Task 1
Review of the state of the art and establishment of the baseline for the FACTS project

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Executive Summary

FreshAircraft (FACTS) is a research study funded by the Directorate-General for Mobility and Transport (DG-MOVE). The motivation of the project comes from concerns among 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. The over-arching objective of the FACTS project is to ascertain potential safety and/or long- and short-term health risks resulting from the contamination of bleed air in both routine and cabin/cockpit air contamination (CAC) event, originated in flight conditions. The FACTS project is organised by the following tasks:

- Task 1. Review of the State of the Art and Establishment of the Baseline for the Work
- Task 2. Exposure Monitoring: Identification of the causes of bleed air contamination and assessment of the impact on the quality of cockpit/cabin air
- Task 3a. Toxicological risk assessment
- Task 3b Health risk assessment
- Task 4. Engineering controls- Risk mitigation strategy
- Task 5. Conclusions and recommendations

This report describes the work performed under Task 1: Review of the State of the Art and Establishment of the Baseline for the Work. The report provides an overview of existing knowledge ("what has been done") regarding cabin air quality regarding the following research themes within the FACTS project:

1. Chemical inventory of potential bleed air contaminants (Task 2)
   - Experimental laboratory investigations
   - In-flight measurement campaigns
2. Toxicology (Task 3A)
   - in vitro neurotoxicity tests (cell line exposure and biochemical assays)
   - in vivo neurotoxicity and behavioral tests (animal testing)
   - Human biomonitoring and biomarkers
3. Health risks (Task 3B)
   - Epidemiological based health risk assessment
   - Toxicological based health risk assessment

The results and conclusions for each of these Themes are summarized here. The topic of Risk mitigation strategy will be addressed in a separate report (D6).

Theme Task 2: Exposure Monitoring

GAP analysis

Both laboratory and in-flight measurements have greatly contributed in gathering knowledge about chemical composition and degradation of chemical substances, respectively compositions of applied engine oil, and oil vapors, and the impact of the emissions of bleed air contaminants on the cabin air quality. Knowledge should be brought on the level of trying to understand one distinct issue: cabin/cockpit air contamination (CAC) due to potential leaks, which results in short-term peaks of cockpit/cabin air contamination. The intrinsic quality of the cockpit/cabin air in normal flight operating conditions have been subject for many in-flight monitoring campaigns covering a good general view of the air quality on board of aircraft. More important is to investigate possibilities with respect to air quality management and air quality monitoring (marker components to be measured) for aircraft in order to gain a good assessment of cabin air quality. After the review of the available literature, the following data gaps are observed:

- Chemical mapping in-between LP and HP section and cockpit/cabin is lacking. There is currently not enough knowledge about what happens underway with certain uncontrolled chemical emissions entering the cockpit/cabin.
- There is little knowledge about the chemical nature and character of a CAC event.
- There is a need for a chemical survey of the surface contamination in the PACKs and ducts as well as of the filter material used in the recirculation air system;
- International inter-laboratory studies on sampling and analysis of TCPs are lacking, resulting in poor validation data on method performance characteristics. Data comparison for TCPs is difficult to make between monitoring studies
- Most of the in-flight measurement campaigns differed in experimental design, analytical approaches and the way of expressing results statistically. This makes it difficult to make comparisons. Analytical standardisation processes are needed in order to harmonize air quality monitoring.
- It is not fully understood which processes in the entire air supply system influence the chemical emissions starting from the LP and HP sections via the ECS packs-and ducts to the cabin aircraft
- It is unclear what components are possibly formed during the route from source to flight deck and passenger cabin.
- It is not clear what are suitable marker components for judgment of cabin air quality
- Spraying aircraft with de-icing agents and the effects on cabin air quality is described as a serious contaminant. Yet, few measurement campaigns for de-icing have been carried out. Many cases were reported of glycols are entering cabin, but poor data has been collected about this phenomena.

Conclusions

Continuation of air quality monitoring projects in-flight is not considered as constructive since encountering a real CAC-event is very unlikely considering the low number of engine oil triggered incidents. In order to answer some of the questions raised above, it seems useful to use appropriate simulations. Simulation of bleed air contamination on ground in real aircraft cabin air environment and during test flights in real aircraft may shed light on this gap of knowledge. Important is to investigate the black box which consists of ECS packs ducting and HEPA filters in order to map possible accumulation of bleed air contaminants in the air system.

Theme Task 3A: Toxicological Risk Assessment

Gap analysis

The central question is whether exposure to neurotoxic substances formed during fume events could be the cause of neuronal damage as observed in cases of so-called aerotoxic syndrome. One of the main problems in the risk assessment of cabin air quality is the lack of neurotoxicity hazard data for the majority of substances present in fumes. Even more, in an aircraft there is potential exposure to a complex mixture of a large number of different substances during a fume event, in highly variable concentrations.

In-vitro and *in-vivo* toxicity testing of mixtures has hardly been performed in the context of fume contaminants. There is a need for testing the combined toxicity of all substances present in fumes of engines, as a lot remains unknown about the hazard and potential health effects of exposure to this type of mixtures (Harrison and Ross, 2016).

For toxicity screening, integrated cellular testing approaches combining different organ systems, as well as whole organism test systems are needed, to allow more realistic simulation of organ interaction, and to include metabolic competence in the test system. In the context of fume exposure, few experiments have been done on studying the translation of in-vitro to in-vivo results.

It is necessary to study biomarker formation, stability and half-life under conditions of controlled exposure, allowing investigating the relationship between fume mixtures and internal exposure doses, without interference of other sources influencing the biomarker levels. It is therefore needed to screen for, and semi-quantify, selected/targeted biomarkers in animals exposed to realistic and characterized fumes.

Conclusions

Integrated *in vitro* testing strategies are needed combining a realistic exposure scenario (inhalation exposure) with a relevant readout (neuronal function). *In vitro* (cellular) research allows for detailed studies of toxicity but often lacks important aspects such as metabolism. The proposed combination of *in vitro* cellular research using MEA and ALI, the whole-organism *in vitro* study with zebrafish and an *in vivo* rodent inhalation study with neurobehavioural and biomarker analysis therefore fills several of the gaps identified above. These studies with human relevant model studies might guide decision support on - temperature/pressure conditions and fuel/hydraulic/de-icing fluids used during flights and associated - fume events which are most at risk to human, and which should be considered for future mitigation procedures.

Theme Task 3B: Health risks

Gap analysis

Despite all available information on cabin air quality in relation to chemicals, many data gaps still exist and prevent decisive conclusions on the possible relationship between health-related problems and exposure to chemicals in cabin air of airplanes.

After a first screening of the available literature, the following data gaps are observed:

- Hazard information on many individual chemicals is lacking or incomplete. Information on the potential neurotoxicity of chemicals is often not available or limited, for example for the non-ortho isomers of TCP. Further, long-term toxicity data that take into account accumulation of chemicals and chronic low-dose effects, especially about neurological effects, are not always available.
- No stand-alone standardized test is available to determine the neurotoxicity after long term exposure to a mixture of chemicals
- Toxicity of chemicals and health-based limit values are based on standard conditions. The effect of deviant conditions in cabin air, such as hypoxic conditions, on toxicity of chemicals needs further investigation.
- Exposure information is often limited to a small set of chemicals, mainly TCP. Limited information is available on mixture toxicology and how to address neurological effects after exposure to a mixture of chemicals in a health risk assessment.
- Exposure information is related to standard conditions during normal flights. Measurements during fume events are not or hardly available. Prospective monitoring of cabin air, e.g. for measuring during fume events, is difficult and expensive.
- Information on clinical effects or toxicity and information on exposure are obtained either independently or at the same time, but never in a prospective cohort design. As a result, a causal relationship between the symptoms reported by flight crew and exposure to chemicals has never been demonstrated.
- No suitable biomarkers exist to determine the exposure or mark potential health effects

Conclusions

This descriptive overview provides the baseline information about health risk assessment in the context of cabin air quality identified in the available literature at the consortium partners. Over the years, various studies have investigated, at least partially, the relationship between cabin air quality and its impact on health in flight crew. Although numerous studies have been performed, several data gaps are identified relating to hazard information of specific chemicals, exposure measurement, health effects measurement and study designs. Some of these data gaps are not easy to fill, for instance, the broad range of reported symptoms makes it difficult, if not impossible, to clearly define the so-called aerotoxic syndrome. Exposure to different chemicals, potential mixture toxicology, infrequent fume events and an undefinable syndrome (health effects) leads to too much complicity in an appropriate epidemiological design to establish causality.

Further research needs to be designed carefully in order to start filling the data gaps, including hazard information focussed on neurological effects, mixture toxicology, exposure measurement

during fume events, influence of the cabin conditions in relation to limit values, and identification of suitable biomarkers.

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1 Introduction

1.1 The FACTS project

FreshAircraft (FACTS) is an EU research study funded by the Directorate-General for Mobility and Transport (DG-MOVE). Background of the project are the concerns among the international governments, pilots, cabin crew and passengers and other stakeholders of commercial jet aircrafts about possible health risks associated with reports of the presence of fumes in the air supplied to aircraft cabins. The over-arching objective of the project is to ascertain potential safety and/or long- and short-term health risks resulting from the contamination of bleed air in both routine and cabin/cockpit air contamination (CAC) event, originated incident flight conditions. The FACTS project is organised by the following tasks:

- Task 1. Review of the State of the Art and Establishment of the Baseline for the Work
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- Task 4. Engineering controls- Risk mitigation strategy
- Task 5. Conclusions and recommendations

The main activity for this report consisted of a scan of publicly available literature regarding Cabin Air Quality, including peer-reviewed as well as non-reviewed papers. It was beyond the scope of the project to perform a thorough scientific review. Instead, the scope was to identify the types of research (“what has been done?”) and their main findings. In this respect, the project elaborated on two recent studies (EASA 2017a; EASA 2017b), which included extensive literature reviews.

Besides the literature scan, interviews with subject-matter-experts were carried out to collect information about possible work is not (yet) available in the open literature.

1.2 Task 1: review of the state of the art and establishment of the baseline of the work

The main objective of Task 1 is to perform a review of the state of the art and establishment of the baseline for the work described in tender proposal MOVE/C2/2016-363,’’ Investigation of the quality level of the air inside the cabin of large transport aeroplanes and its health implication’’, This report provides an overview of existing knowledge (“what has been done”) regarding cabin air quality, as well as a gap analysis (“what has not been done”). The results of the gap analysis will be used as a baseline for the technical tasks in the FACTS project. This report describes the results and conclusions from the review and gap analysis.

1.2.1 Task 1: general approach and methodology

The main activity for this report consists of a scan of publicly available literature regarding Cabin Air Quality, including peer-reviewed as well as non-reviewed papers. It was beyond the scope of the project to perform a thorough scientific review. Instead, the scope was to identify the types of research ("what has been done") and their main findings. In this respect, the project elaborated on two recent studies (EASA 2017a; EASA 2017b), which included extensive literature reviews. Task 1 will also include up to date relevant literature, which may be published during the execution of the project. Besides the literature scan, interviews with subject-matter-experts will be carried out during the project, this in order to collect up to date information on cabin air quality related issues.

Based on the available reviews, an analysis has been conducted in order to identify possible gaps, to identify unaddressed issues in literature, which represent opportunities and research needs for the following research themes of this project:

Chemical inventory of potential bleed air contaminants (Task 2)
- experimental laboratory investigations
- In-flight measurement campaigns

Toxicology (Task 3A)
- in vitro neurotoxicity tests (cell line exposure and biochemical assays)
- in vivo neurotoxicity and behavioral tests (animal testing)
- Human biomonitoring and biomarkers

Health risks (Task 3B)
- epidemiological based health risk assessment
- toxicological based health risk assessment

Regarding Task 4- Risk mitigation strategy, a literature review of grey and peer-reviewed literature as well as interviews with subject-matter-experts will be executed during the project. The results will be presented in a separate Deliverable (intermediate report D6). Reason for this, is that gathering information on this subject is more time consuming compared to the reviews of Task 2 and 3 and therefore the outcome of this review will be included as an integral part of Task 4,''Risk mitigation strategies''.

2 Theme: Task 2- Exposure monitoring, identification of causes of bleed air contamination and assessment of impact on quality of cockpit/cabin air

2.1 Description of theme

The aim of this theme is, to gather information from previous literature reviews in order to define state of the art and certain gaps in knowledge on:

- Laboratory research of engine oil characterization and measurements.
- In-flight measurement campaigns.

The literature review is intended to get a better understanding of what has been done yet in the field of chemical composition and concentration of bleed air contaminants and their impact on cabin and cockpit air quality and more important to identify potential needs for research for further improvement of knowledge in air quality monitoring.

The advantage of experimental lab studies is that measurements are conducted under controlled conditions whereby parameters easily can be adjusted. These experiments give opportunities to look into depth to composition, formation and degradation of chemical substances in oil and oil vapors. The disadvantage of experimental lab studies is how to make the translation of laboratory environment to real circumstances in an aircraft for flight idle conditions, during taxi and climb or descent operations.

Because of the large variation in chemical and physical properties of the chemical substances, it is not always clear if emissions of certain substances found in laboratory experiments will ever end up in the cabin environment. In other words, it is not fully understand which processes in the entire air supply system influences the chemical emissions starting from the LP and HP sections via the ECS packs-and ducts to the cabin aircraft directly or by recirculation of the air.

The advantage of conducting in flight cabin air quality measurements is that assessment of chemical substances takes place directly in the environment where passengers, pilots and cabin crew may be exposed to contaminants. Disadvantage is that seeking correlation of engine bleed air conditions and chemical substances found in cabin air quality in relation to fume events or normal engine flight conditions remains difficult. Even more difficult is the approach in finding a proper official definition for CAC events or accepted air quality levels for aircraft cabins.
For the baseline assessment both laboratory and in flight measurements will be taken into account.

2.2 Baseline (What has been done)

2.2.1 Laboratory research of engine oil characterization and measurements

The available literature (grey-and peer reviewed) provide an overview of laboratory studies of chemical components, pyrolysis products and gases found in engine oil. The overview is quite extensive and therefore the most important studies are summarized in table 1 of Appendix A.

The early laboratory research on engine oils focused mostly on volatile organic compounds (VOCs), aldehydes-ketones, and gases (CO, CO_2, Ozone). At a later stage research was focused more and more on organophosphates which are used as additives in most engine- and hydraulic oils, wherein Tricresyl phosphates (TCPs) plays an important role because of their neurotoxic properties. In most recent studies only four out of 10 isomers of TCPs are detected in engine oil itself and in oil vapor namely, T(m,m,m)CP, T(m,m,p)CP, T(m,p,p)CP and T(p,p,p)CP. In most studies T(o,o,o)CP is rarely found or below detection limits of the applied analytical method.

Many chemical surveys in oil and oil vapor were conducted whereby a broad spectrum of chemical substances and pyrolysis products were identified. These studies include the chemical analysis in oil liquids and vapors, resulting in identification and quantification of volatile organic compounds, aldehydes, organophosphates, CO, CO_2 and various pyrolysis products.
Chemical substances, which are found in engine oil vapor, can be systematically classified in the following groups: alkanes, alkenes, amines, esters, ethers, fatty acids, alcohols, aromatic hydrocarbons, polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, terpenes, aldehydes, ketones, and organo phosphates (triaryl and trialkyl phosphates). It is obvious that the variety of chemical substances in (pyrolysed) oil is a complex mixture. It is hardly impossible to measure all substances as a standard approach for assessing cabin air quality and therefore it is important to make choices in pointing out well-defined marker substances to measure. In two studies, suggestions were made to use tricresyl phosphates and specific aldehydes-and VOCs as good markers for assessing cabin air quality.

Typical pyrolysis products like trimethylolpropane phosphate (TMPP) were found in several investigations as a byproduct of the reaction of TCP and trimethylolpropane at high temperatures of 440 °C or higher. TMPP was mainly found in the scapings of the inner walls of the ducts or in condensate. It is mentioned unlikely that TMPP, because of the low vapor pressure, will end up in the cockpit and cabin. Pyrolysis of triarylphosphates leads to the formation of phosphabenzene, isophosphinoline and phosphinoline. On basis of one study, it was suggested that lubricant based of fatty acid methyl-ethyl esters produces less hazardous by-products than triaryl-phosphates ones exposed to thermally pyrolytic conditions.

Temperatures and pressure of jet engines during operations are variable but under extreme temperature and pressure conditions there is a potential to alter the composition of the original oil during use, and may create degradation products like alkylated cresyl phosphates (xylenyl-and ethylphenyl phosphates). Other studies mentioned the reaction of alkenes and monoterpenes with ozone resulting in the formation of formaldehyde.

Carbon monoxide is frequently found in oil vapor as a result of the combustion process.
Laboratory studies were carried out for different temperature conditions (range from 250-600°C) at normal atmospheric pressure conditions without the presence of ozone, which is a normal constituent of the atmospheric and tropospheric air.

Important to mention is the fact that laboratory conditions are difficult to extrapolate to typical real aircraft engine environment conditions simply due to the absence of a real engine and ECS. Therefore, it is difficult to predict how chemical substances found in laboratory experiments will chemically act during the route between engine leak-bleed air system-ducts to cockpit/cabin in terms of concentration and occurrence. Only one serious research investigation (VIPR study) has been recently carried out in order to simulate a rare engine upset failure condition on a real time operating aircraft. It was demonstrated that during a simulated rare engine upset condition, oil and associated chemical products such as TCP isomers may be present in the bleed air system of the affected aircraft. Although contaminants were detected during the evaluation, the concentrations observed were well below levels known to be hazardous to human health, as noted by the OSHA and ACGIH standards.

Hardly any (peer-reviewed or grey) literature regarding experimental laboratory studies on hydraulic oil and de-icing fluids was found. One study from Rosenberger (2014) mentioned concentrations in the cabin air up to 2.5 mg/m^3 of propylene glycol and 1.5 mg/m^3 1-hydroxyacetone, which is a possible decomposition product of propylene glycol.

2.2.2 Initiatives for European standardization

CEN TC 436,''Cabin air quality on commercial aircraft
Within the European standardisation organisation CEN, a Technical Committee TC436 was established in 2014 dealing with the cabin air quality on commercial aircraft. The scope of the TC defines requirements and recommendations dealing with the quality of the cabin air on civil aircraft concerning chemical agents potentially originating from but not limited to the ventilation air supplied to the cabin and flight deck. A special emphasis is on the bleed air contaminants potentially brought into the cabin. It covers civil aircraft in operation from the period that is defined as when the first person boards the aircraft until the last person leaves the aircraft. It will in particular focus on the presence of and means to prevent exposure to chemical agents including those that could cause adverse effects taking into account the Precautionary Principle'. Within TC436, the following four Task groups were established:

- TG1: Identify suitable chemical agent marker compounds and regulatory requirements on hazards and safety
- TG2: Identification of sampling and measurement methods
- TG3: Sensors and training
- TG4: Exposure control measures'

The publication of the European standard is expected in 2019.

ISO TC146,''Air Quality
ISO TC 146,''Air Quality''', subcommittee 6 (Indoor air) working group 18 developed an ISO standard 16000-31:2014,'' Measurements of flame retardants and plasticizers based on organophosphorous compounds-Phosphoric acid ester'', the version is suitable for measuring chlorinated and non chlorinated trialkyl and triaryl phoshates in indoor air. At present, the standard is under revision. The new version will incorporate the sampling and analysis of different tricresyl phosphate isomers, which is of importance for applying this standard for aviation use.
International interlaboratory studies on sampling and analysis of TCPs are lacking, resulting in poor validation data of method performance characteristics.

2.2.3 In-flight measurements

In the past decades, numerous in-flight measurements have been conducted for different type of aircraft and different type of chemical substances. Table 1 of Appendix B, provides a brief overview of in-flight measurements carried out from 1988 until 2017. Taken into account the major in-flight measurement campaigns conducted since 2011, it is clear that predominantly organophosphates is the most important target compounds measured in the air of aircraft cabin. The major in-flight measurement campaigns were conducted in the period 2011-2017.

Crump et al. (2011) analysed in the so-called Cranfield study aircraft 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 sampling. Besides total VOC and ultrafine particles numbers, the following target compounds were included in this study: ooo-TCP, the other TCP isomers, TnBP, toluene, m- and p-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 VOC 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 or ooo-TCP was detected. Highest ooo-TCP level of 22.8 µg/m^3 was observed during climb of a B757 (overall mean 0.07 µg/m^3) – with no event being reported. The overall mean total TCP level was 0.14 µg/m^3 (with a maximum of 28.5 µg/m^3). The highest TnBP level 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 events did not contain elevated levels of any of the target compounds included in this study.
(Comment: The suitability of the sampling (duration, adsorbent) and analysis method (TD-GC-MS) applied for TCP determination was questioned as in the flight phases before and after no ToCP was detected).

Denola et al. (2011) monitored TCP in cockpit and cabin air of three different aircraft types from Australian Defence Force (ADF): fighter trainer, cargo transporter and fighter bomber, in total 46 aircraft. Long duration air sampling was performed with 0.06 g of Porapak-Q glass tube at 2 L/min. Short term air sampling was performed with metricel filters (GN; 0.8 µm) at 36 L/min. Mono, di or tri(o,o,o)-cresyl phosphate were below LOD in all collected samples. mmm, mmp, mpp and ppp TCP isomers were detected 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 ranging 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. During the flight with the highest concentration of 51.3 µg/m^3 observed smoke and odour was reported. However, the report of smoke and odour did not necessarily correlate with TCP concentrations in other incidents. In two other flights smoke was reported, however no TCP was detected above the LOQ. Results of this study indicate a low health risk from TCP exposure. Other peaks were present in the gas chromatogram with similar retention times of TiBP, TnBP and TPhP.

Solbu (Thesis 2011, recapping four previous papers) developed a sampling method and measured the exposure to organophosphates (OPs) from jet engine oils and hydraulic fluids among aircrew members. 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. Four airline companies in Norway were included in this study. In total 40 aircraft were sampled consisting of jet engine airplanes, propeller airplanes and helicopters. The samples were collected in the cockpit and the passenger cabin. 95 within-day OP samples were collected from cabin air in 47 flights (jet airplanes, propeller airplanes and helicopters). TCP (sum of tri(m,m,m,)-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 ranging 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 at 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 ranging from 24 to 4100 ng/m^3. BDPP was only detected in the jet engine and propeller airplanes with levels ranging 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). 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. 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 six HEPA filters from the jet engine airplanes TCP was detected with levels ranging from 1.1 to 4.3 ng/g per hour. Again, no ortho-isomer of TCP was detected. 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 (5.1 µg/m³) compared to after engine replacement. Ortho-isomers of TCP were not detected in any of the samples collected in this study. Wipe sampling 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 to lower LOQs and higher extraction recovery. There was no difference in concentration observed between sampling the cockpit versus the passenger cabin.

Spengler et al. (2012) monitored aircraft cabin air of 83 commercial flights in two Airbus and four Boeing models on U.S. domestic routes or on international routes. Temperature, relative humidity, cabin pressure and sound levels as well as CO, CO2, O3, VOCs, SVOCs, particles and TCP were investigated. The five carbonyls formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde were also determined, but not on all flights. Acetaldehyde was found in 81% of 70 samples, acetone in 79%, acrolein in 71%, formaldehyde in 49% and propionaldehyde in only 17% of the samples. Concentrations of many VOCs detected were lower in the cabin than what is typically reported for offices and residences. TCP was detected in only one of 71 samples at 0.1 ppt. ToCP was not detected in any of the samples.

Houtzager et al. (2013) investigated 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 four B737-700s, three B737-800s, three B737-900s and from two B737-700s and 800s 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 flights. 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.

Rosenberger et al. (2013) investigated the presence of TCP in cabin air of two airplanes. 90 air samples were collected during 26 flights 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) at 2 L/min. The total TCP concentration in the air samples ranged from 17 to 167 ng/m^3. In 15% of the samples o,o,o-TCP was detected with levels ranging from 2 to 65 ng/m^3. This was during normal flight conditions. 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. In the engine oils (Mobil jet oil II) the ortho-TCP levels were < 20 µg/kg.

Rosenberger et al. (2014) also investigated organic compounds in cabin air after de-icing since some odour complaints and health impairments occurred on a particular aircraft. Since measurement data from that respective flight was not available, the de-icing procedure was repeated with that aircraft, followed by test flights (without passengers) and measurement equipment being on-board. VOCs and organo phosphates were determined. Up to 2.5 mg/m³ of propylene glycol was found in the cabin during test flights after de-icing, also 1-hydroxyacetone, a possible degradation product, was detected at 1.5 mg/m³. Significant contamination with organophosphates was not observed. TCP levels detected were below 0.17 µg/m³, ortho-isomers were not present. Tetrachloroethylene was found on the control flight before de-icing at levels up to 230 µg/m³ and assigned to previous dry cleaning.

Guan et al. (2014a and b) conducted in-flight measurements on 107 commercial flights between August 2010 and August 2012 on domestic China and some international routes on several different aircraft types focussing on VOCs, species, levels, influencing factors and sources. In total 346 VOCs were detected, 59 of which in each flight. These were 41% alkanes and alkenes, 15% esters and alcohols, 11% ketones and aldehydes, 6% halides, 20% aromatics and 6% other VOCs. Occurrence of tetrachloroethene was assigned to dry cleaning of fabrics, 1,4-dichloro-benzene to e. g. disinfectant, deodorant or lavatories. Many VOCs such as benzene, toluene, xylene, ketones and aldehydes (e.g. acetone), esters and alcohols and alkanes and alkenes are present in aircraft cabins – and in buildings. 6-Methyl-5-hepten-2-one likely originates from the reaction of ozone with unsaturated hydrogen compounds and was only found in aircraft cabins. Most VOCs concentrations varied in the three different flight phases investigated, peaking before take-off or during cruise.

Wang et al. (2014) investigated the presence of VOCs in cabin air of aircraft to identify possible emission sources. 84 air samples were collected on Tenax-TA tubes during 14 flight on a B737-800. Flight duration ranged from 80 to 190 minutes. Samples of 1000 ml at 200 ml/min were drawn minimum during 5 flight phases. In total 19 VOCs were detected in air samples collected during the 14 China domestic flights. Highest levels were found for d-limonene (median of 31 µg/m^3), followed by decanal (median of 24 µg/m^3), nonanal (18 µg/m^3), toluene (13 µg/m^3), acetic acid (11 µg/m^3), benzene (10 µg/m^3) and 6-methyl-5-hepten-2-one (9 µg/m³). A receptor model using positive matrix factorization coupled with information related to VOC sources was applied to identify the major sources in the cabin. 29% of the total VOC emission was attributed to services or humans, followed by chemical reactions (15%), fuels (13%), materials (12%), combustion (12%), non-fuel oil (9%), cosmetics and perfumes (5%), and cleaning agents (4%). Non-fuel oil contributed to 69% of total benzene concentration, to 20% of acetone concentration, to 11% of acetic acid concentration and to 10 % of octanal concentration.

Guan et al. (2015) used CO2 and TVOC data of 6 flights performed in domestic China in 2013 to model and estimate net in-cabin emission rates of VOCs and contributions from outside and inside the aircraft cabin. TVOC concentrations were measured during different flight phases and were mostly in the range of 0.20 to 0.40 mg/m³. Based on CO2 and ventilation information air ratios and

air flows were calculated. Results indicated that during cruise phase TVOC in cabin air came mostly from cabin interiors (90%). Contributions from outside became more significant during taxiing on ground, ascent and descent phases

In 2016 Rosenberger et al. published measurement data on 14 airborne aldehydes in cabin air of commercial aircraft determined by HPLC with UV absorbance detection of 2,4-dinitrophenyl-hydrazones sampled in-flight during normal operation and reported smell events. 353 samples were drawn in the flight deck of 26 aircraft (11 A380 and 15 A321) on 108 flights (64 on A380 and 44 on A321) and analysed. LOD levels of the method were between 0.3 and 0.6 µg/m³. No unusual or noticeable aldehyde pollution was observed. 15 smell events were reported by crew members on the 64 A380 flights, however measurement data from phases with smell events did not differ from phases without smell event. Besides very low concentrations of most aldehydes, mainly formaldehyde at levels between 0.4 and 44 µg/m³ (median 4.9 µg/m³) and acetaldehyde between 0.3 and 90 µg/m³ (median 4.6 µg/m³), were detected. Aldehyde concentrations in the cabin are similar or even lower than in other indoor environment such as schools or dwellings. The influence of ozone was investigated by sampling with and without an ozone absorption unit before the trapping cartridge and found to be negligible.

Shehadi et al. (2016) used databases from the Federal Aviation Administration (FAA), NASA, and other sources to determine the frequency of bleed air contamination incidents. The frequency was examined based on aircraft models currently in service by major US airlines and normalized by the number of aircraft, number of flights, and flight hours for each model to account for the large variations in the number of aircraft of di□erent models. Incidents examined included those related to smoke, oil odors, fumes, and any symptom that might be related to exposure to such contamination, reported by crew members of US-based carriers between 2007 and 2012. In addition, authors tried to identify propulsion engines and auxiliary power units associated with aircrafts that had higher frequencies of incidents. Results were thought to be useful for future monitoring studies on eye-catching aircraft/engines and the low statistical probability to encounter a contamination event. Substantial variations were found in the frequency of incidents and contamination events were widely distributed across nearly all common models of aircraft. The average incident frequency was 2.1 per 10000 flights, the highest for one aircraft model was 7.8 per 10000 flights. Consequently, for capturing a meaningful number of incidents for characterisation tens of thousands of flights would have to be monitored.

Schuchardt et al. (2017a and b) performed measurements on 69 commercial flights, 8 of which on B787 in 2015 and 2016. Samples were taken at defined flight phases (taxi-out, take off and climb, descent and landing, complete flight), while climate data, total volatile organic compounds, carbon dioxide, carbon monoxide and ozone were recorded continuously. Total volatile organic compounds (VOC) concentrations ranged from 0.024 – 2.1 mg/m³ and 0.012-0.489 mg/m³ on B787. Low amounts of formaldehyde (range 0.03-48 µg/m³ and 0.02 - 17 µg/m³ on B787), acetaldehyde (range 0.02-42 µg/m³ and 0.01- 15 µg/m³ on B787) and other aldehydes mostly at trace levels were detected. Only traces of meta- and para-TCP-isomers were detected, up to 1.515

µg/m³ and up to 0.403 µg/m³ on B787. No ortho isomers were detected. The most prominent airborne organophosphorous compounds were tri-n-butyl phosphate (TBP) which amounted from 0.037 to 2.484 µg/m³ and in the B787 from 0.037 to 1.482 µg/m³, and tris(chloro-isopropyl)phosphate, which amounted from 0.023 to 9.977 µg/m³ and from 0.041 to 2.633 µg/m³ on B787. Other organo phosphates were detected in trace amounts in most samples.
Concentration levels found were similar or even lower compared to findings of other indoor environments such as offices, schools, kinder gardens or dwellings because of the high air exchange rate required in the cabin, which leads to a quick dilution of contaminant concentrations ("thinning effect").
Previously described reaction products of VOCs with ozone or oil pyrolysis products such as certain alkanoic acids or increased concentrations of n-alkanes were not detected. Occasional increased levels of propylene glycol in cabin air in winter were explained by the use of de-icing fluid. Few VOCs may be assigned to individual passenger actions, in-flight service or use of moth balls. Occurrence of an unknown fraction of iso-alkanes (at non-critical concentrations) especially during the take-off has caught the attention of the researchers. With regard to occupational exposure limits or indoor air guideline values the cabin air monitored in this study were of good quality and no object of any concerns. No single detected contamination reached critical or unusual concentrations for indoor environments.
Results are consistent with findings of other previously performed in-flight/on-board measurement campaigns. For the first time in-flight measurements on Boeing 787 aircraft have been published. TCP has been proven suitable as a marker for engine oil contamination in bleed air – provided that a sensitive analytical method is used. TCP could also be detected on the non-bleed air operated B787 and suggest ubiquitous cabin air contamination in all aircraft types. Randomly occurring higher TCP levels (with no reported CAC event) may be assigned to secondary contamination by e. g. oil deposits in the bleed air or air conditioning system, which may also be responsible for reported smell events, caused by non-toxic odourous compounds release.

2.3 GAP analysis

Both laboratory and in-flight measurements have greatly contributed in gathering knowledge about chemical composition and degradation of chemical substances, respectively compositions of applied engine oil, and oil vapors, and the impact of the emissions of bleed air contaminants on the cabin air quality. Knowledge should be brought on the level of trying to understand one distinct issue: cabin/cockpit air contamination (CAC) due to potential leaks, which results in short-term peaks of cockpit/cabin air contamination. The intrinsic quality of the cockpit/cabin air in normal flight operating conditions have been subject for many in-flight monitoring campaigns covering a good general view of the air quality on board of aircraft. More important is to investigate possibilities with respect to air quality management and air quality monitoring (marker components to be measured) for aircraft in order to gain a good assessment of cabin air quality. After the review of the available literature, the following data gaps are observed:

- Chemical mapping in-between LP and HP section and cockpit/cabin is lacking. There is currently not enough knowledge about what happens underway with certain uncontrolled chemical emissions entering the cockpit/cabin.
- There is little knowledge about the chemical nature and character of a CAC event.

- There is a need for a chemical survey of the surface contamination in the PACKs and ducts as well as of the filter material used in the recirculation air system;
- International inter-laboratory studies on sampling and analysis of TCPs are lacking, resulting in poor validation data on method performance characteristics. Data comparison for TCPs is difficult to make between monitoring studies
- Most of the in-flight measurement campaigns differed in experimental design, analytical approaches and the way of expressing results statistically. This makes it difficult to make comparisons. Analytical standardisation processes are needed in order to harmonize air quality monitoring.
- It is not fully understood which processes in the entire air supply system influence the chemical emissions starting from the LP and HP sections via the ECS packs-and ducts to the cabin aircraft
- It is unclear what components are possibly formed during the route from source to flight deck and passenger cabin.
- It is not clear what are suitable marker components s for judgment of cabin air quality
- Spraying aircraft with de-icing agents and the effects on cabin air quality is described as a serious contaminant. Yet, few measurement campaigns for de-icing have been carried out. Many cases were reported of glycols are entering cabin, but poor data has been collected about this phenomena.

2.4 Conclusions

Continuation of air quality monitoring projects in-flight is not considered as constructive since encountering a real CAC-event is very unlikely considering the low number of engine oil triggered incidents. In order to answer some of the questions raised above, it seems useful to use appropriate simulations. Simulation of bleed air contamination on ground in real aircraft cabin air environment and during test flights in real aircraft may shed light on this gap of knowledge. Important is to investigate the black box which consists of ECS packs ducting and HEPA filters in order to map possible accumulation of bleed air contaminants in the air system.

2.5 Overview of considered literature

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3 Theme: Task 3A- toxicology and human biomonitoring

3.1 Description of theme

The complexity of the mixture of contaminants present in fumes, at relatively low concentration pose a challenge for toxicological testing and also human biomonitoring. The current hypothesis assumes acute, chronic or delayed neurotoxicity of cabin fumes. Since fume events are short, there is not that much information on the actual concentration of engine compounds (oils, hydraulic fluids) or combustion products after such events. *In vitro* and *in vivo* studies were done in previous studies to assess the hazard of the air contaminants. Human biomonitoring focuses on biomarkers of exposure to these compounds, as well as nervous system damage.

3.2 Baseline (''What has been done'')

Several test systems were used for hazard assessment of cabin air contaminants. The individual compounds present in engine oil, include organophosphorous (OP) compounds, phenyl naphthylamine (PAN), acrolein, amines, carboxylic acid, carbon monoxide, polycyclic aromatic hydrocarbons (PAH), formaldehyde, toluene, and xylene and are known toxic compounds, that are individually tested and listed in toxicity databases, such as HSDB[1], INCHEM[2]. Some of the compounds need to be metabolised to generate the toxic metabolite (e.g. TCPs, PAHs).
Those compounds occur as a mixture in cabin air during fume events, but the profile of compounds present either a parent or metabolite, and their concentration can differ as a function of e.g. temperature, pressure, type of engine oil. Some of the fume contaminants (OPs, solvents) have neurotoxic properties, but also chemicals that may be formed by combining compounds in the mixture are of concern (e.g. neurotoxin trimethylolpropane phosphate (TMPP) formed from TCP and trimethylporopane ester at high temperatures) (Harrison and Ross, 2016; Megson et al, 2016). Also, the neurotoxicity of certain PAHs, such as benzo[a]pyrene (see e.g. Chepelev et al, 2015 for a brief review), should be taken into account.

3.2.1 In vitro neurotoxicity tests (cell line exposure and biochemical assays)

In vitro assays (both cell based- and cell-free assays) have been used or proposed as valuable tools for (pre)screening of oils, anti-wear additives, de-icing fluids.

Neuronal network test systems
The neurotoxic potential of non-ortho tricresylphosphate (TCP) isomers, TCP mixtures, and the metabolite of tri-ortho-cresyl phosphate (ToCP)(CBDP: 2-(o-cresyl)-4H-1,3,2-benzodioxaphosphoran-2-one) was tested up to 48h in primary rat cortical neurons for effects on cell viability, mitochondrial activity, spontaneous electrical activity and neurite outgrowth. These combined *in vitro* neurotoxicity data showed that the different TCPs were roughly equipotent, with no-observed-effect concentrations (1µM) well above estimated systemic levels, based on recent measurements of cabin air concentrations of TCPs (Duarte et al., 2017).

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[1] *HSDB: Hazardous Substances Database (https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm)*
[2] *INCHEM : International Program on Chemical Safety, Chemical Safety Information from Intergovernmental Organizations as IPCS, WHO, CCOHS (http://www.inchem.org/)*

Recently, potential neurotoxicity of turbine engine oil pyrolysis products has been tested in an *in vitro* set up combining exposure of an *in vitro* model of the human lung via the air in an Air Liquid Interface (ALI) and subsequent analysis of the ALI effluent on primary rat cortical neurons mounted on a multi-electrode array (MEA system). Testing of the neuronal network function in the MEA system allows for evaluation of toxicity in an integrated spontaneously active neuronal network. The presence of TCP isomers was confirmed although the ortho isomer was not detected. The study concluded that neuroactive products were present in pyrolysis products of aviation engine oils at relatively low exposure levels. However, the presence of an intact lung barrier effectively reduced the toxicity level in the MEA system (EASA, 2017).

Biochemical assays

Baker et al. (2013) performed an *in vitro* assay on triaryl organophosphate anti-wear lubricant additives (TAPs). The assay involved TAP bioactivation with liver microsomes and NADPH, followed by incubation with human butylcholine esterase (BChE) and measurement of BChE activity[3]. Of 19 TAPs tested, tert-butylated isomers produced the least BChE inhibition. The metabolite of ToCP, CBDP, inhibited a recombinant catalytic domain of the neuropathy target esterase (NTE) (rNEST) *in vitro*, but with an IC_{50} value almost 6-times higher than for inhibition of BChE. Makhaeva et al. (2016a, 2016b) presented *in vitro* compared to *in vivo* studies on biochemical measurements of serine esterases (acetylcholinesterase(AChE); BChE; carboxylesterase (CaE) and NTE) in mice brain homogenates and blood samples after exposure to OP compounds. Except for brain CaE, a good correlation was observed between *in vitro* and *in vivo* measures of potency of 2 OP compounds (Makhaeva et al., 2016b). Furthermore, a good agreement between brain and blood enzyme sensitivity was seen for OP compounds (o,o-di-1-propyl-o-2,2-dichlorvinyl phosphate, PrDChVP and o,o-di-1-butyl-o-(1-trifluoromethyl-2,2,2-trifluoroethyl) phosphate, diBu-PFP). Therefore, the mouse blood NTE was considered a biochemical marker of exposure to neuropathic OP compounds. It was suggested that relative inhibition of blood NTE and AChE by OP compounds, allow prediction of cholinergic and/or delayed neuropathic effects (Makhaeva et al., 2016a).

3.2.2 In vivo neurotoxicity and behavioral tests (animal testing)

Rodent studies

Rodent studies have been performed in which plasma AchE activity was shown to be targeted by OP compounds (Duysen and Lockridge 2011). However, reports on rodent tests of additives are limited, and studies assessing the effects of engine oils and let alone fumes of engine oils are hardly available.

Baker et al. (2013) showed that inhibition of BChE by bioactivated anti-wear lubricant additives (TAPs) *in vitro* correlated well with inhibition of other serine active-site enzymes *in vivo* in mice. In contrast to the cell-free *in vitro* enzyme assays (see above), no inhibition of brain AChE and/or NTE was observed in mice following single exposure to those TAPs.

Other animals

A number of toxicity studies have been performed using hens as an experimental animal. These studies demonstrated the absence of an inter-species difference in sensitivity between hen and

[3] Acteylcholinesterase (AChE) enzyme is present in human red blood cells, nervous tissues and skeletal muscles. Butyrylcholinesterase (BuChE) is synthesized in the liver and is present in serum.

human with regard to doses of TCP necessary to produce organophosphorus-induced delayed neurotoxicity (OPIDN) (Mackerer et al., 1999). This potentially severe toxicological syndrome, (described in humans, cat, hen after exposure to TCP mixtures), is characterized by a 7-to 28-d delay period prior to the onset of clinical signs, such as lack of voluntary coordination of muscle movements (ataxia), reduced muscle tone (flaccid paralysis), and paralysis (Mackerer et al. 1999). In the review of Abou-Donia it was reported that subclinical doses produce apoptotic neuronal cell death and involve oxidative stress (2003). Subcutaneous exposure to OP compounds was demonstrated to cause inhibition of neurotoxic esterases and OPIDN in hens (e.g. Carrer and Abou-Donai, 1988).
Mackerer reported (1999) that jet engine oil containing 3% TCPs caused a non-significant increase in brain- NTE in the rat and hen (with the hen being more sensitive). Subchronic (10 weeks) exposure to this oil (but not to jet engine oil with 1% TCP) at 2g/kg/day, resulted in the development of OPIDN. Winder and Balouet (2002), referred to a short-term repeated-dose study in hens, and also other studies done in the nineties, suggesting that engine oil containing 1% TCP, i.e. TCP equivalent of 20 mg/kg/day, was considered a no-observable effect level.

Zebrafish embryo/larvae: an alternative model to screen neurological effects
The high degree of genetic conservation among vertebrates (Howe et al., 2013) and their similar morphological and molecular basis of tissue and organ development shared with other vertebrates, including humans, makes zebrafish an excellent model organism for studying complex biological processes (Garcia et al., 2016; MacRae & Peterson, 2015).
The zebrafish embryo-larvae model is according to EU legislation an ethical accepted non-animal alternative when studied within 5 days post fertilisation (dpf) and practical advantages such as daily production of hundreds of fertilised eggs and the transparent nature of the embryo/larva, which facilitate the ability to screen this whole organism in a microwell plate format while using small amount of compounds. The added value of zebrafish as an alternative, compared to cell culture models, is its **metabolic competence** because liver morphogenesis is completed by 2 dpf and fully functioning by 3dpf with presence of homologs of mammalian lipid metabolizing enzymes (Isogai et al., 2001; Chng et al., 2012).

Zebrafish studies on neurodevelopmental disorders and neurodegenerative diseases have provided insight into cellular, circuit and behavioural level mechanisms (Kozol et al., 2016) and numerous methods to study motor activity in response to toxicant exposure exist (Legradi et al., 2015). A few studies on acute organophosphate exposure, more specific pesticides, with the zebrafish embryo and larvae model have shown significant effects on motor activity, AChE inhibition and axon degeneration (Jacobson et al., 2010; Selderslaghs et al., 2010; 2013; Witters et al., in preparation).

For a number of specific OPs which are known to occur in cabin air after fume events, e.g. tricresyl phosphate, a mixture of para, meta, and ortho isomers (TCP, cas 1330-78-5), tri-o-cresyl phosphate (ToCP)) and tributyl phosphate (TBP)) neurodevelopmental studies including behavioural analysis with zebrafish embryo/larvae are reported (Behl et al. 2015; Jarema et al., 2015; Noyes et al., 2015). TCP exposure was shown to decrease motor activity in the µM range (Jarema et al., 2015; Noyes et al. 2015). Even though ToCP is a component of TCP mixture, the developmental and acute behavioral effects were distinct with ToCP giving result into a narrow range of behavioural changes and embryotoxic effects (Jarema et al., 2015). Exposure to TBP resulted into hypo activity in 5dpf larvae (Noyes et al., 2015). These studies do not report analysis of biomarkers in the zebrafish.

3.2.3 Human biomonitoring and biomarkers

The observed health effects seen in studies on flight crew members are central nervous system dysfunction, and symptoms related to gastrointestinal, respiratory, dermal and possibly the immune system. The advantage of human biomonitoring is certainly its potential to assess exposure assessment of fume events, after such an event. The biomarkers that are used for human biomonitoring of flight crew members or technical personnel exposed to fuels and oils are either exposure or biological effective dose markers.

Exposure markers

Urinary metabolites
Schindler et al. (2013, 2014) analysed urinary OP-metabolites, and reported a slight occupational exposure to TBP, TPP (both potentially originating from hydraulic fluids), TCEP (emission from plastics, foams of seats), but not to TCP (present in oils) in 332 pilots and crew members. Besides TCP, the cabin air may contain a number of other organophosphates like TBP and TPP, for example, from hydraulic fluids (Solbu et al. 2011) as well as TCEP or TCPP (tris-(2-chloropropyl) phosphate) being released from foams of seats and other plastics.
With regard to urinary PAH metabolites, a lot of data have been published within the framework of tobacco-smoke related biomonitoring (see, e.g. Yuan et al, 2014 for a review).

Protein Adducts
Most of the biomonitoring work related to persistent biomarkers (e.g., protein adducts) of aerotoxic syndrome has been carried out on TCPs present in the lubricant oil, mainly because of the fact that the ToCP is metabolized *in vivo* to the toxic CBDP, a potential inhibitor of enzymes involved in neurotransmission. Bioactivation of ToCP to CBDP by human P450's has been studied in detail by Reinen et al. (2015). CBDP is an inhibitor of various esterases, including BChE, able to form covalent adducts which are expected to be persistent in vivo (average half-life time of native BChE *in vivo* is 10 days, with BChE adducts presumably having a similar half-life time). CBDP has also been shown to bind to albumin (Schopfer et al., 2010; average half-life time of native albumin *in vivo* is 20 days, with albumin adducts presumably having a similar half-life time).
It should be stressed that adduct formation to BChE does not explain the toxicity of CBDP (see Schopfer and Lockridge, 2012 and Marsillach et al., 2013 for a general overview of organophosphate adduct analysis). However, adduct formation to this protein is indicative for potential binding to more toxicologically relevant proteins, such as various serine hydrolases, tubulin, and NTE. Inhibition of NTE by CBDP has recently been advocated to be the most relevant mode of action with regard to ToCP-induced neurotoxicity (Zhu et al., 2016; Hausherr et al., 2017). The possible role of NTE inhibition in aerotoxic syndrome is to some extent supported by clinical measurements in patients (Heutelbeck et al., 2016).

Schopfer et al. (2010, 2014) and Johnson et al. (2015) developed an assay for quantitation of CBDP adducts to BChE in human serum, based on uHPLC-MS/MS analysis. In addition, Schopfer et al. (2010) developed an assay based on LC-tandem MS analysis of the o-cresyl phosphate adduct of CBDP to the Tyrosine-411 residue in albumin. Liyasova et al (2012) demonstrated that CBDP forms covalent adducts with histidine, lysine and tyrosine residues in human serum albumin. Marsillach et al (2011) developed assays for mass spectrometric analysis of CBDP adducts to BuChE and red cell acylpeptide hydrolase (APH).

In this respect, it is interesting to note that the levels of the toxic ortho-substituted TCP isomers are usually extremely low, as recently exemplified for Mobil Jet Oil II (Megson et al., 2016). The same authors, however, identified a number of alkylated cresylphosphates formed in the engine at elevated temperatures which might display similar toxicity as ToCP and which according to them should be on a shortlist of compounds of interest for monitoring purposes.

Application of adduct-based assays to real samples
Tacal & Schopfer (2014) demonstrated that BChE-CBDP adducts could not be identified in blood samples of healthy F-16 pilots, indicating non-exposure to ToCP. Liyasova et al. (2011) showed that jet airplane passengers had detectable amounts of the CBDP-BuChE adduct, indicating a very low exposure to ToCP. However, these subjects did not display toxic symptoms.

Biological effective dose markers
Biological effective dose monitoring covers monitoring at the subcellular level, such as for example changes in enzymatic activity, antibody formation, expression of genes, oxidative stress markers, and DNA damage. These markers are useful to study OP exposure (Kapka-Skrzypczak et al., 2011). Gene expression changes in e.g. oxidative stress, immune response, vascular cell adhesion molecule related genes are interesting targets studied in the context of air pollution/traffic and fume exposure (Hemmingsen et al., 2011; Totlandsdal et al., 2010). In the context of aviation engine oil and fume exposure, so far, only little biological effect markers were studied.

Autoantibodies against proteins associated with degeneration of nerve cells were increased in flight crew members compared to controls (Abou-Donia et al.2013).

Changes in activity of enzymes are used as indicators for exposure to neurotoxic compounds. Organophosphorus compounds have three distinct neurotoxic actions. The primary action is the irreversible inhibition of AChE, resulting in the accumulation of acetylcholine and subsequent overstimulation of the nicotinic and muscarinic acetylcholine receptors, resulting in cholinergic effects. *AChE* (that regulate neurotransmitter concentrations) *and NTE* (an enzyme needed during neuronal differentiation) were measured in flight crew members as potential biomarkers for effects of OP. Schopfer et al. (2014) demonstrated inhibition of BChE in human plasma, as a result of covalent adduct formation of CBDP, the metabolite formed after ToCP exposure. Heutelbeck et al. (2016) reported on a likely inhibition of NTE activities (5 days after the alleged exposure) in crew members displaying symptoms of intoxication after experiencing a fume event, which might be caused by ToCP metabolites. These findings warrant further investigations.

3.3 Gap analysis

The central question is whether exposure to neurotoxic substances formed during fume events could be the cause of neuronal damage as observed in cases of so-called aerotoxic syndrome. One of the main problems in the risk assessment of cabin air quality is the lack of neurotoxicity hazard data for the majority of substances present in fumes. Even more, in an aircraft there is potential exposure to a complex mixture of a large number of different substances during a fume event, in highly variable concentrations.

In-vitro and *in-vivo* toxicity testing of mixtures has hardly been performed in the context of fume contaminants. There is a need for testing the combined toxicity of all substances present in fumes

of engines, as a lot remains unknown about the hazard and potential health effects of exposure to this type of mixtures (Harrison and Ross, 2016).

For toxicity screening, integrated cellular testing approaches combining different organ systems, as well as whole organism test systems are needed, to allow more realistic simulation of organ interaction, and to include metabolic competence in the test system. In the context of fume exposure, few experiments have been done on studying the translation of in-vitro to in-vivo results.

It is necessary to study biomarker formation, stability and half-life under conditions of controlled exposure, allowing investigating the relationship between fume mixtures and internal exposure doses, without interference of other sources influencing the biomarker levels. It is therefore needed to screen for, and semi-quantify, selected/targeted biomarkers in animals exposed to realistic and characterized fumes.

3.4 Conclusions

Integrated *in vitro* testing strategies are needed combining a realistic exposure scenario (inhalation exposure) with a relevant readout (neuronal function). *In vitro* (cellular) research allows for detailed studies of toxicity but often lacks important aspects such as metabolism. The proposed combination of *in vitro* cellular research using MEA and ALI, the whole-organism *in vitro* study with zebrafish and an *in vivo* rodent inhalation study with neurobehavioural and biomarker analysis therefore fills several of the gaps identified above. These studies with human relevant model studies might guide decision support on - temperature/pressure conditions and fuel/hydraulic/de-icing fluids used during flights and associated - fume events which are most at risk to human, and which should be considered for future mitigation procedures.

3.5 Overview of considered literature

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Yuan et al. Urinary tobacco smoke-constituent biomarkers for assessing risk of lung cancer. Cancer Res. 74(2):401-11, 2014.

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4 Theme: Task 3B- health risk assessment

4.1 Description of theme

This theme describes literature about health risk assessment on cabin air quality. The concept of health risk assessment can broadly defined as the assessment that tries to identify the relationship between exposure to certain substances or exposure conditions and the subsequent health consequences. Two different approaches can be defined. The first approach is an epidemiological based health risk assessment in which a causal relationship between exposure and health effect in humans during normal exposure conditions are identified. These human dose-response relations are used to predict the health effects caused by a certain exposure in order to assess whether measured exposure conditions would lead to undesired health effects.

The second approach is the toxicological based health risk assessment, which is based on controlled animal experiments. The controlled experiments in animals generate a dose-response function at a higher exposure to the substance compared to humans. In addition, animal studies also identifies exposure levels without any effects, which are used to estimate human no-effect exposure levels. These estimated no-effects exposure levels are compared with exposure levels during normal conditions to assess health risk.

Both approaches have the common aim of health risk assessment and both have pro's and con's.

Toxicological approach		**Epidemiological approach**	
Advantages	*Disadvantages*	*Advantages*	*Disadvantages*
Controlled experiment	Unrealistic exposures levels	Realistic exposures levels	Very expensive study design needed for identification of causal relationship
Protocols available	Uncertainty about translation animal data to human relevance	Realistic pathways and clinical effects	Ethical consideration
	Single substances	Realistic exposure conditions (mixture toxicology and interaction effects are taken into account)	No standardized study protocols, difficulties in interpretation of less optimal study designs
			Confounders

In the baseline assessment, both approaches will be taken into account.

4.2 Baseline (''What has been done'')

Comprehensive reviews

The available comprehensive reviews provide an overview of the information of most elements relating to cabin air quality. This includes overviews of reported symptoms, findings from

epidemiological studies, hazard information on chemicals in e.g. engine oil and pyrolysis products, and exposure information during normal flights.

In the CAA paper, the toxicity of lubricant pyrolysis products has been compared to cabin air quality incidents. Symptoms reported in incidents were listed. Data from Marshman were used to list chemicals formed during pyrolysis. The CAA paper analyzed corresponding hazard data for these chemicals. Furthermore, this paper has made an analysis of aircraft air-conditioning duct contaminants by listing the chemicals present in ducts; comparing new and used ducts and linking odour of ducts to odour of chemicals.

The COT (2007) and EPAAQ (2011) reviews are quite elaborate and have summarized much of the knowledge on cabin air quality and their impact. Main findings related to health risk assessment are:

- So far, there is no causal relation between cabin air exposures (either general or following incidents) and ill health in commercial aircraft crews, though an association was plausible. The information available on the association between specific contaminants and symptoms appeared more frequently conjectural rather than definitive.
- Exposure measurement should address the widest possible range of potential contaminants from oil/hydraulic fluid that can be analysed and should not focus on only a single chemical group or compound. In addition, validated methods are needed for exposure measurements.
- Exposure monitoring needs a link to data recorded by airlines with regard to engineering status of the aircraft and reports of odours and adverse symptoms by pilots.
- While sampling cabin air during normal operation was feasible and equipment has been available, identifiable contamination events were infrequent and unpredictable. This reduced the opportunity to capture an event and analyse for the presence and level of possible contaminants at the time of exposure. An extensive environmental monitoring programme conducted during smoke/fume events would be necessary to clarify whether the exposures of concern exist before making recommendations for change.
- Mixture toxicity investigation may be needed, e. g. structure-activity equation.
- Currently insufficient evidence is available to the COT to recommend additional epidemiological research on any acute health effects, but additional research on neuropsychological impairment of pilots is desirable. The most appropriate epidemiological approach to study the neuropsychological health-status in commercial pilots would be a cross-sectional study to investigate how the prevalence of reported neuropsychological symptoms and the results of neuropsychological testing differ between pilots flying different airframes/engine combinations and between pilots who report, or do not report, air quality incidents, and whether associations differ between countries.
- The EPAAQ had difficulty attributing any clinical outcome to specific exposure because of the large number of potential toxic chemical substances that could be involved, and the lack of data documenting exposure levels in cabin air.
- The EPAAQ concluded that there was insufficient evidence at present to confirm or deny biologically significant exposure to cabin air contamination that would lead to significant absorption by crew or passengers. Butyrylcholinesterase (BChE) inhibition has been the standard biological test used for organophoshate (OP) monitoring but should only be considered a marker of acute exposure. Tests based on measuring tricresylphosphate (TCP) metabolites in blood and/or urine may not be sufficiently sensitive, or able to discriminate

exposures to the more neurotoxic compounds (TOCP, DOCP, MOCP and TMPP). Further research on biomarkers of exposure and markers of contaminated cabin air is continuing in the USA.

- The EPAAQ noted the possibility that genetic polymorphism in metabolism of organophosphates could possibly account for some individuals having an inherent susceptibility to OP-induced toxicity. However, the Panel was unable to draw any conclusions as to whether this factor could explain individual differences between affected and non-affected aircrew because of the lack of definitive evidence.

Articles and reports:

General:

In the available literature, a commonly discussed topic is the potential neurological effects of the long-term low dose exposure to organophosphates (usually from the application of pesticides by farmers). Some papers discuss the induced neurotoxicity and describe so-called Organophosphate-ester induced delayed neurotoxicity (OPIDN)

At present, a more broad general discussion on the existence and definition of the “aerotoxic syndrome” is going on. There are concerns about the health effects of oil leaks and pyrolysis products of engine oil and hydraulic oil based on their components. In many cases, the focus is on TCP. Many papers summarize information on exposure or hazard of single substances and reported symptoms in flight crew in order to highlight potential risks or safety issues. Most papers conclude with recommendations for further research, including effective monitoring and surveillance systems, structured reporting of incidents, more useful diagnostic neurological test, and full large scale epidemiological studies in which objective measures of cabin air quality is assessed together with meaningful measures of neurological damage taken into account individual differences.

Epidemiological approach:

The published literature consists mainly of case reports of self-reported health effects of flight crew, observational studies of self-reported symptoms crew and cabin air measurement (however not during the same flights), description of air quality incidence reports. There is also an assessment of criteria for causality in observational studies (Ramsden 2012, Verbeek 2012).

Toxicological approach:

Papers on risk assessment of TCP levels in cabin air, methodologies for mixture toxicology in risk assessment, and a risk model for ToCP for cockpit crew exist. More comprehensive information is available on the hazard and exposure data for TCP outside the scope of cabin air quality, however this is a bit outdated (WHO 1990). Two papers describe a type of risk assessment on the toxicity of commercial jet oils, including the toxicological profile of components, effects of combustion and

altitude and the exposure scenario (Winder 2002, Winder 2006). Recent risk assessment incorporated mixture toxicology for multiple components in cabin air and office workers to investigate whether there is a link between reported symptoms in office workers and flight crew (Wolkoff 2016).

4.3 Gap analysis

Despite all available information on cabin air quality in relation to chemicals, many data gaps still exist and prevent decisive conclusions on the possible relationship between health-related problems and exposure to chemicals in cabin air of airplanes.

After a first screening of the available literature, the following data gaps are observed:

- Hazard information on many individual chemicals is lacking or incomplete. Information on the potential neurotoxicity of chemicals is often not available or limited, for example for the non-ortho isomers of TCP. Further, long-term toxicity data that take into account accumulation of chemicals and chronic low-dose effects, especially about neurological effects, are not always available.
- No stand-alone standardized test is available to determine the neurotoxicity after long term exposure to a mixture of chemicals
- Toxicity of chemicals and health-based limit values are based on standard conditions. The effect of deviant conditions in cabin air, such as hypoxic conditions, on toxicity of chemicals needs further investigation.
- Exposure information is often limited to a small set of chemicals, mainly TCP. Limited information is available on mixture toxicology and how to address neurological effects after exposure to a mixture of chemicals in a health risk assessment.
- Exposure information is related to standard conditions during normal flights. Measurements during fume events are not or hardly available. Prospective monitoring of cabin air, e.g. for measuring during fume events, is difficult and expensive.
- Information on clinical effects or toxicity and information on exposure are obtained either independently or at the same time, but never in a prospective cohort design. As a result, a causal relationship between the symptoms reported by flight crew and exposure to chemicals has never been demonstrated.
- No suitable biomarkers exist to determine the exposure or mark potential health effects

4.4 Conclusions

This descriptive overview provides the baseline information about health risk assessment in the context of cabin air quality identified in the available literature at the consortium partners. Over the years, various studies have investigated, at least partially, the relationship between cabin air quality and its impact on health in flight crew. Although numerous studies have been performed, several data gaps are identified relating to hazard information of specific chemicals, exposure measurement, health effects measurement and study designs. Some of these data gaps are not easy to fill, for instance, the broad range of reported symptoms makes it difficult, if not impossible, to clearly define the so-called aerotoxic syndrome. Exposure to different chemicals, potential

mixture toxicology, infrequent fume events and an undefinable syndrome (health effects) leads to too much complicity in an appropriate epidemiological design to establish causality.

Further research needs to be designed carefully in order to start filling the data gaps, including hazard information focussed on neurological effects, mixture toxicology, exposure measurement during fume events, influence of the cabin conditions in relation to limit values, and identification of suitable biomarkers.

4.5 Overview of considered literature

Articles and reports:

[1] "2015Neurotoxicology-Anderson letter.pdf"

[2] "2015Neurotoxicology-repsonse de Ree et al.pdf"

[3] "Abou-Donia 2003_OP ester-induced chronic neurotoxicity.pdf"

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[15] "de Boer 2014_Tricresyl phosphate and the aerotoxic syndrome of flight crew members – Current gaps in knowledge.pdf"

[16] "de Graaf 2014_Het aerotoxisch syndroom_feit of fabel.pdf"

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[19] "Ehrich and Jortner_2002_chapter on OP induced delayed neuropathy.pdf"

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[22] "Hooper_2011_Workshop summary and conclusions human sacrifice–road speed–responsibility–economics.pdf"

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[27] "Metcalf 1982_Historical perspective of OP ester-induced delayed neurotoxicity.pdf"

[28] "Michaelis_2011_Contaminated aircraft cabin air.pdf"

[29] "Nicholson_2003_Travel-Medicine-and-Infectious-Disease_The airline passenger-current medical issues.pdf"

[30] "Ramsden 2012_Contaminated aircraft cabin air_aspects of causation and acceptable risk.pdf"

[31] "van Netten 1998_Air Quality and Health Effects Associated with the Operation of BAe 146-200 Aircraft.pdf"

[32] "van Netten 2005_aircraft air quality incidents_symptoms exposures and possible solutions.pdf"

[33] "vd Berg_IRAS_Risk model For tri-ortho-cresyl Phosphate (TOCP) For cockpit crew.pdf"

[34] "Verbeek 2012_when work is related to disease_what establishes evidence for a causal relation.pdf"

[35] "WHO 1990_environmental health criteria 110_TCP.pdf"

[36] "Winder 2002_the toxicity of commercial jet oils.pdf"

[37] "Winder 2005_crew effects from toxic exposures on aircraft.pdf"

[38] "Winder 2006_hazardous chemicals on jet aircraft_case study jet engine oils and aerotoxic syndrome.pdf"

[39] "Winder_2005_Crew Effects from Toxic Exposures on Aircraft.pdf"

[40] "Wolkoff 2016_exposure and health symptoms aircrew and office workers_is there a link.pdf"

[41] "Zaharik 2011_safety delayed is safety denied.pdf"

Reviews:

[1] "Book_John Hoyte_2014_Aerotoxic Syndrome.pdf"

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[3] "COT_2007_REVIEW OF THE CAQ, ILL-HEALT IN AIRCRAFT CREWS AND THE POSSIBLE RELATIONSHIP TO SMOKE FUME EVENTS IN AIRCRAFT.pdf"

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Appendix A: Summary of experimental laboratory surveys

Study design, results and conclusions of experimental laboratory studies	reference
This laboratory study investigated temperature ranges from 300 to 600 °C for the evaluation of thermal degradation products from aircraft engine oil carbon monoxide (CO) occurred at 306°C and doubled in concentration when the temperature increased from 350 to 533°C (up to 10600 ppm). It was concluded that the temperature of 400°C is an adequate model for thermal degradation in the turboprop engine. None of the formed pyrolysis products generated was more toxic to rats than the quantity of CO that was formed.	Crane et al. (1983)
This study 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). TMPP was only quantified in the condensate of the pyrolysis engine oils at temperatures higher than 440°C.	Porvaznik et al. (1987)
This study investigated the presence of toxic elements in jet engine lubricant oils. 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 (1999)
This laboratory experiment used a temperature of 525°C to perform pyrolysis experiments on various engine oils. The pyrolysis experiments were performed for 1 min at a temperature of 525°C. Various pyrolysis products were identified It was noted that the identified compound 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 in 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.	Van Netten et al. (2000, 2001) van Netten and Leung (2000) Rubey et al. (1996)
The National Research Council (2002) reports that formaldehyde, acetaldehyde and acrolein could be found in engine oil or could be formed during thermal decomposition of engine oil.	(Nagda et al., 2001).
This study 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)

Winder and Balouet (2002a) report that the commercial product of N-phenyl-1-napthyl-amine 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).	
Laboratory experiments 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)	Winder and Balouet (2002b)
This study reported 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.	CAA (2004)
This study investigated the gas phase thermal degradation, under pyrolytic conditions, of two lubricants of different triaryl phosphates composition and one based on fatty acid methyl-ethyl esters. Experimental results show that, for each sample tested, the degradation starts at 600–700◦C and is complete within 900 and 1000◦C. A great number of by-products, mainly aromatic and poly-aromatic compounds (PAHs), was always observed at temperatures higher than 700◦C. Two mechanisms are proposed for the formation of these products: the first one is directly related to the production of benzene radicals originated from aryl moieties that act as building blocks in consecutive reactions; the second one is related to the occurrence of displacement and cyclization reactions, which lead to the obtainment of aromatic moieties from vinyl radicals. The amount of PAHs coming from the lubricant based on fatty acid methyl-ethyl esters is always much lower than that of the other two samples. Moreover, the pyrolysis of the two triaryl phosphates lubricants leads to the formation of three phosphorus-containing aromatic by-products (phosphabenzene, isophosphinoline and phosphinoline) and a mechanism for their formation was proposed. On the basis on the experimental results found, it is possible to conclude that the lubricant based of fatty acid methyl-ethyl esters produces less hazardous by-products than triaryl-phosphates ones when exposed to thermally severe pyrolytic conditions.	Mascolo, Rausa, Mininni, Tinussi (2003)
A methodology for personal occupational exposure assessment of airborne trialkyl and triaryl organophosphates originating from hydraulic fluids was developed. The combination of Chromosorb 106 and 37 mm glass fiber filter and dichloromethane as desorption/extraction solvent was the best combination for air sampling of organophosphates. Triarylphosphates were recovered solely from the filter, while the trialkyl phosphates were recovered both from the filter and the adsorbent. Sampling efficiency on the combined sampler amounted 92-101%, based on spiking experiments following by pulling air through the sampler and experiments in an exposure chamber with generated oil aerosol.	Solbu et al. (2007)

In this study jet engine oils were screened 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.	De Nola et al. (2008)
This study suggested that the higher ortho-TCP/TCP ratio observed in bleed air compared to the ratio 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.	Ramsden et al. (2013)
Jet oil was heated at 250 and 370°C. The simulation was carried out under an oxygen depleted atmosphere using nitrogen. The emitting components measured in the simulation test were directly measured in the vapour of the investigated jet-oil. The applied Jet-oil contained 4 isomers of TCP: T(m,m,m)CP, T(m,m,p)CP, T(m,p,p)CP and T(p,p,p)CP were observed in the analysis of the oil itself and in the oil vapour. No detectable amounts of T(o,o,o)CP were found in the oil and oil vapour. Emissions of OPEs increased at increasing oil temperature. Besides TCPs, also TBP, TPhP and CDP isomers were quantified in the oil vapour. Due to increase of the temperature (from 250 °C to 370 °C) also the concentration of aldehydes increased significantly.	Havermans, Houtzager and Jacobs (2013)
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 a 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 tri isopropyl phenyl phosphate (TIPP). Whereas others have reported that in the NYCO jet engine oils, TCP is replaced by TPhP.	OHRCA (2014)
An analytical methodology using automatic thermal desorption and GC-MS analysis was optimized and validated for simultaneous determination of a set of components from three different flame retardant chemical classes among than trialkyl-and aryl phosphates. The methodology is based on low volume active air sampling of gaseous and particulate air fractions on mixed-bed (polydimethylsiloxane (PDMS)/ Tenax TA) sorption tubes. The optimized method provides recoveries >88 %; a limit of detection in the range of 7– 41 pg m^{-3} for; a linearity greater than 0.996; and a repeatability of less than 10% for all studied compounds.	Lazarov, Swinnen, Spruyt et al. (2015)
This investigation observed the following amines and organophophates in jet engine oils and hydraulic fluids • 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)	Hildre and Jensen (2015)

In this study, aircraft engine oil was heated well above the permissible temperature range and the evaporation behavior, and the resulting emissions were observed. It turns out that the lubricant does not evaporate completely, but a brown residue or precipitate remains. Especially with severely elevated temperature, significant emissions can be observed. The major parts of those emissions were oxidized compounds, mainly organic acids, aldehydes and ketones. Furthermore, the additives of the lubricant or their degradation products were observed in the emissions. Increasing the temperature of the oil will increase the emissions of alcohols, fatty acids, aldehydes, TCPs and cresols.	S. Mair, C. Scherer, F. Mayer (2015)
The objective of this paper is to share results from bleed air measurements obtained from the NASA Vehicle Integrated Propulsion Research (VIPR) study conducted on a C-17 Globemaster III at Edwards Air Force Base in 2015. This industry-first research has been conducted to simulate a rare engine upset failure condition on a real time operating aircraft. Measurements to identify particulate sizes and concentrations and chemical concentrations of semi-volatile and volatile organic compounds were taken at multiple locations in the bleed air system to map the nature of possible bleed air contaminants. In order to prevent contamination of the C-17 environmental control systems during testing and to facilitate bleed air measurements and sampling, a bleed air extraction sampling system (BAESS) platform designed and built to simulate the aircraft's bleed air and pack systems, was installed outside of the aircraft and integrated into an engine's bleed air ducting. Furthermore, air purification and prototype sensor technologies from various commercial suppliers were installed aboard the BAESS platform for preliminary performance evaluations. Chemical contaminants from the injected engine oil were captured by various types of sampling media located at different stations within the BAESS platform; these products were subsequently analyzed by GC/MS US EPA methods. After analysis, it was found that no concentration of contaminants in the bleed air exceeded established OSHA PEL and STEL. The data and scientific conclusions stemming from the VIPR study will be used to develop future technologies to enhance the performance of airplane bleed air systems.	David R. Space et al. (paper 2015)
This article deals with a review and current gaps in knowledge regarding TCP and the aerotoxic syndrome of flight crew members. Wyman et al., 1993 reported a study reported the formation of trimethylolpropane phosphate (TMPP) from the reaction of TCP and trimethylolpropane ester during elevated temperatures in ship turbines. Temperatures of 550 °C are, however, ideal for the formation of TMPP (Wright, 1996) and those do occur in jet engines. On top of other additives in the motor oil vapors, there may be pyrolized mineral oil constituents and polycyclic aromatic carbons, as the engines reach temperatures of several hundred degrees (Lipscomb et al., 1995). Furthermore, carbon monoxide may be present as well and has, for example, a 2-fold higher toxicity at 2600 m than at sea level (Winder and Balouet, 2002). De Boer concluded that TCP concentrations reported in the literature are much too low to explain health effects Other compounds present from engine oil in combination with TCP and degradation products could pose additional health effects. High altitude and lower air pressure may have an influence on the toxic mechanism of TCP.	Jacob de Boer et al. (2015)
Fresh and used aircraft engine lubricants (Mobil jet Oil II) were analysed using FTICRMS and GC*GC-HRTOFMS with special focus to its TCP content. Only four TCP isomers were present in the fresh oil: mmm-TCP, mmp-TCP, ppm-TCP and ppp-TCP. Results show a significant risk from alkylated cresyl phosphates, which were identified in the used oils. Several xylenyl and ethylphenyl phosphates have been shown to exhibit a similar toxicity to ortho substituted isomers. It was suggested to include these compounds in future aircraft air quality studies.	Megson, Ortiz, Jobst, Reiner, Mulder, Balouet, Chemosphere (2016)

The experimental set-up of the chemical characterization consisted of heating the engine 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 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. 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. It was found that the original oils and the oil vapours contained tri(m,m,m,)-, tri(m,m,p)-, tri(m,p,p) and tri(p,p,p)-cresyl phosphate. Comparison of new oil (A_n) and used oil (A_u) shows no significant differences in composition of the four isomers. 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. Relative high concentrations of formaldehyde and acetaldehyde were found in the oil vapours. In the basic oil patterns, without heating, 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. 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. 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. 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.	Houtzager, Havermans, Bos et all (AVOIL 2017)

Appendix B: Summary of in-flight measurement campaigns

Study design, results and conclusions of in-flight measurement campaigns on cabin air quality (CAQ) and cabin air contamination (CAC)	Reference
Kelso et al. (1988) were tasked with the identification of the chemical composition of the odorous vapours in the environmental control system of 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. Four aircraft were sampled during flight. One of the four was also sampled on ground. From each airplane 5 samples were collected. Additional pyrolysis experiments 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. Exxon 2380 oil 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) were 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 compounds derived from the oil additives 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)
Van Netten (1998) 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 2 h 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 was sampled for 1.5 h. 2 min after take-off an oily smell was reported. During test flight number of ascents 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	Van Netten (1998)

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 before take-off and after 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.	
Waters et al. (2002) measured cabin air quality on 36 commercial transport flight segments on 11 different aircraft with flight durations from 42 min to 14 h 23 min with 34 to 100% passenger occupancy. At two coach locations temperature, relative humidity, total particulates, barometric pressure as well as VOCs, nitrogen oxides, CO, CO2, O3 were monitored over whole flight durations. CO2 levels depended on flight duration (the shorter, the more), occupancy (the higher, the more), air ratio (more recirc. air, more CO2) and size (higher CO2 levels on narrow-body aircraft). Major VOCs found by the integrative sampling method were ethanol at up to 2.4 ppm, toluene at up to 130 ppb, limonene at up to 12 ppb. Few other VOCs were present at trace levels. VOC levels were rather low and not atypical for indoor environments, however more time resolved investigations and research trying to correlate crew complaints with cabin air quality are suggested.	Waters et al. (2002)
Nadga & Rector (2003) reviewed six monitoring studies performed between 1997 and 2001, in total 71 flights on 18 aircraft types. Parameters monitored included humidity, CO, CO2, VOC, SVOC and particulate matter. About 20 detected compounds were attributed to different origins. Neither TMPP nor ToCP was determined. At normal operating conditions, contaminant levels in aircraft cabins are mostly similar to those in residential and office buildings. Only ethanol and acetone were found in higher concentrations in aircraft.	Nadga & Rector (2003)
Spicer et al. (2004, report on ASHRAE project 1262-1) developed a battery powered under-seat system and protocol for on-board aircraft measurements including temperature, relative humidity, light intensity, acceleration, ozone, CO, CO2, fine particulate matter, VOCs and SVOCs, for on-line monitoring as well as for spot and long-term sampling for off-line analyses. The system was tested on four commercial flights, on two MD-80, one B757-200 and one B737-800. Samples of VOC spot measurements were taken by evacuated 1 L canisters over 30 minutes during cruise and descent phases. A grab sample of the bleed air (with recirc. air being shut off for about 15 min) was collected from a gasper in the cabin during cruise phase. Off-line GC-MS analyses targeted 54 VOCs at ppb level. Main compounds in the cabin on all 4 flights were ethanol at concentrations up to 1220 ppb and acetone at up to 22 ppb. Sources for ethanol are alcoholic beverages served on-board, acetone is a human bio-effluent. Many other common VOCs such as benzene, toluene, ethylbenzene, xylenes were found at levels much lower than normally detected in ambient air. Traces of dichloromethane were likely residues from adsorbent tube cleaning, traces	Spicer et al. (2004, report on ASHRAE project 1262-1)

of tetrachloroethene were likely brought in by dry cleaned passenger clothing, dichlorobenzene was attributed to a lavatory deodorizer. VOC passive sampling devices were also applied to collect air during the whole duration of the flight, but shown to be less suitable. SVOC analyses after trapping 1.4-2.3 m³ of cabin air on a XAD-2/quartz fiber filter cartridge over 180-285 min. revealed naphthalene at 809 ng/m³, acenaphthene at 20 ng/m³, phenanthrene at 17 ng/m³ and fluorene at 11 ng/m³ (average concentrations) as major compounds. TCP at a LOD of 9 ng/m³ could not be detected. No fume event occurred. In 2008, MacGregor et al. published results of the same study in a separate Journal (ASTM Vol. 5 No. 8, ID JAI101639).	
Crump et al. (2011) analysed in the so-called Cranfield study aircraft 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 sampling. Besides total VOC and ultrafine particles numbers, the following target compounds were included in this study: ooo-TCP, the other TCP isomers, TnBP, toluene, m- and p-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 VOC 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 or ooo-TCP was detected. Highest ooo-TCP level of 22.8 µg/m^3 was observed during climb of a B757 (overall mean 0.07 µg/m^3) – with no event being reported. The overall mean total TCP level was 0.14 µg/m^3 (with a maximum of 28.5 µg/m^3). The highest TnBP level 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 events did not contain elevated levels of any of the target compounds included in this study. (Comment: The suitability of the sampling (duration, adsorbent) and analysis method (TD-GC-MS) applied for TCP determination was questioned as in the flight phases before and after no ToCP was detected).	Crump et al. (2011)
Denola et al. (2011) monitored TCP in cockpit and cabin air of three different aircraft types from Australian Defence Force (ADF): fighter trainer, cargo transporter and fighter bomber, in total 46 aircraft. Long duration air sampling was performed with 0.06 g of Porapak-Q glass tube at 2 L/min. Short term air sampling was performed with metricel filters (GN; 0.8 µm) at 36 L/min. Mono, di or tri(o,o,o)-cresyl phosphate were below LOD in all collected samples. mmm, mmp, mpp and ppp TCP isomers were detected 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 ranging 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. During the flight with the highest concentration of 51.3 µg/m³ observed smoke and odour was reported. However, the report of smoke and odour did not	Denola et al. (2011)

necessarily correlate with TCP concentrations in other incidents. In two other flights smoke was reported, however no TCP was detected above the LOQ. Results of this study indicate a low health risk from TCP exposure. Other peaks were present in the gas chromatogram with similar retention times of TiBP, TnBP and TPhP.	
Solbu (Thesis 2011, recapping four previous papers) developed a sampling method and measured the exposure to organophosphates (OPs) from jet engine oils and hydraulic fluids among aircrew members. 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. Four airline companies in Norway were included in this study. In total 40 aircraft were sampled consisting of jet engine airplanes, propeller airplanes and helicopters. The samples were collected in the cockpit and the passenger cabin. 95 within-day OP samples were collected from cabin air in 47 flights (jet airplanes, propeller airplanes and helicopters). TCP (sum of tri(m,m,m,)-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 ranging 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 at 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 ranging from 24 to 4100 ng/m^3. BDPP was only detected in the jet engine and propeller airplanes with levels ranging 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). 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. 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 six HEPA filters from the jet engine airplanes TCP was detected with levels ranging from 1.1 to 4.3 ng/g per hour. Again, no ortho-isomer of TCP was detected. 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 (5.1 µg/m^3) compared to after engine replacement. Ortho-isomers of TCP were not detected in any of the samples collected in this study. Wipe sampling 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 to lower LOQs and higher extraction recovery. There was no difference in concentration observed between sampling the cockpit versus the passenger cabin.	Solbu (Thesis 2011, recapping four previous papers)

Spengler et al. (2012) monitored aircraft cabin air of 83 commercial flights in two Airbus and four Boeing models on U.S. domestic routes or on international routes. Temperature, relative humidity, cabin pressure and sound levels as well as CO, CO2, O3, VOCs, SVOCs, particles and TCP were investigated. The five carbonyls formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde were also determined, but not on all flights. Acetaldehyde was found in 81% of 70 samples, acetone in 79%, acrolein in 71%, formaldehyde in 49% and propionaldehyde in only 17% of the samples. Concentrations of many VOCs detected were lower in the cabin than what is typically reported for offices and residences. TCP was detected in only one of 71 samples at 0.1 ppt. ToCP was not detected in any of the samples.	Spengler et al. (2012)
Houtzager et al. (2013) investigated 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 four B737-700s, three B737-800s, three B737-900s and from two B737-700s and 800s 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 flights. 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.	Houtzager et al. (2013)
Rosenberger et al. (2013) investigated the presence of TCP in cabin air of two airplanes. 90 air samples were collected during 26 flights 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) at 2 L/min. The total TCP concentration in the air samples ranged from 17 to 167 ng/m^3. In 15% of the samples o,o,o-TCP was detected with levels ranging from 2 to 65 ng/m^3. This was during normal flight conditions. 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. In the engine oils (Mobil jet oil II) the ortho-TCP levels were < 20 µg/kg.	Rosenberger et al. (2013)
Rosenberger et al. (2014) also investigated organic compounds in cabin air after de-icing since some odour complaints and health impairments occurred on a particular aircraft. Since measurement data from that respective flight was not available, the de-icing procedure was repeated with that aircraft, followed by test flights (without passengers) and measurement equipment being on-board. VOCs and organo phosphates were determined. Up to 2.5 mg/m³ of propylene glycol was found in the cabin during test flights after de-icing, also 1-hydroxyacetone, a possible	Rosenberger et al. (2014)

degradation product, was detected at 1.5 mg/m³. Significant contamination with organophosphates was not observed. TCP levels detected were below 0.17 µg/m³, ortho-isomers were not present. Tetrachloroethylene was found on the control flight before de-icing at levels up to 230 µg/m³ and assigned to previous dry cleaning.	
Guan et al. (2014a and b) conducted in-flight measurements on 107 commercial flights between August 2010 and August 2012 on domestic China and some international routes on several different aircraft types focusing on VOCs, species, levels, influencing factors and sources. In total 346 VOCs were detected, 59 of which in each flight. These were 41% alkanes and alkenes, 15% esters and alcohols, 11% ketones and aldehydes, 6% halides, 20% aromatics and 6% other VOCs. Occurrence of tetrachloroethene was assigned to dry cleaning of fabrics, 1,4-dichloro-benzene to e. g. disinfectant, deodorant or lavatories. Many VOCs such as benzene, toluene, xylene, ketones and aldehydes (e.g. acetone), esters and alcohols and alkanes and alkenes are present in aircraft cabins – and in buildings. 6-Methyl-5-hepten-2-one likely originates from the reaction of ozone with unsaturated hydrogen compounds and was only found in aircraft cabins. Most VOCs concentrations varied in the three different flight phases investigated, peaking before take-off or during cruise.	Guan et al. (2014a and b)
Wang et al. (2014) investigated the presence of VOCs in cabin air of aircraft to identify possible emission sources. 84 air samples were collected on Tenax-TA tubes during 14 flight on a B737-800. Flight duration ranged from 80 to 190 minutes. Samples of 1000 ml at 200 ml/min were drawn minimum during 5 flight phases. In total 19 VOCs were detected in air samples collected during the 14 China domestic flights. Highest levels were found for d-limonene (median of 31 µg/m^3), followed by decanal (median of 24 µg/m^3), nonanal (18 µg/m^3), toluene (13 µg/m^3), acetic acid (11 µg/m^3), benzene (10 µg/m^3) and 6-methyl-5-hepten-2-one (9 µg/m³). A receptor model using positive matrix factorization coupled with information related to VOC sources was applied to identify the major sources in the cabin. 29% of the total VOC emission was attributed to services or humans, followed by chemical reactions (15%), fuels (13%), materials (12%), combustion (12%), non-fuel oil (9%), cosmetics and perfumes (5%), and cleaning agents (4%). Non-fuel oil contributed to 69% of total benzene concentration, to 20% of acetone concentration, to 11% of acetic acid concentration and to 10 % of octanal concentration.	Wang et al. (2014)
Guan et al. (2015) used CO2 and TVOC data of 6 flights performed in domestic China in 2013 to model and estimate net in-cabin emission rates of VOCs and contributions from outside and inside the aircraft cabin. TVOC concentrations were measured during different flight phases and were mostly in the range of 0.20 to 0.40 mg/m³. Based on CO2 and ventilation information air ratios and air flows were calculated. Results indicated that during cruise phase TVOC in cabin air came mostly from cabin interiors (90%). Contributions from outside became more significant during taxiing on ground, ascent and descent phases	Guan et al. (2015)

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Schuchardt et al. (2017a and b) performed measurements on 69 commercial flights, 8 of which on B787 in 2015 and 2016. Samples were taken at defined flight phases (taxi-out, take off and climb, descent and landing, complete flight), while climate data, total volatile organic compounds, carbon dioxide, carbon monoxide and ozone were recorded continuously. Total volatile organic compounds (VOC) concentrations ranged from 0.024 – 2.1 mg/m³ and 0.012-0.489 mg/m³ on B787. Low amounts of formaldehyde (range 0.03-48 µg/m³ and 0.02 - 17 µg/m³ on B787), acetaldehyde (range 0.02-42 µg/m³ and 0.01- 15 µg/m³ on B787) and other aldehydes mostly at trace levels were detected. Only traces of meta- and para-TCP-isomers were detected, up to 1.515 µg/m³ and up to 0.403 µg/m³ on B787. No ortho isomers were detected. The most prominent airborne organophosphorous compounds were tri-n-butyl phosphate (TBP) which amounted from 0.037 to 2.484 µg/m³ and in the B787 from 0.037 to 1.482 µg/m³, and tris(chloro-isopropyl)phosphate, which amounted from 0.023 to 9.977 µg/m³ and from 0.041 to 2.633 µg/m³ on B787. Other organo phosphates were detected in trace amounts in most samples. Concentration levels found were similar or even lower compared to findings of other indoor environments such as offices, schools, kinder gardens or dwellings because of the high air exchange rate required in the cabin, which leads to a quick dilution of contaminant concentrations (“thinning effect”). Previously described reaction products of VOCs with ozone or oil pyrolysis products such as certain alkanoic acids or increased concentrations of n-alkanes were not detected. Occasional increased levels of propylene glycol in cabin air in winter were explained by the use of de-icing fluid. Few VOCs may be assigned to individual passenger actions, in-flight service or use of moth balls. Occurrence of an unknown fraction of iso-alkanes (at non-critical concentrations) especially during the take-off has caught the attention of the researchers. With regard to occupational exposure limits or indoor air guideline values the cabin air monitored in this study were of good quality and no object of any concerns. No single detected contamination reached critical or unusual concentrations for indoor environments. Results are consistent with findings of other previously performed in-flight/on-board measurement campaigns. For the first time in-flight measurements on Boeing 787 aircraft have been published. TCP has been proven suitable as a marker for engine oil contamination in bleed air – provided that a sensitive analytical method is used. TCP could also be detected on the non-bleed air operated B787 and suggest ubiquitous cabin air contamination in all aircraft types. Randomly occurring higher TCP levels (with no reported CAC event) may be assigned to secondary contamination by e. g. oil deposits in the bleed air or air conditioning system, which may	Schuchardt et al. (2017a and b)

also be responsible for reported smell events, caused by non-toxic odourous compounds release.	

