: Reading disorder
DST: Disturbances × smell and taste
LDM: Learning disability in mathematics

Table A.5. Symptoms reported by 35 flight crew during 4 months period (van Netten, 1998).

Symptom	No. of Individuals
Headache	29
Burning eyes	27
Burning throat	48
Watery eyes	6
Sinus congestion	6
Light headedness	6
Nausea	9
Chest pains	7
Dizziness	7
Disorientation	16
Breathing problems requiring oxygen	2
Gagging, coughing	3
Blurred vision	1
Tingling of nose and lips	3
Numbness	2

Five aircraft, involving 35 flights each. Total individuals with symptoms = 112; total flight crew present = 200.

Table A.6. Symptoms summary of seven case studies (Winder and Balouet, 2001).

Symptom/Symptom cluster	Case Study No							Tot
	1	2	3	4	5	6	7	
Loss of consciousness, "grey out"		✓		✓	✓			3
Ataxia, seizures				✓				1
Narcosis, somnolence		✓	✓					2
Vertigo	✓	✓						2
Loss of balance	✓	✓			✓	✓		4

Figure A.9. Symptoms summary of 34 flight crew members (Abou-Donia et al., 2013).

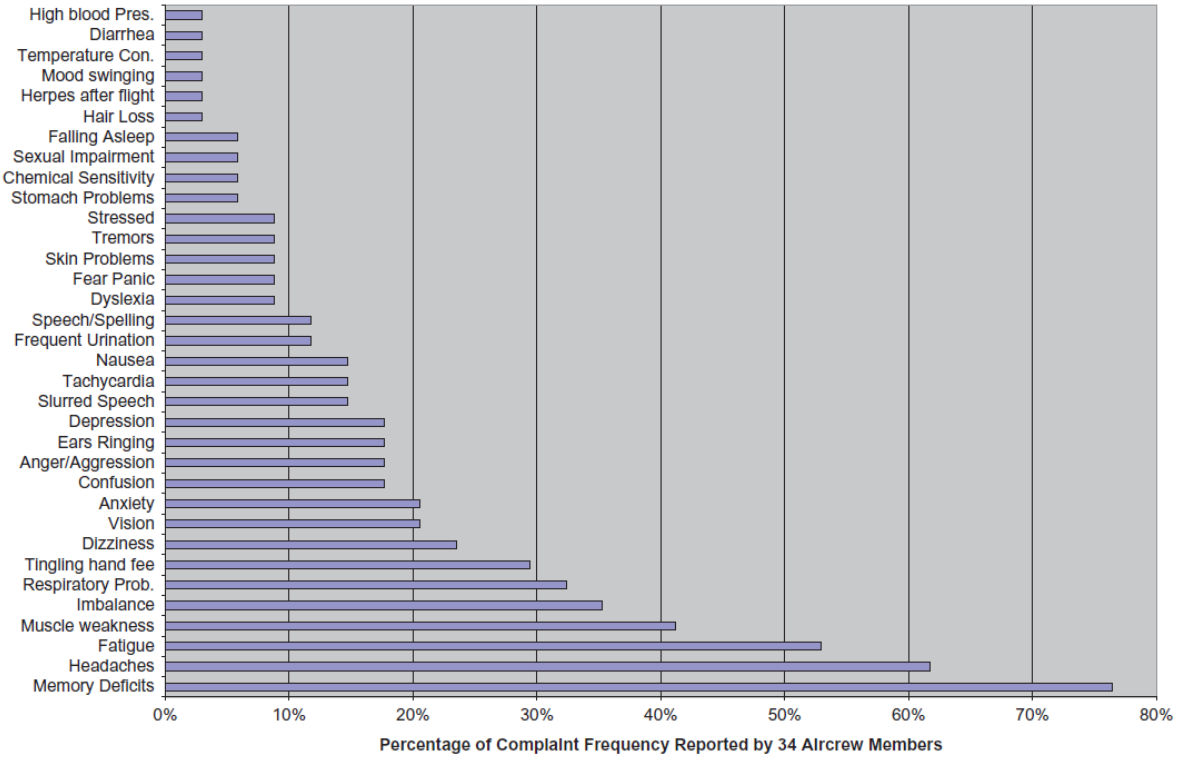
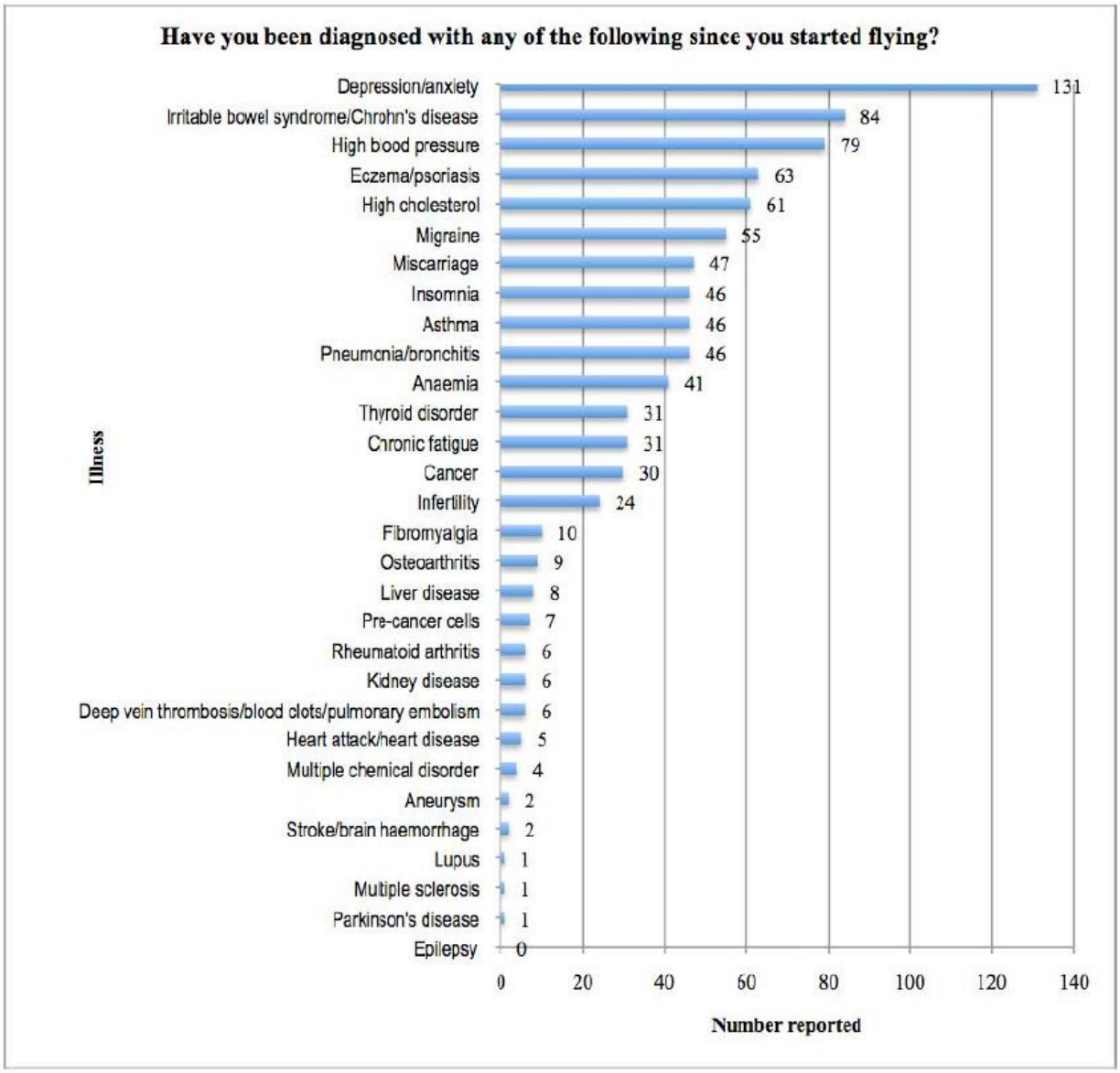


FIGURE 2. Frequency of complaints reported by 34 flight crew members (color figure available online).

Table A.7. Health symptoms reported by at least 15% of the flight attendants (OHRCA, 2014).

	Percentage of flight attendants with 95% confidence intervals	Number
A. ***FREQUENT SYMPTOMS: lasting 5-7 days (OVER PAST WEEK)***		
Sinus congestion	29.0% (27.6 – 30.5)	3,789
Bloating	25.2% (23.8 - 26.6)	3,750
Fatigue	27.3% (25.9 - 28.7)	3,817
Anxiety	20% (18.7 – 21.3)	3,778
Back pain	27.7% (26.3 – 29.1)	3,787
Foot pain	28.5% (27.1 – 30.0)	3,775
Shoulder/elbow/wrist/hand pain	29.4% (28.0 – 30.9)	3,792
Generalized muscle aches	23.3% (21.9 – 24.7)	3,775
B. NOTABLE CONDITIONS: needing medical attention (OVER PAST 12 MONTHS)		
Reactive airways/sinusitis/allergies	54.7% (53.1 - 56.2)	3,850
Shortness of breath/reduced lung capacity	15.5% (14.4 – 16.7)	3,787
Other respiratory symptoms	14.6% (13.4 – 16.7)	3,436
Severe headache	23.4% (22.1 – 24.7)	3,804
Numbness/tingling of extremities	17% (15.8 – 18.2)	3,801
Dizziness/lightheadedness	19.4% (18.1 – 20.6)	3,796
Memory loss/Lack of concentration	14.7 (13.6 - 15.8)	3,783
Fatigue	36.8% (35.3 – 38.3)	3,809
Muscle weakness	16.3% (15.1 – 17.5)	3,778
Joint aches/pains	33.3% (31.8 – 38.8)	3,813
Rashes/hives	15.5% (14.3 – 16.6)	3,805
C. DIAGNOSED CONDITIONS: told by a care provider (EVER)		
High blood pressure	16.7% (15.5 – 17.8)	3,882
Chronic bronchitis	15.6% (14.5 – 16.7)	3,910
Migraines	19.4% (18.2 – 20.6)	3,934
Hearing loss	17 % (15.9 - 18.2)	3,853
Low back pain	52.6 % (51.0 – 54.2)	3,861
Sleep disturbances	33.7 % (32.2 – 35.2)	3,852
Depression/Anxiety	36.3 %(34.8 – 37.8)	3,851
Allergies	39.0 % (37.5 – 40.6)	3,831

Figure A.10. Health symptoms and frequency reported in survey of 640 aircrew by Toxic Free Airlines (EPAAQ, 2011).



Appendix 3 Order of magnitude of measurements of oil compounds found in a realistic setting in an aircraft.

Table A.8. OP levels detected in air samples collected during flight (Solbu et al., 2011).

Table 5 Organophosphate concentrations on long-term (1–3 months) samples (wipe sampling and sampling on activated charcoal cloths), collected in cockpit and galley aft during commercial flights

	Model A airplanes ($k^a = 14$, $n^b = 32$)					Model C airplanes ($k = 6$, $n = 12$)					Model D airplanes ($k = 6$, $n = 12$)				
	TiBP[c]	TnBP[f]	DBPP[g]	TPP[h]	TCP[i]	TiBP	TnBP	DBPP	TPP	TCP	TiBP	TnBP	DBPP	TPP	TCP
Wipe samples															
%>LOQ	6%	38%	50%	66%	31%	0%	58%	67%	75%	92%	0%	17%	0%	92%	8%
Median[c]	*d*	*d*	*d*	0.37	*d*	*d*	0.20	0.25	0.61	2.3	*d*	*d*	*d*	0.90	*d*
Minimum[c]	<0.05	<0.05	<0.05	<0.05	<0.05	<0.10	<0.13	<0.13	<0.13	<0.18	<0.07	<0.07	<0.07	<0.3	<0.07
Maximum[c]	0.42	19	20	15	1.3	<0.24	2.0	1.7	2.6	4.1	<0.3	0.32	<0.3	2.3	8.3
90th percentile[c]	*d*	0.87	1.4	1.6	0.77	*d*	1.0	0.79	2.5	3.8	*d*	0.29	*d*	1.9	*d*
Charcoal cloth															
%>LOQ	100%	100%	100%	47%	6%	100%	100%	100%	50%	0%	100%	100%	83%	75%	0%
Median[c]	96	970	210	*d*	*d*	41	860	190	*d*	*d*	76	110	8.3	2.0	*d*
Minimum[c]	5.9	330	59	<1.3	<1.3	26	490	120	<2	<2	40	56	1.7	<0.8	<0.9
Maximum[c]	390	16 000	970	7.6	270	68	1400	280	4.5	<4	120	500	140	4.7	<4
90th percentile[c]	260	3 100	410	6.2	*d*	63	1 300	250	4.1	*d*	110	480	76	4.3	*d*

[a] Number of unique aircrafts. [b] Number of measurements (wipe/cloth). [c] The unit is mass of compound per area per installation time (ng dm^{-2} per day). [d] Median/90th percentile concentration level was <LOQ. [e] Triisobutyl phosphate. [f] Tri-*n*-butyl phosphate. [g] Dibutylphenyl phosphate. [h] Triphenyl phosphate. [i] Tricresyl phosphates.

Table A.9. VOCs detected in air samples collected during 14 domestic flights (Wang et al., 2014)

Species	Samples	First quartiles	Mean	Median	Third quartiles	Fourth quartiles	S.D.[a]	C.V.[b]
Benzene	84	6.43	18.24	10.01	17.53	145.46	23.72	130%
Toluene	84	7.13	30.66	13.41	44.43	237.32	40.69	133%
Ethylbenzene	84	0.57	6.35	3.76	8.09	45.12	8.66	136%
p-Xylene	84	1.45	4.53	2.55	4.74	42.03	6.94	153%
o-Xylene	84	0.45	4.33	3.70	6.35	31.98	5.42	125%
Decanal	84	19.32	25.75	24.43	31.13	57.16	8.86	34%
Nonanal	84	14.69	18.35	17.78	21.46	35.84	6.18	34%
Dodecane	84	3.08	5.94	4.73	7.29	27.48	4.74	80%
Undecane	84	0.23	2.55	2.21	4.16	11.15	2.53	99%
Octanal	84	5.26	6.55	6.79	8.27	17.12	3.56	54%
1-Hexanol, 2ethyl-	84	4.58	6.91	6.13	9.55	19.88	3.91	57%
Tetrachloroethylene	84	1.78	2.79	2.57	3.64	12.90	1.97	71%
Benzaldehyde	84	0.15	5.91	6.26	8.83	31.96	5.23	89%
D-Limonene	84	9.40	62.86	31.21	56.02	660.12	111.07	177%
Acetic acid	84	0.61	9.89	11.43	15.18	22.56	7.07	72%
5-Hepten-2-one, 6-methyl-	84	1.88	8.79	8.71	13.80	21.15	6.32	72%
Styrene	84	0.09	2.38	1.23	2.80	14.14	3.36	142%
Menthol	84	0.06	3.47	2.50	4.79	23.91	4.19	121%
Acetone	84	0.46	4.29	0.46	8.50	21.39	4.98	116%
Sum of VOCs[c]	84	162.65	236.48	205.03	262.63	1048.82	144.78	61%

[a] S.D. = standard deviation.
[b] C.V. = coefficient of variation = S.D./mean value.
[c] Sum of VOCs = sum of 19 VOCs.

Appendix 4 Identification of various compounds in oil vapours (GC/MS/TD)

Compounds were identified based on peak deconvolution with AMDIS followed by a target library search. The target library contains over a 1000 compounds with spectra and retention indices. The identification was based on the NET match factor, a combination of the match factor and the retention index, with a minimum NET match factor of 80. Compounds that were not identified with the target library search were tentatively identified by a search in the NIST library with a minimum match factor of 80.

Table A.10. Identification of various aldehydes and ketones in oil vapour.

Oil type		A_n	A_n	A_u	A_u	B_n	B_n
Temperature range (˚C)		20-350	375±25	20-350	375±25	20-350	375±25
Test duration (min)		30	60	30	60	30	60
Compounds	CAS						
Aldehydes							
heptanal	111-71-7	X	X		X	X	X
nonanal	124-19-6		X		X	X	X
2-methylpropanal	78-84-2	X	X				
2-propenal	107-02-8	X	X	X	X	X	X
2-butenal	4170-30-3	X	X		X	X	X
2-pentenal	1576-87-0	X	X		X	X	X
trans-2-hexenal	6728-26-3		X		X		X
2-heptenal	57266-86-1		X		X		X
2-octenal	2548-87-0						X
2-methyl-2-propenal	78-85-3	X	X	X	X	X	X
2-methyl-2-butenal	1115-11-3				X		X
2-ethylpropenal	922-63-4	X	X		X		X
3-furaldehyde	498-60-2		X		X		X
Ketones							
2-butanone	78-93-3	X	X	X	X	X	X
2-pentanone	107-87-9	X	X	X	X	X	X
2-heptanone	110-43-0		X		X	X	X
2-octanone	111-13-7				X		
3-hexanone	589-38-8		X		X		X
3-heptanone	106-35-4		X		X		X
4-heptanone	123-19-3		X		X		X
5-nonanone	502-56-7		X		X	X	X
2,3-butanedione	431-03-8					X	
2,5-hexanedione	110-13-4	X	X		X	X	X
cyclopentanone	120-92-3		X		X		
3-methylcyclohexanone	591-24-2		X		X		X
1-buten-3-one	78-94-4	X	X	X		X	X
1-penten-3-one	1629-58-9	X				X	X
1-hexen-3-one	1629-60-3		X				X
1-hepten-3-one	2918-13-0		X				X
3-penten-2-one	625-33-2	X	X		X	X	X
3-hexen-2-one	763-93-9		X		X		X
5-hexen-2-one	109-49-9	X	X		X		X
2-methyl-2-cyclopenten-1-one	1120-73-6		X		X		X
1-hydroxy-2-propanone	116-09-6		X		X		X
1,4-naphthalenedione	130-15-4					X	X
1,2-naphthalenedione	524-42-5						X

Table A.11. Identification of various alkenes in oil vapour.

Oil type		A_n	A_n	A_u	A_u	B_n	B_n
Temperature range (˚C)		20-350	375±25	20-350	375±25	20-350	375±25
Test duration (min)		30	60	30	60	30	60
Compounds	CAS						
Alkenes							
1-pentene	109-67-1	X	X	X	X	X	
1-hexene	592-41-6	X	X		X	X	X
1-heptene	592-76-7		X		X		X
1-octene	111-66-0	X	X		X	X	X
trans-2-hexene	4050-45-7	X			X	X	X
trans-2-heptene	14686-13-6		X		X		X
cis-2-hexene	7688-21-3		X		X		X
2-methyl-1-pentene	763-29-1	X	X		X	X	
4,4-dimethyl-1-pentene	762-62-9	X	X		X		
2,4,4-trimethyl-1-pentene	107-39-1	X				X	
cyclopentene	142-29-0				X		
cyclohexene	110-83-8		X		X		
1-methylcyclopentene	693-89-0						
1-ethylcyclopentene	2146-38-5		X		X		X
1-methylcyclohexene	591-49-1		X		X		X

Table A.12. Identification of various organic acids and esters in oil vapour.

Oil type		A_n	A_n	A_u	A_u	B_n	B_n
Temperature range (˚C)		20-350	375±25	20-350	375±25	20-350	375±25
Test duration (min)		30	60	30	60	30	60
Compounds	CAS						
Organic acids							
acetic acid	64-19-7	X	X		X	X	
formic acid	64-18-6					X	X
propanoic acid	79-09-4	X			X	X	
butanoic acid	107-92-6		X		X		X
pentanoic acid	109-52-4	X		X		X	
hexanoic acid	142-62-1						X
heptanoic acid	111-14-8	X	X			X	X
octanoic acid	124-07-2		X		X	X	X
decanoic acid	334-48-5		X		X		X
2-methylbutanoic acid	116-53-0				X	X	
3,5,5-trimethylhexanoic acid	3302-10-1	X	X		X		
2-propenoic acid	79-10-7	X				X	
3-butenoic acid	625-38-7						X
Esters							
acetic acid, methyl ester	79-20-9	X	X		X	X	X
pentanoic acid methyl ester	624-24-8	X	X		X	X	X
heptanoic acid methyl ester	106-73-0		X		X	X	X
octanoic acid methyl ester	111-11-5					X	
decanoic acid, methyl ester	110-42-9				X	X	X
2-methylbutanoic acid, methyl ester	868-57-5						X
actic acid, propyl ester	109-60-4		X		X		
pentanoic acid, 2-propenyl ester	6321-45-5	X	X		X		X
acetic acid, ethenyl ester	108-05-4		X		X		X
2-propenoic acid, methyl ester	96-33-3	X	X		X		X
3-butenoic acid, methyl ester	3724-55-8		X		X		X

Table A.13. Identification of various alkenes in oil vapour.

Oil type		A_n	A_n	A_u	A_u	B_n	B_n
Temperature range (˚C)		20-350	375±25	20-350	375±25	20-350	375±25
Test duration (min)		30	60	30	60	30	60
Compounds	CAS						
Alcohols							
tert-butanol	75-65-0	X	X		X		
2-propen-1-ol	107-18-6						X
Furanes							
furan	110-00-9		X		X	X	X
2-methylfuran	534-22-5		X		X	X	X
2-ethylfuran	3208-16-0				X		X
2,5-dimethylfuran	625-86-5		X		X		X
2-ethyl-5-methylfuran	1703-52-2		X		X		X
2-n-propylfuran	4229-91-8				X		
tetrahydrofuran	109-99-9	X	X		X	X	X
2-methoxytetrahydrofuran	13436-45-8		X		X		X
Various components							
trans-1,2-dimethylcyclopentane	822-50-4	X	X		X	X	X
tetrahydro-2-methylpyran	10141-72-7	X	X		X	X	X
methyl 2-oxopropanoate	600-22-6	X	X				X
phthalic anhydride	85-44-9				X	X	X
trans-2,3-dimethyl oxirane	21490-63-1				X	X	X
N-phenylformamide	103-70-8					X	X
o-isopropenyltoluene	7399-49-7				X		
3- or 4-methylphenol	108-39-4	X	X		X	X	X

Appendix 5 2D TIC chromatogram of TENAX samples

2D TIC chromatogram of TENAX samples obtained from pyrolysis of the oils in the various flight phases (Horizontal plots respectively Tax, Climb, Cruise, Descent) and under inert and oxygenated conditions (Vertical plots; N_2 duplicate and O_2). Each plot shows the peak height shown by color gradient against the: 1st dimension time (sec, x-axis) and 2nd dimension time (sec, y-axis)

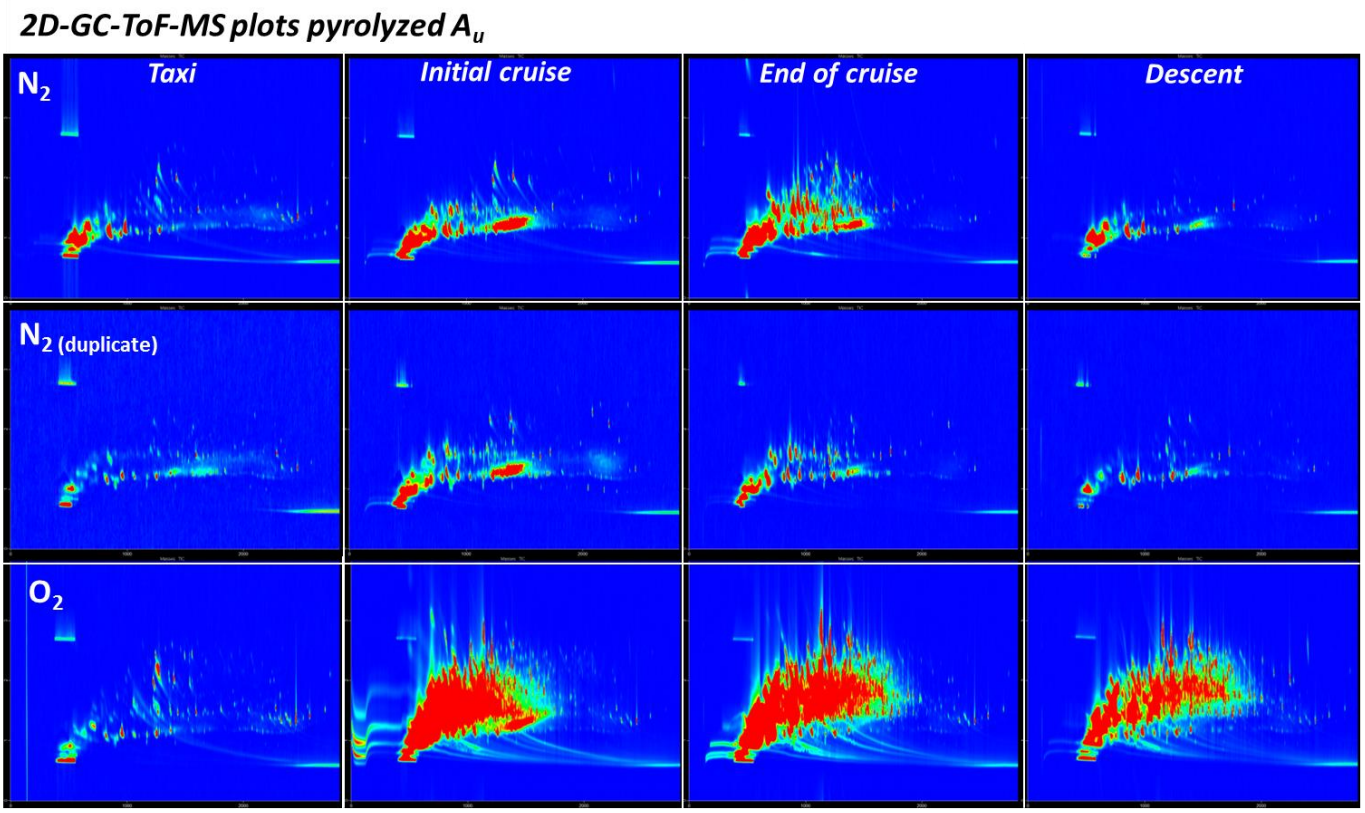


Figure A.10: Oil A_u

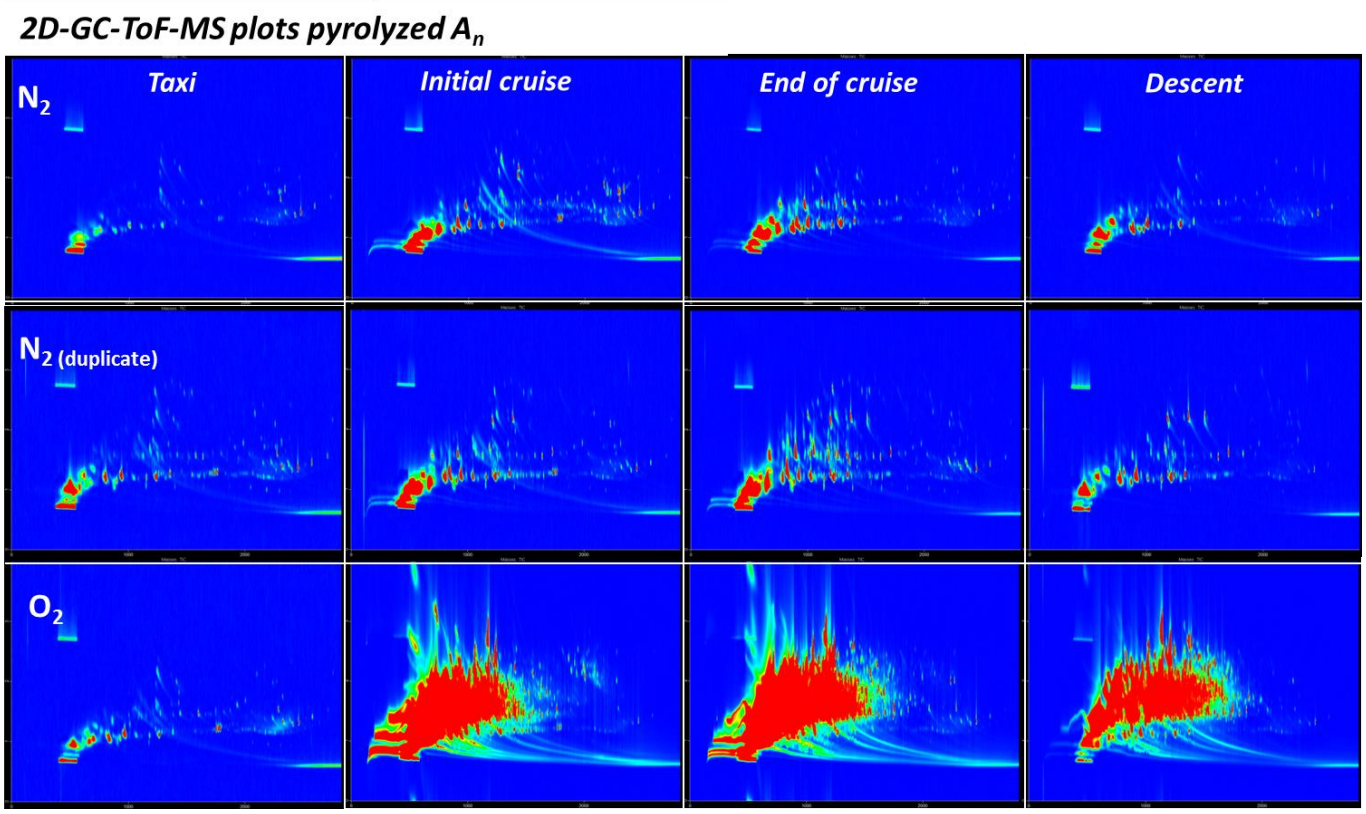


Figure A.11: Oil A_n

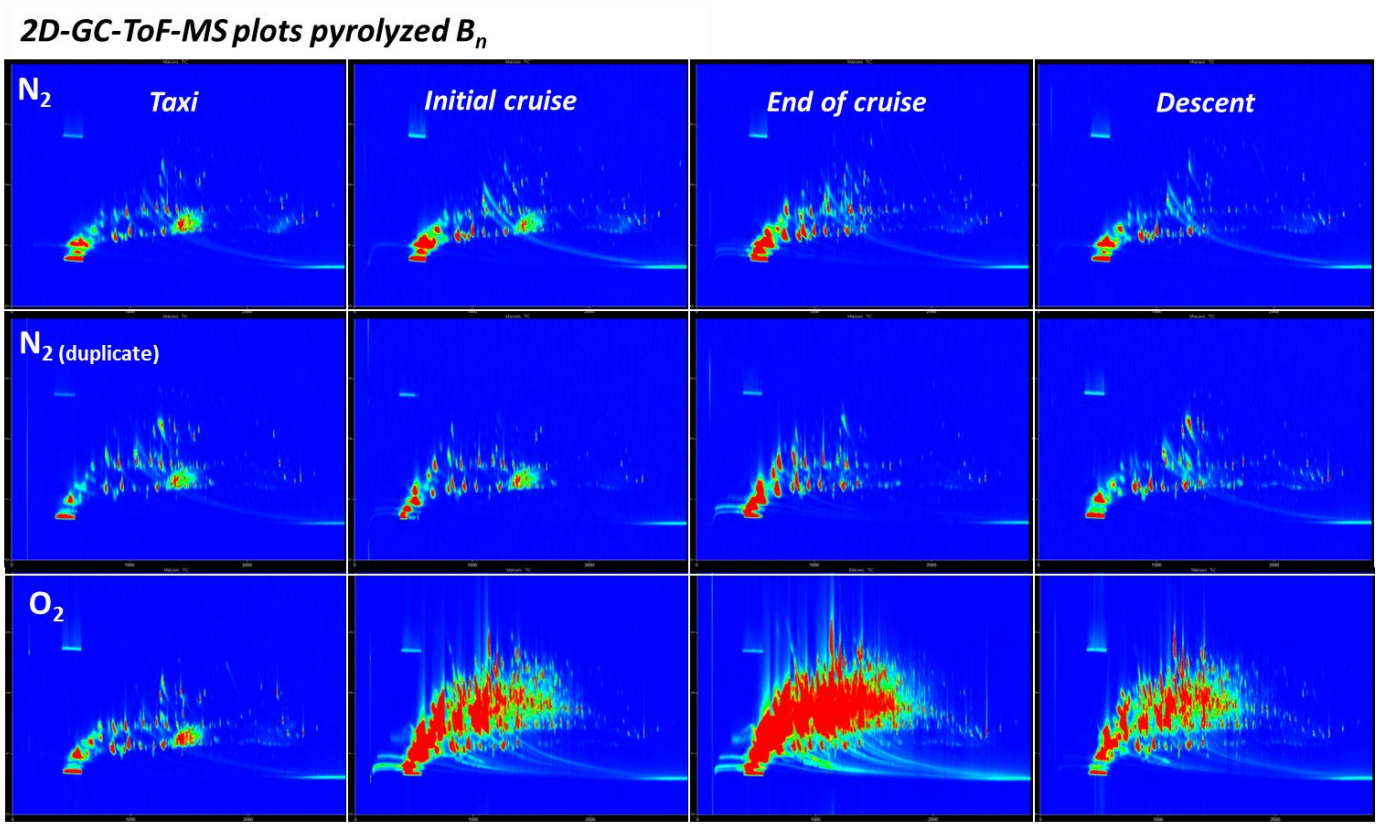


Figure A.12: Oil B_n

Appendix 6 Classification of chemicals list

The following table describes the hazard profile of the list of compounds (Table 5.6) identified under both nitrogen and oxygen conditions, in all oils and during different flight stages. The hazard profile, or classification, of a substance is an indication of its intrinsic toxicity. A substance is classified, e.g. assigned a notation for a specific toxicity, according to an internationally agreed classification criteria. The classification can distinguishes between the various types of toxicity (reprotoxic, carcinogenic, irritant), the potency (4 (less potent) =>1 (most potent), the frequency needed to exert the toxicity (Single Exposure (SE) vs Repeated Exposure (RE)) and the level of evidence (1A, 1B and 2) available (for more information, see the Guidance on the criteria for Classification and Labelling under REACH).

In most cases, the manufacture or importer classifies the substance without any review by an independent organisation, the so-called self-classification. Different manufacturers classify the same substance differently. Harmonized classification means the classification is agreed upon in a scientific committee and that classification is mandatory for every manufacturer or importer.

The substances are run through the Classification and Labelling database of the European Chemical Agency (ECHA) database. This database contains all classification and labelling information on notified and registered substances received from manufacturers and importers in the European Union. Manufacturers and importers need to notify a substance to the Classification and Labelling (C&L) Inventory if they intent to place the substance on the EU market.

Compound #	Name	CAS	Harmonized classification	Self-classification*
1	Diethyl Phthalate	84-66-2		NC
2	1-Nonene, 4,6,8-trimethyl-	54410-98-9		
3	2-Ethylhexyl salicylate	118-60-5		Skin Irrit. 2
4	Acetophenone	98-86-2	Acute Tox. 4 Eye Irrit. 2	
5	Benzaldehyde	100-52-7	Acute Tox. 4	
6	Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4	NR	NR
7	Heptane, 4-methyl-	589-53-7	Asp. Tox. 1 Skin Irrit. 2 STOT SE 3	
8	Nonanal	124-19-6		NC
9	2,4-Dimethyl-1-heptene	19549-87-2		Asp. Tox. 1
10	Decanal	112-31-2		Eye Irrit. 2
11	Dodecanoic acid, isooctyl ester	84713-06-4		NC
12	Heptadecane, 2,6,10,14-tetramethyl-	18344-37-1	NR	NR
13	Octanal	124-13-0		Skin Irrit. 2 Eye Irrit. 2
14	Dodecane, 4,6-dimethyl-	61141-72-8	NR	NR
15	Heptane	142-82-5	Asp. Tox. 1 Skin Irrit.2 STOT SE 3	
16	5,9-Undecadien-2-one, 6,10-dimethyl-	689-67-8		Skin Irrit. 2
17	Benzene	71-43-2	Asp. Tox. 1 Skin Irrit. 2 Eye Irrit. 2 Muta. 1B Carc. 1A STOT RE 1	

Compound #	Name	CAS	Harmonized classification	Self-classification*
18	Glycidol	556-52-5	Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 3 STOT SE 3 Muta. 2 Carc. 1B Repr. 1B	
19	Nonane, 2,6-dimethyl-	17302-28-2	NR	NR
20	2-Propanol, 2-methyl-	75-65-0	Eye Irrit. 2 Acute Tox. 4 STOT SE 3	
21	Decane, 2,3,5,8-tetramethyl-	192823-15-7	NR	NR
22	Nonane	111-84-2		Eye Irrit. 2
23	Octane	111-65-9	Asp. Tox. 1 Skin Irrit. 2 STOT SE 3	
24	Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4		Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1
25	2,5-Hexanediol, 2,5-dimethyl-	110-03-2		Eye Dam. 1
26	Acetic acid, octadecyl ester	822-23-1	NR	NR
27	Undecane	1120-21-4		Asp. Tox. 1
28	2-Heptanone, 4-methyl-	6137-06-0	NR	NR
29	Hexane, 2,3,4-trimethyl-	921-47-1	NR	NR
30	1,2-Benzenedicarboxylic acid, di-2-propenyl ester	131-17-9	Acute Tox. 4	
31	1-Iodo-2-methylundecane	73105-67-6	NR	NR
32	Isopropyl Palmitate	142-91-6		NC
33	n-Decanoic acid	334-48-5		Skin Irrit. 2 Eye Irrit. 2
34	Tridecane	629-50-5		Asp. Tox. 1
35	TCP isomer	563-04-2		Acute Tox. 4
36	1-Propanol, 2-methyl-	78-83-1	Skin Irrit. 2 Eye Dam. 1 STOT SE 3	
37	1-Tridecanol	112-70-9		NC
38	5-Hepten-2-one, 6-methyl-	110-93-0		Eye Irrit. 2
39	Decane	124-18-5		Asp. Tox. 1
40	Pentane	109-66-0	Asp. Tox. 1 STOT SE 3	
41	Pentanoic acid, methyl ester	624-24-8		NC
42	Amylene Hydrate	75-85-4	Skin Irrit. 2 Acute Tox. 4 STOT SE 3	
43	Glycerin	56-81-5		NC
44	Heptanal	111-71-7		Skin Irrit. 2 Eye Irrit. 2
45	Heptanoic acid, methyl ester	106-73-0		Skin Irrit. 2

Compound #	Name	CAS	Harmonized classification	Self-classification*
46	Octane, 3,5-dimethyl-	15869-93-9		NC
47	Phenol	108-95-2	Acute Tox. 3 Skin Corr. 1B Muta. 2 STOT RE 2	
48	Propane, 2-ethoxy-2-methyl-	637-92-3		STOT SE 3
49	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	84-69-5	Repr. 1B	
50	2-Butanone	78-93-3	Eye Irrit. 2 STOT SE 3	
51	Butylated Hydroxytoluene	128-37-0		NC
52	Decanoic acid, 2-ethylhexyl ester	73947-30-5		NC
53	Diazene, dimethyl-	503-28-6		NC
54	Dodecane, 2-methyl-	1560-97-0	NR	NR
55	Methacrolein	78-85-3		Acute Tox. 3 Skin Corr. 1B Eye Dam. 1 Acute Tox. 2
56	Pentadecane	629-62-9		Asp. Tox. 1
57	Benzaldehyde, 4-methyl-	104-87-0		Acute Tox. 4 Eye Irrit. 2
58	Phenol, 3-methyl-	108-39-4	Acute Tox. 3 Skin Corr. 1B	
59	2H-Pyran-2-one, tetrahydro-	542-28-9		Eye Dam. 1
60	Benzene, (1,1,2-trimethylpropyl)-	26356-11-6	NR	NR
61	Cyclopropyl carbinol	2516-33-8		Acute Tox. 4 Eye Irrit. 2
62	Hexadecen-1-ol, trans-9-	64437-47-4	NR	NR
63	Hexanal	66-25-1		Eye Irrit. 2
64	Isobutane	75-28-5	NC	
65	Octanoic acid, methyl ester	111-11-5		Skin Sens. 1
66	(2-Aziridinylethyl)amine	4025-37-0	NR	NR
67	1,4-Dioxane-2,5-dione, 3,6-dimethyl-, (3S-cis)-	4511-42-6		Eye Irrit. 2
68	1-Octene, 3,7-dimethyl-	4984-01-4		NC
69	2-Butene	107-01-7		NC
70	Cyclobutylamine	2516-34-9		Skin Corr. 1B
71	n-Hexadecanoic acid	57-10-3		NC
72	Tridecane, 3-methyl-	6418-41-3	NR	NR
73	1-Octene	111-66-0		Asp. Tox. 1
74	2(5H)-Furanone, 3-methyl-	22122-36-7		Skin Irrit. 2 Eye Irrit. 2 STOT SE 3
75	Pentanal	110-62-3		Skin Sens. 1 Eye Irrit. 2 Acute Tox. 4 STOT SE 3
76	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	4376-20-9		Skin Irrit. 2 Eye Irrit. 2

Compound #	Name	CAS	Harmonized classification	Self-classification*
77	2,5-Furandione, dihydro-3-methyl-	4100-80-5		Skin Irrit. 2 Eye Irrit. 2 STOT SE 3
78	Benzeneacetaldehyde	122-78-1		Acute Tox. 4 Skin Sens. 1
79	Dodecanoic acid	143-07-7		Eye Dam. 1
80	Methylglyoxal	78-98-8		Acute Tox. 4 Skin Sens. 1B Eye Dam. 1 Muta. 2
81	Pentadecane, 2,6,10-trimethyl-	3892-00-0	NR	NR
82	2-Hexanone	591-78-6	STOT SE 3 Repr. 2 STOT RE 1	
83	Hydroxyurea	127-07-1		Muta. 1B Repr. 2
84	(S)-2-Hydroxypropanoic acid	79-33-4		Skin Irrit. 2 Eye Dam. 1
85	1,3,5,7-Cyclooctatetraene	629-20-9		Asp. Tox. 1 Skin Irrit. 2 Eye Irrit. 2 STOT SE 3
86	2-Propanone, 1-hydroxy-	116-09-6		NC
87	Butyrolactone	96-48-0		Acute Tox. 4 Eye Dam. 1 STOT SE 3
88	Cyclopentanone	120-92-3	Skin Irrit. 2 Eye Irrit. 2	
89	Dodecane	112-40-3		Eye Irrit. 2
90	Eicosane	112-95-8		NC
91	Heptadecane, 2,6-dimethyl-	54105-67-8	NR	NR
92	l-Pantoyl lactone	5405-40-3		Eye Dam. 1
93	Pentanoic acid	109-52-4	Skin Corr. 1B	
94	Phthalic anhydride	85-44-9	Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 Eye Dam. 1 Resp. Sens. 1 STOT SE 3	
95	trans-3-Decene	19150-21-1	NR	NR
96	Undecanal	112-44-7		Skin Irrit. 2
97	1-Hexene	592-41-6		Asp. Tox. 1
98	1H-Indene, 1-methylene-	2471-84-3	NR	NR
99	1-Pentene	109-67-1		NC
100	Acetone	67-64-1	Eye Irrit. 2 STOT SE 3	
101	Decane, 3,7-dimethyl-	17312-54-8	NR	NR
102	Formamide, N-methyl-	123-39-7	Acute Tox. 4 Repr. 1B	

Compound #	Name	CAS	Harmonized classification	Self-classification*
103	Hexane	110-54-3	Asp. Tox. 1 Skin Irrit. 2 STOT SE 3 Repr. 2 STOT RE 2	
104	Octane, 1-chloro-	111-85-3		Asp. Tox. 1
105	1,3,5-Cycloheptatriene	544-25-2		Asp. Tox. 1 Acute Tox. 3 Skin Irrit. 2 Eye Irrit. 2 STOT SE 3
106	1-Hexadecanol	36653-82-4		NC
107	3-Undecene, (Z)-	821-97-6	NR	NR
108	Benzonitrile	100-47-0	Acute Tox. 4	
109	Cyclooctane, 1,4-dimethyl-, cis-	13151-99-0	NR	NR
110	Dodecane, 2,7,10-trimethyl-	74645-98-0	NR	NR
111	Heptane, 3-ethyl-	15869-80-4	NR	NR
112	1-Pentene, 2,4,4-trimethyl-	107-39-1	NC	
113	1-Pentene, 2-methyl-	763-29-1		NC
114	1-Propene, 2-methyl-	115-11-7	NC	
115	2(3H)-Furanone, dihydro-5-methyl-	108-29-2		NC
116	2-Heptanone	110-43-0	Acute Tox.4	
117	2-Hexenal	505-57-7		Acute Tox. 4 Acute Tox. 3 Skin Sens. 1
118	2-Pentanone	107-87-9		Acute Tox. 4 Eye Irrit. 2 STOT SE 3
119	2-tert-Butyltoluene	1074-92-6		NC
120	Acetic acid	64-19-7	Skin Corr. 1A	
121	cis-2-Nonene	6434-77-1		Asp. Tox. 1 Skin Irrit. 2 Eye Irrit. 2 STOT SE 3
122	Decane, 1-chloro-	1002-69-3		Carc. 2
123	Heptanoic acid	111-14-8	Skin Corr. 1B	
124	Isopropyl Myristate	110-27-0		NC
125	Tetradecanoic acid	544-63-8		NC
126	1-Pentene, 4-methyl-	691-37-2		Asp. Tox. 1 Or Skin Irrit. 2 Eye Irrit. 2 STOT SE 3
127	2-Cyclopenten-1-one	930-30-3		NC

* according to the largest number of notifiers

NC = not classified for human health effects

NR = not registered under REACH

Acute Tox = Acute toxicity
Asp. Tox. = Aspiration toxicity
Eye Irrit. = Eye irritation
Eye Dam. = Eye Damage
Skin Irrit. = Skin irritation
Skin Corr. = Skin corrosion
Skin Sens. = Skin sensitisation
Resp. Sens = Respiratory sensitisation
STOT SE = Specific Target Organ Toxicity Single Exposure
STOT RE = Specific Target Organ Toxicity Repeated Exposure
Muta. = Mutagenicity
Carc. = Carcinogenicity
Repr.= Reprotoxicity

Appendix 7 Chemical analysis of the vapour generated for the in vitro exposures.

Sample description	Generation into air-liquid interface	Generation into air-liquid interface	Generation into air-liquid interface	Generation into air-liquid interface
Oil type	Oil A_n	Oil A_n	Oil B_n	Oil B_n
Component	$\mu g/m^3$ (oil vapor)	$\mu g/m^3$ (oil vapor)	$\mu g/m^3$ (oil vapor)	$\mu g/m^3$ (oil vapor)
TiPP	0,03	0,02	0,06	0,03
TBP	0,90	0,72	0,74	0,37
TCEP	0,04	0,04	0,03	0,03
TCPP-1	0,05	0,08	0,05	<
DBPP	<	0,02	<	<
TPhP	0,02	0,03	0,02	0,02
DCP-1	0,05	<	0,02	<
DCP-2	0,04	<	0,02	<
T(m,m,m)CP	3,3	2,6	5,7	4,6
T(m,m,p)CP	7,4	5,6	15	11
T(m,p,p)CP	6,5	5,1	13	9,8
T(p,p,p)CP	1,9	1,5	3,4	2,5

Appendix 8 Abbreviations

ADSE	:	Aircraft Development and Systems Engineering
AMDIS	:	Automated Mass Spectral Deconvolution and Identification System
A_n	:	New oil of brand A
ASE	:	Accelerent solvent extraction
ASTM	:	American Society for Testing and Materials
A_u	:	Used oil of brand A
AVOIL	:	Aviation oil
B_n	:	New oil of brand B
CO	:	Carbon monoxide
DCM	:	Dichloromethane
DNPH	:	Dinitrophenylhydrazine
FTIR	:	Fourier transform infrared spectroscopy
GC-FID	:	Gaschromatography-Flame ionization detector
IRAS	:	Institute for Risk Assesment Sciences
ISO	:	International Standardisation Organisation
NEN	:	Dutch Normalisation Institute
NEN-EN	:	Dutch Standard-European Standard
NIOSH	:	National Institute for Occupational Safety and Health
NIST	:	National Institute of Standards and Technology
NM	:	Nautical miles
NRC	:	National Research Council
OPCW	:	Organisation for the prohibition of chemical wapons
OPE	:	Organo Phosphate Esters
PAH	:	Polycyclic Aromatic Hydrocarbons
PNC	:	Particle number concentrations
RIVM	:	National Institute for Public Health and the Environment
TCN	:	Tetrachloro naphthalene
TCP	:	Tricresyl phosphate
TMPP	:	Trimethylolpropane phosphate
TNO	:	Netherlands organisation for Applied Science
ToF-MS	:	Time-of-Flight Mass Spectrometer
UME	:	Unidentified complex mixtures
VOC	:	Volatile Organic Compounds
VU	:	Vrije Universiteit Free University

EASA
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European Aviation Safety Agency

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