An Assessment of Chemical Exposure in Aviation.

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An Assessment of Chemical Exposure in Aviation.

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Declaration

I am responsible for the conception and creation of all contents within this manuscript that are not otherwise credited within the submission.

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LIST OF ABBREVIATIONS

100LL	Leaded Aviation Fuel (See also: AVGAS)
6-MHO	6-Methyl-5-hepten-2-one
AChE	Acetylcholinesterase
ACN	Acetonitrile
ADBI	Celestolide
AHE	Adverse Health Effects
ALS	Amyotrophic Lateral Sclerosis
AOPA	Aircraft Owners and Pilots Association
APCI	Atmospheric Pressure Chemical Ionization
APU	Auxillary Power Unit
ASHRAE	American Society of Heating, Refrigerating and Air-Conditioning Engineers
ASTM	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry
AVGAS	Aviation Gasoline
BAFDP	Bisphenol AF Bisdiphenyl Phosphate
BChE	Butyrylcholinesterase
BLK	Blank
CAQ	Cabin Air Quality
CBDP	Cresyl Saligen Phosphate
CI	Chemical Ionization
CNS	Central Nervous System
CO	Carbon Monoxide
DBPP	Dibutyl Phenyl Phosphate

DBPP	Dibutylphenyl Phosphate
DCM	Dichloromethane
DFP	Diisopropylfluorophosphate
DKLRI	Deuterated Kovats Lee Retention Index
DPEHP	2-ethylhexyl diphenyl Phosphate
EASA	European Union Aviation Safety Agency
ECS	Environmental Control System
EHDP	2-ethylhexyl diphenyl Phosphate (See also: DPEHP)
EI	Electron Ionization
EPA	Environmental Protection Agency (USA)
FAA	Federal Aviation Administration
GC	Gas Chromatography
GCxGC ToFMS	Two-dimensional Gas Chromatography coupled with Time of Flight Mass Spectrometry
HEPA	High Effciency Particulate Air (Filter)
HPLC	High -Performance Liquid Chromatography
ICP OES	Inductively Coupled Plasma Optical Emission Spectroscopy
INEF	International Network of Environmental Forensics
IPA	Isopropyl Alcohol
IS	Internal Standard
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MeOH	Methanol
MRU	Mount Royal University

ND	Non-Detect
NDTPI	N-dimethylaminomethyl-tert-butyl-isopropylphosphine
NIOSH	National Institute for Occupational Safety and Health (USA)
NIST	National Institute of Standards and Technology
NRC	National Research Council
NTE	Neuropathy Target Esterase
OPEs	Organophosphate Esters
OPIDN	Organophosphate Induced Delayed Neurotoxicity
OPs	Organophosphates
PBDE	Polybrominated diphenyl ethers
PCDL	Personal Compound Database and Library
PETO	Pentaerythritol Oleate
PFHE	Plate-Fin Heat Exchangers
PM	Particulate Matter
PON-1	Paraoxonase Enzyme
POPs	Persistant Organic Pollutants
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
QA/QC	Quality Assurance and Quality Control
QToF-MS	Quantitative Time of Flight Mass Spectrometer
RF	Radio Frequency
RH	Relative Humidity
RPM	Rotations per Minute
RSD	Relative Standard Deviation
RT	Retention Time

SDS	Safety Data Sheet
SVOCs	Semi-Volatile Organic Compounds
TBEP	tris(butoxyethyl) phosphate
TCEP	Tris(chloroethyl) Phosphate
TCP	Tricresyl Phosphate
TCPP	Tris(chloropropyl) Phosphate
TDCPP	Tris(1,3-dichloro-2-propyl) Phosphate
TEHP	Tris(ethylhexyl) Phosphate
TEL	Tetraethyl Lead
TEP	Triethyl Phosphate
TLVs	Threshold Limit Values
TmCP	Tri-meta-cresyl Phosphate
TMMP	Tris(methyl-phenyl) Phosphate (See also: TCP)
TnAP	Triamyl Phosphate
TnBP / TBP	Tributyl Phosphate
ToCP	Tri-ortho-cresyl Phosphate
TpCP	Tri-para-cresyl Phosphate
TPhP	Triphenyl Phosphate
TVOCs	Total Volatile Organic Compounds
TXP	Trixylenyl Phosphate
VOA	Volatile Organic Analysis
VOCs	Volatile Organic Compounds
WHO	World Health Organization

DEDICATION

To Meghan.

For your patience and never-ending support. I hope to one day possess a modicum of the grace that you so freely display to the world. I love you.

To Finn and Wade,

You probably don’t know at this point how much time, that should have belonged to you, that you gave to me, to complete this work. I will make it up to you somehow. I love you both.

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It is a great privilege to get to do research that you want to do, with people that you want to do it with. I feel that I have been exceptionally privileged in this respect.

GENERAL ABSTRACT

Aircraft pneumatic systems are pressurized by air bled from the engines. This air and engine interaction can result in contamination of the air with intact, degraded, and pyrolyzed oil. Additionally, other pneumatically pressurized aircraft components, such as the hydraulic reservoirs, can contribute to chemical fouling of the air. The pneumatic system then facilitates the transport of this contaminated air to locations that allow for interactions with human receptors; most commercial aircraft use this bleed air to pressurize the cabin, and all use it to pressurize the onboard potable water systems. Human interactions with these contaminants, coupled with an increased prevalence of neurological illness and injury, anecdotally reported and described in cohorts of those who have careers in aviation, has led to our description of an associated occupational risk (**Chapter 1**). In this chapter, we systematically examine the available literature and describe several gaps in knowledge that, if addressed, could better qualify the chemical exposures and verify the pathways of contaminants from source to receptor. Of those knowledge gaps, three were the most pressing.

Historical exposure to known contaminants of concern, in particular organophosphates like tricresyl phosphate, had been theorized to potentially contribute to pilot illness. The mechanisms of this historical exposure had yet to be explored. We completed an elemental assessment on new and used oil of small single-engine aircraft; this aircraft type is often used for training pilots prior to commercial flying careers (**Chapter 2**). In this oil, we found an enrichment of organophosphates in the used product, indicative of the addition of contaminants of concern as a fuel or oil additive. We also found elevated concentrations of lead in the used oil product (average concentration 5.77g kg-1), which described a significant enrichment from its source of leaded aviation fuel and is at concentrations capable of exposure-induced neurological injury. Additionally, in this chapter we examine a number of turboprop and jet aircraft oils and noted that phosphorus is lost from the new oil product with use at rates that exceed oil attrition. This validates the contaminant source determination of aircraft engine oil for organophosphates; the loss of the phosphorus from the oil implies its availability to the bleed air systems of the aircraft as either intact or degraded compounds.

The second pressing knowledge gap identified described a previously unexamined contaminant pathway that originated in the engines and hydraulic reservoirs of aircraft

and resulted in contamination of the potable water system due to the pneumatic connection with all aforementioned systems (**Chapter 3**). We qualitatively assessed the potable water onboard aircraft, utilizing liquid chromatography coupled with high-resolution mass spectrometry. We detected hydraulic oil (tributyl phosphate) in more than half of the unconcentrated water samples taken from flights and tentatively identified several additional organophosphates also in relatively high percentages (tris(chloropropyl) phosphate: 20%; triphenyl phosphate: 10%; tributoxyethyl phosphate: 10%). This confirmed the contamination pathway from the pneumatic system to the potable water and described a new exposure route for contaminants of concern to receptors that was undescribed by prior research.

The contaminants known to be present on aircraft make up only a small fraction of the actual exposures that one experiences in the cabin environment. This is because previous assessments of the cabin had largely been targeted, sampling or monitoring the cabin for specific chemical compounds or classes, and completed with instrumentation (typically conventional Gas Chromatography Mass Spectrometry) that lacked the chromatographic resolution to detect and identify unknown compounds. We conducted the first non-targeted assessment of the aircraft cabin that was not restricted to a particular chemical class, and used this analytical technique to compare bleed air-pressurized and non-bleed air-pressurized aircraft (**Chapter 4**). Our analysis demonstrated increased contamination of bleed air pressurized aircraft in terms of compound number and abundance and tentatively identified contaminants associated with the degradation of aircraft engine oil (short-chain organic acids), exclusively on bleed air pressurized flights. This non-targeted assessment, contrary to previous comparison of aircraft in the literature, seems to demonstrate a marked difference in pressurization system-associated contamination, and helps verify cabin pressurization via the bleed air pneumatic system as a contaminant exposure pathway.

When examining the results of the thesis as a whole, we have, through literature review, demonstrated a probable occupational risk and, through experimentation, shown that the risk may extend to training prior to employment in the commercial occupation. We have shown that the primary contaminant source, as described in the literature (aircraft engine oil), loses the element associated with the principal contaminant class of concern (phosphorus) with use, allowing the contaminant to enter bleed air systems as theorized. We have identified and validated a new source-to-receptor pathway involving the pneumatic and potable water systems on aircraft. And we have demonstrated, with a non-targeted qualitative chemical comparison with bleed-free aircraft, that pressurization of the cabin via bleed air from the engines is an important source of contamination, strengthening that pathway's validity. While we have closed some of the knowledge gaps associated with chemical exposure and occupational risk in aviation, some remain to be answered. We hope that the contents of this thesis can influence decisions to mitigate some of this risk and inform future experimentation and research.

THESIS INTRODUCTION, AIMS, AND STRUCTURE

The majority of modern jet aircraft utilize air bled from the engines to perform several tasks essential for high altitude flight; cabin and hydraulic system pressurization are completed in this manner, as well as, engine cowl and wing de-icing, windshield rain and ice protection, engine thrust reversers, and pressurizing the potable water and waste systems on the aircraft (Moir and Seabridge, 2008). Within the engines on jet aircraft, air is drawn into the turbofan, entering a number of compression stages prior to the addition of fuel and the combustion of the mixture. Pressures and temperatures within the compression sections are highly variable, depending on the location within and the operating conditions of the engines; at ground idle temperatures of approximately 180°C and pressures of 50psi can be expected, while during takeoff pressures exceeding 400psi and temperatures above 500°C are experienced (Moir and Seabridge, 2008). Due to this variation air is bled from the engines at different locations depending on stage of flight. Typically, two locations are utilized, with a high-pressure port placed further back in the engine used when the engines are operating at low settings such as ground idle / taxi, and during the later stages of cruise, as well as portions of the landing, and a lower pressure port, located closer to the front of the engine, which is utilized when the engines are operating at high settings, such as take-off, and the majority of cruise (Fielding, 1999; NRC, 2002; Moir and Seabridge, 2008). With this system a relatively consistent pressure of “fresh” air can be supplied to the aircraft at any engine speed, and the ease, efficiency, and availability of this hot, pressurized gas, makes it invaluable for the numerously mentioned functions (Moir and Seabridge, 2008). However, there is a concern with the quality of air provided by the aircraft pneumatic system to pressurize the cabin (Michaelis et al, 2017).

Bleed air contamination can occur when seals, bearings, and hydraulic components fail, allowing intact and/or pyrolyzed oil and additives to enter the cabin (Michaelis, 2018). Additionally, these components are typically designed to “leak” at low levels, with permitted consumption of oil falling between 95 and 475 milliliters per hour of engine operation (Michaelis, 2018). The pathway from the engines to the cabin and cockpit of the aircraft is typically not filtered and is not normally monitored for contamination (Hunt et al, 1995; Harrison and Mackenzie Ross, 2015). Mobile Jet Oil II is used by a large percentage of the aircraft fleet for the lubrication of these components; it is a synthetic product consisting of primarily of fatty acid esters of pentaerythritol and dipentaerythritol (Winder and Balouet, 2002). Additionally, the product contains isomers of Tricresyl Phosphate (TCP), used as an anti-wear agent and flame retardant at approximately 3% by volume, 1% phenyl-α-naphthylamine, and benzamine (Winder and Balouet, 2002).

Tricresyl phosphates, in particular the ortho- substituted isomers, are known to be neurotoxic; first intentionally synthesized in 1854 by Alexander Williamson, less pure mixtures unknowingly containing TCP isomers were used in the treatment the treatment of Tuberculosis under the brand name Phosote earlier in that decade (Petroianu, 2016). By the late 1800's these organophosphates were known to cause neuropathy. Camille Lorot described loss of peripheral nerve function in patients prescribed the medications in 1899 and suggested that large doses of the organophosphates were responsible (Petroianu, 2016). This is further exemplified in prohibition era America when Jamaican Ginger Extract was intentionally adulterated with Lindol, the brand name of the tri-ortho isomer of TCP; this resulted in the mass poisoning of thousands and left the victims with debilitating neurological damage coined the Jake Leg, known today as Organophosphate Induced Delayed Neurotoxicity (OPIDN) (Petroianu, 2016). It is the cumulative exposure of pilots and flight attendants to these compounds found in the engine oil, as well as, other potentially hazardous chemicals found within the deicing fluid, hydraulic fluids, and flame retardant materials on board aircraft that lead to concern of a possible occupational illness coined Aerotoxic Syndrome (Winder and Balouet, 2000).

Symptoms that have been associated with Aerotoxic Syndrome include irritation in the eyes, nose, and throat, disorientation, headaches, dizziness, numbness, and tremors (Winder and Balouet, 2000). The neurological nature of many of the symptoms has led to a focus on ortho-isomers of TCP as one of most likely candidates (Winder and Balouet, 2000, 2002; Liyasova et al, 2011; Ramsden, 2013; Schindler et al, 2014). In response to the concern about the neurotoxicity of these compounds, present formulations of the turbine oil have decreased the amount of ortho isomers present; this is exemplified by no ortho isomers of TCP being detectable in new or used aircraft oil by highly sensitive gas chromatographic mass spectroscopy techniques (Winder and Balouet, 2002; Megson et al, 2016).

Ortho-TCP is not the only risk to flight crews; occupational hazards include increased dosage of ionizing radiation, sleep issues related to traveling across time zones, hypoxia, low humidity, increased ozone concentration and reaction products, and a host of Volatile Organic Compounds (VOCs) (Wilson et al, 2003; Waters et al, 2009; Harrison and Mackenzie Ross, 2015; Wolkoff et al, 2015). Several of these risk factors are innately associated with the act of flying. Radiation doses and hypoxia are related to altitude and the limitations of the aircraft component materials; pressure differentials are too great to be pressurized to sea level at altitude, and flying low enough to limit radiation dose is too expensive to allow for economical flight (NRC, 2002; Matthia et al., 2015).

While many of the health risks of flying are generally accepted, there is no consensus on the impact of the chemical exposure to the flight crews. Ortho-TCP and VOC concentrations on monitored flights are typically well below traditional safety guidelines, often falling below limits of detection, and this has led to the belief that Aerotoxic Syndrome may not, in fact, be an occupational illness (Wolkoff et al., 2015; de Ree et al, 2014). However, many avenues of exposure have not been explored. TCP has a high boiling point and low vapor pressure; this implies that the compound is likely to settle onto the surfaces onboard the aircraft or in the air-conditioning packs when the temperature of the gas is reduced upon leaving the engine (Wolkoff et al., 2015; Chaturvedi, 2011). It is possible that the primary route of exposure investigated, namely the inhalation of gas phase or entrained contaminants, provides only a portion of the dose of these organophosphates and other semi-volatile compounds.

Monitoring of aircraft and aircrew is limited, especially when considering acute exposures. Schindler et al. (2012) collected 332 urine samples from pilots and flight attendants of commercial aircraft after the presence of visible smoke, mist, or unusual smells were reported; these events of potential greater exposure are referred to as fume events. No ortho-TCP metabolites were found to exceed limits of detection, but metabolites of other compounds were detected and associated to the flame retardant fixtures aboard the aircraft and the aircraft hydraulic fluid. This study’s findings have been disputed, stating that the three metabolites that were examined were inappropriate/insufficient and that no fume events were officially documented for any of the flights described, among other issues (Anderson, 2014). Likely, the closest that anyone has come to sampling during a reported fume event occurred during a study conducted by (Solbu et al., 2011). A propeller-driven aircraft was grounded following a fume event; testing then took place, operating the faulty engine at full thrust for 30 minutes prior to its replacement. During this period, a series of thermal desorption tubes, oil aerosol and organophosphate sampling devices were placed on the aircraft. The results demonstrated an order of magnitude elevation in TCP concentration when the engine was running with the leak as compared to after the engines replacement. A mean concentration of 5.1µg/m^3 total TCP was determined; no ortho isomers of TCP were detected (Solbu et al, 2011).

As described, the aircraft environment is chemically complex. Potential risks to human health have been associated with chemical pollution in the aircraft cabin. This has been linked to the pressurization process, as in most cases, the mechanism requires air bled from the engines to perform the task. This creates a potential pathway from the engines, to the pneumatic system, and ultimately to the cabin and human receptors. On-aircraft sampling and subsequent chemical analysis have shown this is likely to be a viable pollution pathway, however many questions still remain. The aforementioned bleed air pathway has not been fully validated, there are many scientific papers published on the subject but the findings are conflicting. A systematic review is required to assimilate this wealth of information and form informed conclusions. An examination of the theorized contamination source, namely aircraft engine oil and the additives within, has been completed on fresh oils. But a detailed assessment has yet to be completed on oils that have actually been used on aircraft, this would allow for questions related to the effectiveness of transfer of the contaminants to the pneumatic system. Other potential exposure pathways to the receptors have not been examined, such as pneumatic system contamination of potable water on board aircraft. Additionally, the majority of studies on the cabin environment conducted to date have involved targeted analysis for specific pollutants. There is a need to undertake non-targeted analysis to better understand the chemical exposome. Bleed-free aircraft have now been in operation for several years and so comparison of the chemical environment in bleed free and bleed air pressurized cabins would help validate if bleed air is a significant pathway for exposure.

This work aims to understand better what chemical exposures individuals working in the aircraft cabin are subject to, the implications of these exposures from an

occupational health and safety perspective, and whether these exposures can be associated with pathways from aircraft systems to human receptors.

To achieve these aims, four distinct, but interrelated segments of scholarship comprise the thesis. This takes the form of one systematic literature review chapter and three data chapters. Each of these chapters is written in the form of a scientific manuscript with the intention of publication, and as such, is composed of succinct, self-contained documents with their own methods section, discussion, and conclusions. These chapters within the thesis meet the overarching aims as follows:

Chapter 1 –

This chapter is composed of a systematic review of available literature discussing the complexity of the aircraft exposome and implications of existing on-aircraft sampling, biomonitoring studies, benchtop assessments, and largescale cohort studies of pilots and flight attendants. This examination of the academically published literature allows us to determine the foundational work that has been completed and allows us to identify gaps in knowledge. Completing a critical examination of the literature allows us to examine occupational risk of chemical exposures within aviation, and doing so in a systematic way allows us to avoid and assess bias that is very prevalent in the field.

Chapter 2 –

This chapter contains an elemental assessment of new and used aircraft engine oil. This research demonstrates a loss with use of contaminants of concern, by proxy, from the theorized contaminant source. It also identifies new potential exposure routes, to known and previously unaddressed contaminants of concern, that could occur during recreational flight, aircraft maintenance, or pilot training. The findings of this chapter change the understanding of how and when exposures to contaminants of concern in aviation can occur, and help us to realize previously unaddressed occupational risk.

Chapter 3 –

This chapter examines and validates a novel exposure pathway tied to aircraft pneumatic systems but unrelated to cabin pressurization. We examine samples drawn from the potable water system onboard aircraft with high-resolution liquid chromatography-mass spectrometry. This suspect screening demonstrates a viable exposure pathway and tentatively identifies contaminants of concern not previously identified on aircraft. Although preliminary, this chapter alters a paradigm of the safety of potable water on aircraft being related to primarily microbiological contamination to one that should also consider chemical infiltration. The interconnectedness of the pneumatic systems on aircraft is demonstrable in this chapter of creating a contaminant pathway to human receptors that was previously unrealized.

Chapter 4 –

In the final data chapter, a comparison between bleed air-pressurized aircraft and non-bleed air-pressurized aircraft utilizing non-targeted analytical techniques is completed. This comparison allows for further validation of the bleed-air cabin pressurization exposure pathway and enhances the understanding of the chemical contaminants present within the aircraft exposome based on the pressurization method used. This information can then be applied to the aircraft. Mitigation methods may be established for known contaminants, and known contaminants that are unique to a particular pressurizations system could be beneficial to future work involving cohorts of pilots and flight attendants who flew on respective aircraft types.

Chapter 5 -

Following the data chapters, conclusions and future work are presented. This chapter discusses the successes of the work completed in meeting the aims as described, and details the additional research that will be conducted in the near future to satisfy questions raised while completing this work.

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RESEARCH DISSEMINATION

Presentations and publications

Presentations

1. **Hayes, K.,** Megson, D., and O'Sullivan, G. (2019). Aerotoxins and wipe sampling workshop. *International Network of Environmental Forensics Conference*. Honolulu, Hawaii, USA. June 12th. Oral Presentation.

2. **Hayes, K.** (2022). Source delineation of organo-metallic/ phosphorus contaminants posing occupational risk in aviation. *42nd International Symposium on Halogenated POPs – Dioxin*. New Orleans, Louisiana, USA. October 10-12th. Poster Presentation.

3. **Hayes, K.** (2023). Contextualizing occupational risk of organophosphates and other chemical and radiative exposure in the aircraft cabin: A systematic review. *Symposium National Advisory Group Cabin Air: Nationale Adviesgroep Cabinelucht.* Virtual – Amsterdam, Netherlands. (Oral Presentation.)

4. **Hayes, K.** (2024). Chemical characterization of the aircraft cabin environment utilizing GCxGC-ToFMS and hard and soft ionization. *15th Multidimensional Chromatography Workshop*. Los Angeles, California, USA. January 10th. Oral Presentation.

Publications

1. Boegelsack, N., **Hayes, K.,** Sandau, C., Withey, J., McMartin, D., and O'Sullivan G. (2021). Method development for optimizing analysis of ignitable liquid residues using flow-modulated comprehensive two-dimensional gas chromatography. *Journal of Chromatography A.* DOI:10.1016/j.chroma.2021.462495

2. **Hayes, K.,** Megson, D., Doyle A., and O'Sullivan G. (2021). Occupational risk of organophosphates and other chemical and radiative exposure in the aircraft cabin: A systematic review. *Science of the Total Environment.* https://doi.org/10.1016/j.scitotenv.2021.148742 (**Chapter 1**)

3. **Hayes, K.** O'Sullivan, G., Doyle, A., and Megson, D. (2021) Reply to letter to the editor RE: Hayes K, Megson D., Doyle A, O'Sullivan G. Occupational risk of

organophosphates and other chemical and radiative exposure in the aircraft cabin: A systematic review. *Science of the Total Environment.* DOI: 10.1016/j.scitotenv.2022.155509

4. O'Sullivan, G., **Hayes, K.,** Ursino, A., and Hart, E. (2023). Variation in metal concentration across tree organs and stands: implications for reference specimen development. *Journal of Environmental Exposure Assessment.*

5. **Hayes, K.,** O'Sullivan, G., McKendry, D., Boegelsack, N., Doyle, A., and Megson, D. (Under Review). Analysis of fresh and used aircraft oil: An indication of exposure pathways to inorganic and organic pollutants. *Journal of Occupational and Environmental Hygiene.* (**Chapter 2**).

6. **Hayes, K.,** Megson, D., Fries, E., Sühring, R., Roberts, G., Doyle, A., and O'Sullivan, G. (Under Review). Hydraulic oil infiltration into potable water through aircraft pneumatic systems: A qualitative assessment of chemical contamination. *Environmental Science: Processes and Impacts.* (**Chapter 3**).

7. **Hayes, K.,** O'Sullivan, G., Doyle, A., and Megson D. (Under Review). Bleed air-pressurization of aircraft demonstrates increased chemical contaminant loading: Preliminary results of a quasi-non-targeted analysis. *Science of the Total Environment,* (Short Communication). (**Chapter 4 – Supplementary Information**)

1.0 A SYSTEMATIC REVIEW OF LITERATURE

Author Contributions Statement

- Conceptualization: Kevin Hayes, David Megson, Gwen O’Sullivan
- Methodology: Kevin Hayes
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This chapter was published in Science of the Total Environment in 2021, titled: Occupational risk of organophosphates and other chemical and radiative exposure in the aircraft cabin: A systematic review. PRISMA 2009 was selected as the systematic review protocol for this chapter because other systematic reviews in related fields (insecticides on aircraft, sleep patterns of aircraft workers, etc.) were conducted using this system (Pang et al., 2020; Bendak and Rashid, 2020). The final search terms, as they appear in the chapter, went through several iterations. The structure of the search terms (Keywords and Boolean operators) were selected so as to be in agreement with each of the databases queried. Each search term was optimized to deliver the maximum number of relevant manuscripts with the help of a research librarian and each of the co-authors. The databases were queried without specified fields and without restrictions other than those noted within the systematic review.

Limitations of this systematic process are still evident. Our search terms were unable to identify all relevant manuscripts within the field, including twenty-two papers that were added for completeness. Additional papers beyond those twenty-two could have also been included should we have optimized our search terms further or widened our scope to additional databases. In hindsight, this completeness issue may have been partially mitigated by compiling a list of target papers and comparing our search recovered manuscripts against them; this is a required completion step for an alternative systematic review process, ROSES, and would have allowed us to mathematically assess the completeness of our review.

1.1 Abstract

Occupational exposure to oil fumes, organophosphates, halogenated flame retardants, and other volatile and semi-volatile contaminants is a concern within the aviation industry. There is no current consensus on the risk attributed to exposure to these chemical classes within the aircraft cabin. Contaminant concentrations rarely exceed conventional air quality guidelines, but concerns have been raised about these guidelines' applicability within the aircraft environment. This systematic review, the largest and most comprehensive completed to date on the subject matter, aims to synthesize the existing research related to chemical and other exposures inside the aircraft cabin to determine the occupational risk that may be attributed said exposure, as well as, determine knowledge gaps in source, pathway, and receptor that may exist. The Science Direct, Scopus, and Web of Science databases were queried with five search terms generating 138 manuscripts that met acceptance criteria and screening. Several potential areas requiring future examination were identified: Potable water on aircraft should be examined as a potential source of pollutant exposure, as should air conditioning expansion turbines. Historical exposure should also be more fully explored, and non-targeted analysis could provide valuable information to comprehend the aircraft cabin exposome. Occupational risk under typical flight scenarios appears to be limited for most healthy individuals. Contaminants of concern were demonstrated to be extant within the cabin, however the concentrations under normal circumstances do not appear to be individually responsible for the symptomologies that are present in impacted individuals. Questions remain regarding those that are more vulnerable or susceptible to exposure. Additionally, establishing the effects of chronic low dose exposure and exposure to contaminant mixtures has not been satisfied. The risk of acute

exposure in mitigatable fume events is substantial, and technological solutions or the replacement of compounds of concern for safer alternatives should be a priority.

1.2 Introduction

The International Labour Organization, a specialized agency under the United Nations umbrella, estimates that a worker dies directly, or resultant from, toxic chemical exposure every twenty seconds (United Nations, 2018). Globally, occupational health and safety legislation provide workers and employers with rights and responsibilities for safe work. For example, the Canadian Occupational Health and Safety Act (S.N.B. 1983, c. O-0.2) legislates three rights that workers have regarding their employment; the right to refuse what is perceived to be unsafe, the right to participate in safety-related decision making, and the right to be informed regarding potential or actual dangers present in the workplace (Government of Canada, 2021). In the United States of America, the 1970 Occupational Safety and Health Act (91-596) asserts that employers must provide “employment and a place of employment which are free from recognized hazards that are causing or are likely to cause death or serious physical harm to his employees” (Occupational Safety and Health Administration, 2004). It is evident that occupational hazards are associated with flight for pilots and flight attendants: these include increased dosage of cosmic radiation, circadian rhythm disruption, mild hypoxia, low humidity, increased ozone concentration with associated reaction products, and potential exposure to a host of volatile organic compounds (VOCs) (Wilson et al., 2003; Waters et al., 2009; Harrison and Mackenzie Ross, 2015; Wolkoff et al., 2015). Many of these risks are innately coupled with flying at altitude, such as increased exposure to cosmic radiation. Others are similar to working in other indoor environments, such as exposure to a quantity of VOCs. However, it is theorized that the method of pressurization of most aircraft cabins, bleed air systems, may add occupational risk not innately linked to flight or work in other environments. It is principally for this that an assessment must be made regarding the chance of aircrew members becoming injured or ill.

The majority of modern jet aircraft utilize air bled from the engines to perform several tasks essential for high altitude flight. Cabin and hydraulic system pressurization, engine cowl and wing deicing, windshield rain and ice protection, engine thrust reversers, and pressurizing the potable water and waste systems on the aircraft are all completed in this manner (Moir and Seabridge, 2008). On most modern commercial aircraft, the air is drawn into the engine, entering several compression stages before adding fuel and the mixture's combustion. Pressures and temperatures within the compression sections are highly variable, depending on the location and engine operating conditions (Moir and Seabridge, 2008). It is from this compression section, prior to fuel addition and combustion, that air is drawn into the aircraft. Due to the variable pressures within the compression stages air typically drawn from two stages: A high pressure port is located further back in the engine, which is used when engines are operating at low settings, such as idle, taxi, late cruise, and portions of landing, and a low pressure port is located more forward in the engine and is used during high engine requirement, such as takeoff and the majority of cruise (Moir and Seabridge, 2008; NRC, 2002). Bleed air entering the aircraft is then mixed with recirculated air and then introduced to the cabin (NRC, 2002). In conjunction with the environmental control system (ECS), the bleed air system provides relatively consistent pressure and quantity of fresh air that can be supplied to the aircraft at any engine speed. The ease, efficiency, and availability of this pressurized gas make it invaluable for the numerous functions. Even so, the interaction between the air and the engines before entering the cabin has been identified as a possible source of contamination (Michaelis, 2017).

Bleed air contamination may occur when seals, bearings, and hydraulic components fail, allowing intact and pyrolyzed oil and additives to enter the cabin (Michaelis, 2018). Additionally, the seals used within aircraft engines are typically designed to “leak” oil at low levels (Michaelis, 2018). The air's pathway from the engines to the aircraft's cabin and cockpit is typically not filtered and is not generally monitored for contamination (Hunt et al., 1995; Harrison and Mackenzie Ross, 2015). Commercial aircraft engine oil often contains isomers of tricresyl phosphate (TCP), used as an anti-wear agent and flame retardant at approximately 3% by volume (Winder and Balouet, 2002). TCP, particularly the ortho-substituted isomers, are known to be neurotoxic (Petroianu, 2016). It is the suspected exposure of pilots and flight attendants to this compound, as well as an unknown aggregation of other contaminants found within the engine oil, deicing fluid, hydraulic fluids, and flame-retardant materials, followed by potentially resultant symptomology, that has led to the coining of the term “Aerotoxic Syndrome” to describe occupational illness on aircraft (Winder and Balouet, 2000). Acute onset and chronic symptoms that have been associated with occupational exposure include irritation of the eyes, nose, and throat, disorientation, headaches, dizziness, numbness, cardiovascular concerns, tremors, and cognitive problems (Winder and Balouet, 2000). In response to the concern about the neurotoxicity of Tri-ortho-Cresyl Phosphate (ToCP), concentrations have been reduced in oil formulations resulting in the absence of detectable levels of ortho isomers of TCP in new or used aircraft oil (Winder and Balouet, 2002; Megson et al., 2016, Megson et al., 2019). ToCP, other TCP isomers, other organophosphates (OPs), and VOC concentrations on monitored flights have been reported at concentrations well below traditional safety guidelines, often falling below limits of detection; this has led to the belief by some that “Aerotoxic Syndrome” may not be an occupational illness (Wolkoff et al., 2015; de Ree et al., 2014).

At issue with this determination is the limited and conflicting data regarding air contamination during fume, smoke, and smell events and the impacts of chronic low dose exposure. Major fume events occur when a seal within an aircraft engine fails in some manner; this allows oil into the compression section of the engine in sufficient quantities to rapidly enter the aircraft through the bleed air system. The majority of studies to date have not been able to sample fume events, although the concentrations of many contaminants have been estimated (Wolkoff et al., 2015; de Ree et al., 2014; Harrison and Mackenzie Ross, 2015). Fume events are expected to produce the highest concentrations of contaminants in the cabin (Solbu et al., 2011), but such events' random occurrence makes practical sampling very difficult. Shehadi et al. (2015) calculated the average frequency of fume events as 2.1 incidences per 10,000 flights, and the maximum reported incidence, by aircraft type, per flight was 7.8 per 10,000. This creates a significant temporal and financial challenge in collecting a statistically relevant number of fume event samples. However, contamination of the cabin and cockpit may occur without a detectable fume event occurring. Several studies imply that low-level contamination of cabin air occurs in the absence of noted fume events. There may be additional TCP sources on aircraft, the impacts of which are still in question (de Ree et al., 2014; Crump et al., 2011). There is no current consensus on the risk attributed to chemical exposure within the aircraft cabin. This review implements a systematic approach to examine, summarize, and critique the available literature; this is completed to determine if a significant occupational risk exists that may be related to the chemical compounds that are extant on aircraft due to manufacturing and safety requirements, including organophosphate/brominated flame retardants and off-gassing of products and/or compounds that are introduced to the aircraft cabin through the bleed air system of the aircraft such as those found within engine oil and pyrolyzed oil products, ozone, and particulate matter. Additionally, other sources of concern and potential confounding variables are examined including relative humidity on aircraft and cosmic radiative dosing. A holistic approach is taken including characterization of the contaminants present on aircraft by direct measurement (Section 3.1), and modeling and laboratory experimentation (Section 3.2). Also assessed are the potential health consequences of exposure in animal and biomonitoring studies (Section 3.3), and the reported health effects reported by aviation employees on an occupational scale (Section 3.4). The review is completed to identify discrete knowledge gaps within this research area and provide a comprehensive understanding of occupational risk as it applies to work within the aircraft cabin.

1.3 Methods

1.3.1 Search parameters and resource identification

The present systematic review follows the 2009 PRISMA guidelines (Moher et al., 2009) to identify research articles on occupational risk in aircraft (Figure 1). The systematic review employed five search terms:

((“aerotoxic” AND aircraft) AND (organophosphate OR occupational OR exposure OR neurotoxic OR psychosomatic OR symptoms)).

((“cabin air quality” AND aircraft) AND (fumes OR smoke OR oil OR mist OR particles OR sulfur OR metals OR “flame retardant” OR pesticide)).

((“aircraft engine oil”) AND (“tricresyl phosphate” OR tcp OR tocp OR tmcp OR tpcp OR bleed air OR hydraulic OR potable OR pyrolyzed OR tnap)).

((“flight crew” AND “chemical exposure” NOT “Space”) AND (inhalation OR absorption OR ingestion OR illness OR complaint OR death OR flight hours OR cohort)).

(“sample collection” AND “aircraft cabin” NOT tobacco).

Each search term reflects themes within the literature, briefly stated as occupational exposure and symptoms, contamination events, source delineation and contaminants of concern, exposure pathway and duration, and sample collection. The words “tobacco” and “space” were excluded within search terms 4 and 5 respectively due to large numbers of irrelevant results generated. Each term was searched for within three databases with no temporal restrictions: Science Direct, Scopus, and Web of Science, generating 285, 212, and 78 results, respectively, for a total of n = 575 manuscripts (Figure 1). Searches were conducted up to January 15, 2021.

1.3.2 Acceptance criteria and screening

Three acceptance criteria were applied; 1) Papers must be in the English language due to author fluency, 2) Full-text availability and 3) Duplicate Removal. Initial screening involved a title review (removing n = 102), abstract review (removing n = 76), and finally, a full-text review (removing n = 23). Each of the full text removals was at the author's discretion (KH, following consultation with DM and GoS), based on resource quality (n = 3), and the relevance to this manuscript (n = 20) Following the screening, commonly cited manuscripts from within the systematic review and the general field were included for completeness (n = 22) (SI-Table 1). Results included a total of 138 manuscripts that are analyzed. Finally, these manuscripts were subdivided into two categories:

Experimental and Review. Experimental manuscripts were further classified into four subgroups (Figure 1). Resources that fit more than one category were placed in each to which they belonged for meta-analysis.

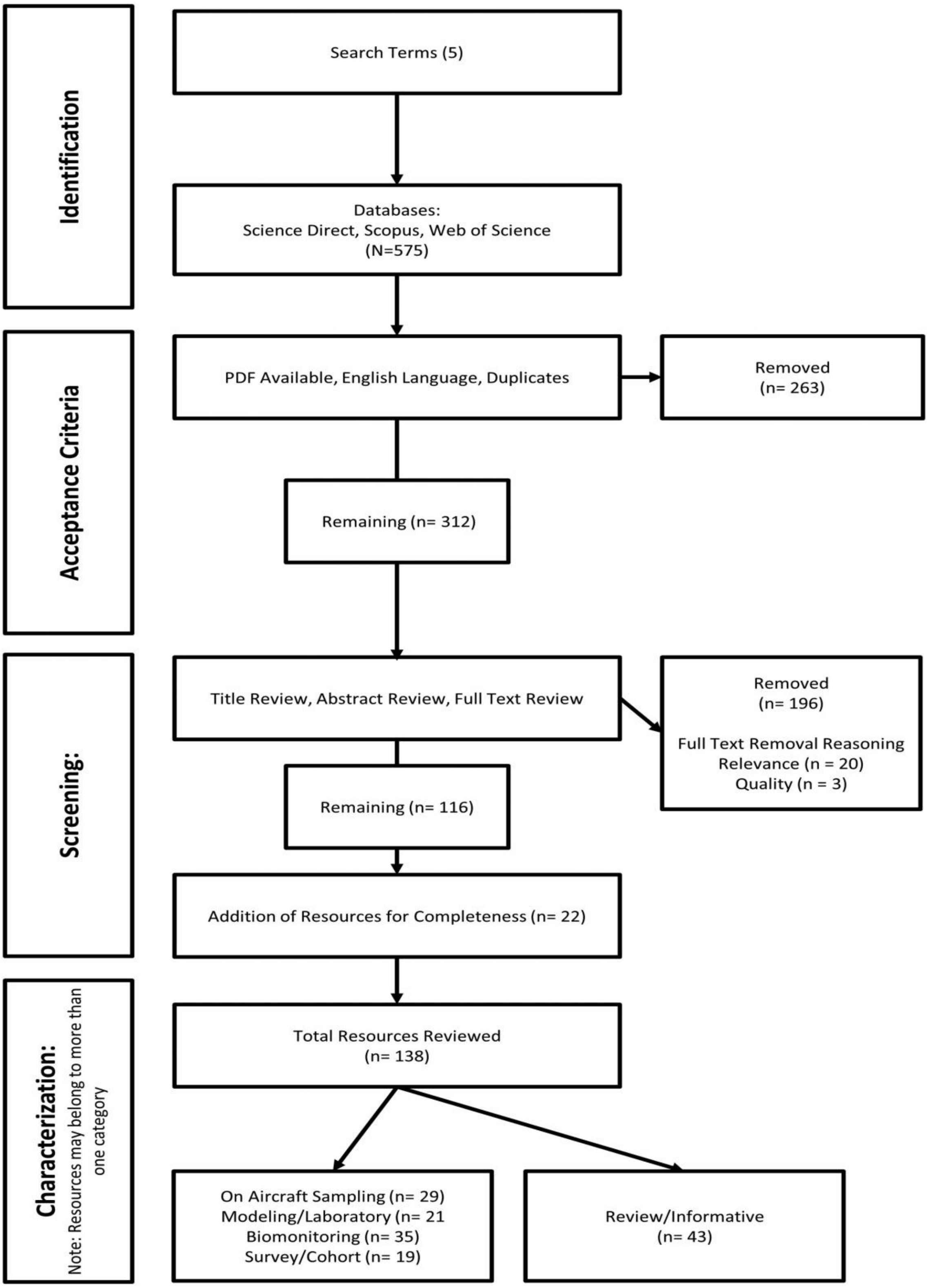


Figure 1. Abbreviated methods describing the acquisition and screening of identified manuscripts.

1.4 Results and Discussion

One hundred thirty-eight manuscripts were reviewed in this study, spanning slightly over three decades of research. Grouping the manuscripts by decade, 1990–1999 (n = 8), 2000–2009 (n = 26), 2010–2019 (n = 88), and 2020–2021 (n = 17), the progression of the field may be observed. The research in the 1990s was principally related to tobacco smoke or radiative dose. The first manuscript considering bleed air as a potential source of contamination on BAe-146 aircraft was Van Netten (1998). In the 2000s, research turned towards symptoms of aircrew and passengers and cabin air quality. The impact on circadian rhythm crossing time zones and ozone in the cabin were also prominent in this period. The first biomonitoring experiment on chromosome aberrations and translocations linked to cosmic radiation exposure was also reported (Heimers, 2000; Yong et al., 2009). This period also included early discussions on jet oils' potential toxicity and the term Aerotoxic Syndrome's coining (Winder and Balouet, 2000, Winder and Balouet, 2002). The 2010–2019 decade saw increases in airplane cabin sampling experiments, biomonitoring studies related to chemical exposure on aircraft, and increased focus on TCP isomers. The most recent research (2020−2021) is largely focused on demonstrating susceptibility to OP exposure via genetic mutation, impacts of exposure, and possibly potential treatments for chronic effects. This review will examine the field's evolution through aircraft cabin sampling, biomonitoring, cohort/ survey studies, and laboratory experiments in upcoming subsections.

1.4.1 Chemical characterization of the on-aircraft cabin environment

Concern has been raised that research has not adequately confirmed that health impacts result from chemical contamination of the aircraft cabin, primarily based upon the intermittency and lack of severity of exposure (Bagshaw and Illig, 2019). In support of this claim are several studies: Wolkoff et al. (2015), Schuchardt et al. (2019), and de Ree et al. (2015); that suggest there is a limited, if any, chemical contribution occupational risk for aircrew. This is based mainly upon threshold values established for industrial work. Some of the manuscript results are described as conclusive or not meeting the definition of occupationally related disease in the study's respective country. However, the prescribed threshold limits cited in these studies are not explicitly designed for, and may not be adequately suited to, the aircraft environment. Watterson and Michaelis (2017) discuss some of the established threshold limits' failings: They do not consider differences in sensitivities or sensitization of workers, atmospheric pressures, and time of exposure. Additionally, the authors state that threshold limit values (TLVs) are for individual compounds and are not suited for complex mixtures. Multiple sources within the Watterson and Michaelis (2017) manuscript are quoted as stating that TLVs or occupational exposure standards are not well suited to the aircraft environment, including the Aerospace Medical Association, ASHRAE, EASA, aircraft manufacturers, and other industry sources.

A wide range of contaminants of concern have been investigated within aircraft cabins, including tobacco, particulate matter, flame retardants, tricresyl phosphates, and other OPs, smoke, fume and smell events, and volatile organics. Table 1 summarizes the measurement of center values, max concentration, and an abbreviated list of citations for the manuscripts reviewed in this study. More detailed information is available in SI-Table 2. In the following sections, we will explore the literature around each of these contaminants of concern.

1.4.1.1 Tobacco

In some of the earliest manuscripts on cabin air quality (CAQ), tobacco smoking and the resulting particulate matter was the principal agent of concern (Lee et al., 2000; Lindgren and Norback, 2002; Nagda et al., 1992; Wieslander et al., 2000). The manuscripts found in this review included information on the sampling of 138 flights and are summarized in SI-Table 2. Following the smoking ban on aircraft, all studies demonstrated a significantly lower respirable particle count and improved CAQ.

1.4.1.2 Particulate matter

Particulate matter exposure risk on modern flights, post-ban on smoking, are described in 5 manuscripts sampling 148 flights (Table 1; SI-Table 2). The manuscripts related increased particle counts to several factors, including particle size, age of the aircraft, flight phase (departure taxi, takeoff, cruise, landing, arrival taxi), weather, human emissions, following aircraft in flight, and unknown causes. The mean range of PM by flight varied dramatically (Table 1), indicating that some of these factors must play an important role. Particle size played a predictable role in particle count, increasing with decreasing diameter. Flight through clouds or trailing aircraft, presumably drawing engine emissions and particles contained within the clouds through the bleed air system, demonstrated large particle count increases. Cruising appears to demonstrate low particle counts, while taxiing causes higher counts. Turbulence also increases particle count, possibly shaking loose particulate that otherwise would remain entrained within the ECS of the aircraft. Particulates that remain airborne within the cabin are likely to be brought through the aircraft's recirculation system, including HEPA filtration, decreasing counts by volume. This, coupled with deposition, is likely responsible for the temporal spike nature of the measurements. Sustained high counts have been demonstrated to exist when a steady source of particulates enters the cabin via the bleed air system. This issue will likely not be resolved on bleed-less aircraft, pending filtration of the pathway, as outside air is still required to pressurize the cabin. For a complete description of the measurement of center values and particle size breakdown, see SI-Table 2. Chemical qualification of the particulate matter was lacking in most cases within the reviewed manuscripts. The sample collection primarily involved continuous monitoring, determining counts by volume but neglecting to determine particle composition (SI-Table 2). Dust and wipe sampling (3.1.3 Flame retardants, 3.1.4 Tricresyl phosphates and other organophosphates) have demonstrated that OPs and brominated flame retardants are present as PM, emphasizing the need for qualification (Table 1; SI-Table 2). Chaturvedi (2011) refers to a 2004 U.K. study in which black carbon-like deposits were found in cabin air supply ducts. This carbon-like material contained many VOCs and semi-volatiles (SVOCs) such as TCP isomers and tris(methyl-phenyl) phosphate (TMPP). It was determined that this substance was easily dislodged; however, the semi-volatiles only became available following solvent extraction. Fouling of the palladium catalyst to remove ozone from the air was also noted (Farrauto and Armor, 2016). Early designs of the catalyst were fouled by elements not expected to exist in the high-altitude air. These included sulfur, phosphorus, silicon, chlorine, sodium, and calcium, positively correlated with increasing flight time (Farrauto and Armor, 2016). Silicon was determined to have originated from o-rings within the engine; sodium and chlorine may be explained by saltwater mist; phosphorus and traces of zinc result from traces of oil vapor used to lubricate equipment. The sulfur collected on the catalyst may be due to the emission from other aircraft or deicing procedures (Farrauto and Armor, 2016). Deicing before takeoff can contribute to elevated contamination within the cabin (Rosenberger, 2018).

1.4.1.3 Flame retardants

Manuscripts by Allen et al. (2013b), Allen et al. (2013a), and He et al. (2018) explore the presence of flame-retardant compounds through the sampling of 83 flights or aircraft

(SI-Table 2). Dust sampling demonstrated a much greater median loading and detection percentage of most PBDE congeners compared to air monitoring (Table 1: SI-Table 2). In general, PBDEs and OP flame retardants' loadings were higher on aircraft than in offices or homes, emphasizing PBDE 209, tributyl phosphate (TnBP), and TMPP (He et al., 2018; SI-Table 2). While air sampling demonstrated median and max values significantly lower than exposure thresholds described in Allen et al. (2013b), the much larger loadings in dust and potentially related exposure routes need to be further investigated to determine potential risk.

1.4.1.4 Tricresyl phosphates and other organophosphates

TCP isomers have been considered important when describing occupational risk on aircraft and were the principal contaminants of concern in eight manuscripts found in this review, summarizing the sampling of 400 flights (Table 1). When conducting active air monitoring, TCP is found rarely within aircraft, and when found, it is within the low µg m−3 range (Table 1; SI-Table 2). Other OPs such as TnBP and dibutyl phenyl phosphate (DBPP), typically linked to hydraulic oil, are more common, found in 100% and 92% of relevant samples in Solbu et al. (2011; SI-Table 2). The tri-ortho cresyl phosphate isomer (ToCP) has been the focus of several studies but is rarely reported in the aircraft environment. This may be because it was largely removed from jet oil formulations, and it is unlikely that engine conditions or catalysis will result in trans-isomerization (Megson et al., 2019). ToCP was reported in only one of the found manuscripts, with a max concentration of 22.8 µg m−3 (Crump et al., 2011). TCPs, excluding ToCP, are more commonly found when sampling passively, via wipe sampling, or sampling filters onboard aircraft (SI-Table 2). This is due to the isomers' low vapor pressure and a tendency to not remain in the air phase within the environment, raising suggestions from de Ree et al. (2014) that the compounds may become fixed within the ducting of the ECS and released sporadically in particulate form (see Section 3.1.2). Another exposure route that is yet to be explored fully is ingestion. Moir and Seabridge (2008) describe that the water systems, including potable onboard aircraft, are pressurized by the bleed air system. Cleaning of the water system is infrequent and may be a reservoir for contaminants. The potable water onboard aircraft is understudied in terms of contaminant of concern loadings. We feel that the fact that the potential pathway exists, unfiltered, from the engines to the storage tank warrants a deeper look into the potential issue. The evidence for further study is indicated by contaminants matching the chemical fingerprint of engine oil being found in the cabin; if contaminants can be found within the cabin, there is no reason to suppose that they should not be able to reach the potable water tanks on many aircraft. It is likely a minor issue for most workers, but hot beverages, sometimes drinking water, and cleaning procedures use this reservoir.

1.4.1.5 Smoke, fume, and smell events

Smoke and smell events indicate increased contaminants but may not be a strong indicator of TCP entering the aircraft. Smoke was seen in several cases within Denola et al. (2011). While an incidence of smoke did lead to maximum TCP loadings of all studies included (Table 1), several other noted smoke events did not generate high loadings; for example, the second-highest TCP loading completed within Denola et al. did not have a smoke event. Smell events are likewise not consistent indicators of TCP contamination. Within Schuchardt et al. (2019), all high TCP loadings' incidences did not correlate to 17 recorded smell events. However, this does not imply that TCP concentrations are not a good indicator of potential oil leaks. In Solbu et al. (2011), an aircraft with a known oil leak was sampled, and TCP concentrations increased a hundred-fold over other TCP loadings within the study. Chaturvedi (2011) describes 15 non-fire aviation incidents with 17 fatalities from 1991 to 98. Some of these are linked to CO poisoning/ incapacitation citing exhaust malfunction as the significant cause; this review also cites a manuscript that describes many of the deaths associated with accidents in 1981 that were suspected of having been caused by contamination of the ECS (turboprop aircraft).

Additionally, TCP found in de Ree et al. (2014) shared a similar signature to the aircraft engine oil, and Schuchardt et al. (2019) also acknowledge that the compounds may be useful in providing evidence of oil leaks on bleed aircraft. Schuchardt et al. (2019) raise questions in that TCP was also found on the bleed-free Boeing 787; the authors tentatively source the contaminants in these cases to the compound's background levels within the aircraft environment. However, nondelineated sources may provide clarity to this claim. Likely the most prominent potential source that has been somewhat overlooked thus far are the air conditioning packs. Wright et al. (2018) describe that air conditioning systems are expected to be removed from aircraft at 18-month intervals for maintenance but require servicing following 3–9 months of use. This is “directly attributed to a fouling buildup on the pack PFHE," indicating that bleed air containing contaminants enters the system where the contaminants are deposited on the plate-fin heat exchangers. This deposition is not the principal issue of concern as it indicates that many of the contaminants will not reach the cabin. The air conditioning system on large commercial aircraft involves air passing through heat exchangers, a compression section, and a turbine used for expansion cooling within the air conditioning pack. Turbines require lubrication; Aviation Structural Mechanic E 1 and C by Arthur R. Paulsen Identifies this oil as meeting Mil-L-23699 specifications. This location is directly before air being sent to the mixing unit and entering the cabin. All air that passes through this section is destined to the aircraft's interior, as opposed to the vast majority of air that passes through the engines without being bled to the pneumatic system. This may imply that a small leak on a malfunctioning air conditioner pack turbine could be significantly more impactful in contamination loading than a similar leak within the engines. Ideally, future study would be able to isolate this system by sampling the air directly up and downstream while in operation. Several aircraft models should be analyzed. This would allow for the quantification of the contribution that the pack expansion turbines may be making to the contaminant loading onboard the aircraft.

The concern of fume and smoke events has not lessened; while the EASA and FAA generally consider cabin air safe for most people, they have acknowledged that risk may be present. The FAA issued an alert for operators in 2018 that calls for enhanced procedures to ensure the safety of flight crew and passengers in the event of fumes or smoke entering the cabin (Michaelis, 2017).

Table 1. An abbreviated description of on aircraft sampling manuscripts from within the systematic review. Mean and Median (denoted by *) composed of the measurement range of all relevant manuscripts. Max value is the maximum value individually within all relevant manuscripts. Unit Changes (denoted by **) used temperature = 25 °C, Pressure = 760 hPa (8000 ft. equivalent).

Contaminent of Concern	Range of Measure of Center	Max Value	Literature
PBDEs (air sampling)	<0.4 - 1.3ng/m3 *	2100ng/m3	Allen et al, 2013
PBDEs (dust sampling)	20 - 495000ng/g*	2600000ng/g	Allen and Stapleton et al, 2013
TVOCs	7ug/m3 - 4ppm*	>10ppm	Crump et al, 2011; Guan et al, 2015; Rosenberger, 2018; Rosenberger et al, 2016; Solbu et al, 2011; Wang et al, 2014
Carbon Monoxide	<LOD - 3ppm	>5ppm	Crump et al, 2011; Lee et al, 2000; Nagda et al, 1992; Rosenberger, 2018; van Netten, 1998
Carbon Dioxide	520 - 2700ppm	5177ppm	Giaconia et al, 2013; Guan et al, 2019; Guan et al, 2015; Lindgren and Norback, 1991; Lee et al, 2000; Li et al, 2014; Lindgren et al, 2007; Nagda et al, 1992; Rosenberger, 2018; van Netten, 1998; Wieslander et al, 2000
Ozone	<LOD - 117ug/m3**	302ug/m3**	Lindgren and Norback, 1991; Lee et al, 2000; Nagda et al, 1992; Rosenberger, 2018; Rosenberger et al, 2016; Spengler et al, 2004
TCPs (air sampling)	<LOD - 2.9ug/m3	51.3ug/m3	Crump et al, 2011; Denola et al, 2011; de Ree et al, 2014; Rosenberger, 2018; Rosenberger et al, 2016, Solbu et al, 2011; van Netten, 2009; van Netten, 1998
TCPs (wipe sampling)	<LOD - 1.15 ng/dm/day	8.3ng/dm/day	de Ree et al, 2014; Solbu et al, 2011
ToCP	<LOD - 0.07ug/m3	22.8ug/m3	Crump et al, 2011; Denola et al, 2011; de Ree et al, 2014; Rosenberger et al, 2016; Solbu et al, 2011
Particulate (UFP) & <1.0um	417 - 100000 counts/cm3	>500000counts/cm3	Crump et al, 2011; Guan et al, 2019; Li et al, 2014; Lindgren et al, 2007; Zhai et al, 2014
Cosmic Radiation	1 - 4 mSv annual	4.69 mSv annual	Lewis et al, 1999; Verhaegen and Poffijn, 2000

1.4.1.6 VOCs

Twelve manuscripts focus on VOC qualification and/or quantification (Crump et al., 2011; Guan et al., 2014a; Guan et al., 2014b; Guan et al., 2015; Lindgren and Norback, 2002; Lindgren et al., 2007; Rosenberger, 2018; Rosenberger et al., 2016; Schuchardt et al., 2019; Van Netten, 1998; Wang et al., 2014). The sampling of 524 flights is represented within this data (SI-Table 2). In general, VOC concentrations were lower inside the aircraft cabin as compared to other indoor locations. Guan et al. (2014b) found only five compounds that exceeded airport terminal concentrations onboard aircraft, namely limonene, nonanal, acetone, tetrachloroethene, and octanal. The low values are emphasized by Schuchardt et al. (2019), who reported that higher VOC and aldehyde concentrations are found in kindergartens. Unlike a typical kindergarten, however, bleed air contamination events provide an intermittent source that may increase VOC concentrations significantly over short periods. Van Netten (1998) noted several VOCs present in an aircraft (BAe-146) grounded due to fume issues that were not present in blanks; Rosenberger (2018) noted VOC concentrations that briefly exceeded German indoor air quality thresholds following a wing deicing procedure. This variability is described in (Table 1) where Total VOCs (TVOCs) measurement of center values range significantly. Unlike particulates, VOC concentrations tend to peak during the cruising phase of the flight; this has been attributed to the passengers on board or food service; Guan et al. (2015) describe that the VOC concentrations within the cabin are only minimally sourced to bleed air during flight (10%). This is contradicted in Wang et al. (2014), in which a source apportionment of the VOCs found indicated that 34% of the compounds were resultant from fuels, non-fuel oil, and combustion products. Additionally, the authors note a significant (15%) contribution from ozone reaction products. VOC reduction appears to be possible. Both Rosenberger (2018) and Schuchardt et al. (2019) noted that activated carbon filters in the recirculation air pathway decrease VOC concentrations on aircraft. For a more complete list of individual VOC loading, see SI-Table 2.

1.4.1.7 Other concerns

Studies also took place to principally determine the humidity (Giaconia et al., 2013), ozone (Spengler et al., 2004), the presence of magnetic fields (Nicholas et al., 1998a, Nicholas et al., 1998b), or cosmic radiation on aircraft (Lewis et al., 1999; Verhaegen and Poffijn, 2000). Relative humidity (RH) on aircraft is generally lower than other indoor environments and is consistently one factor that does not conform to guidelines. It is not uncommon for RH to drop below the 20% recommended by ASHRAE during the cruising phase of flight (Giaconia et al., 2013; Lee et al., 2000). Symptoms such as eye and throat discomfort may be due to this dryness and often improve when humidification is present (Lee et al., 2000; Lindgren et al., 2007). Humidification is not always possible on aircraft as increased weight, and corrosion issues make the prospect prohibitive. The concentration of the reactive gas ozone is enhanced at altitude; commercial aircraft typically fly in the lower stratosphere where ozone concentrations are higher than those in the troposphere; to mitigate this, many aircraft are equipped with catalytic converters to degrade the gas phase molecule (Megson et al., 2019; Farrauto and Armor, 2016). The catalysts may not always function as intended, as demonstrated by Spengler et al. (2004). Approximately one-third of the samples on transcontinental and trans-Pacific flight routes exceeded the EPA 8h recommended concentrations for ozone. Cosmic radiation exposure to flight crews appears to fall within traditional safety guidelines for occupationally exposed workers, with a maximum annualized dose lower than 5mSV (Table 1). Though magnetic fields were found to be elevated in the cockpit of aircraft, the impacts may be inconsequential as Nicholas et al. (1998a) describe that the health effects, if any exist, are unknown.

1.4.1.8 Summary and future work

Gaps identified by de Boer et al. (2015) included limited sampling during fume events, lack of mono-ortho substituted TCP analysis, exploration of other compounds which may be pyrolyzed or otherwise, altitude effects of the compounds, and the possible introduction of contaminants via the APU. While some of these gaps have begun to be filled, it is evident that the understanding of the bleed air contamination on aircraft is very much incomplete. The concentrations of compounds of concern on aircraft are described within most reviewed manuscripts as low; however, the full exposome onboard aircraft is undescribed. The bulk of manuscripts have focused on certain organophosphates and VOCs, often due to the availability of suitable standards (SI-Table 2). However, these substances make up only a portion of what one is potentially exposed to onboard the aircraft (Winder and Balouet, 2002). High-Resolution Mass Spectrometry allows for non-targeted analysis (Kauffman, 2014; Cavanna et al., 2018; Megson et al., 2016). This technique allows for the tentative identification of detectable compounds within the exposome without prior knowledge of the compound's existence (Cavanna et al., 2018). This technique could allow researchers to more fully understand what contaminants exist within the aircraft, such as the multitude of potential pyrolyzed compounds present during a fume event, allowing practitioners the ability to identify contaminants capable of entirely or synergistically contributing to the symptoms of the flight crews that have been unidentified to date.

1.4.2 Laboratory experimentation and modeling contaminant concentrations on aircraft

The following describes the examination of laboratory and modeling experimentation detailed within the identified manuscripts. For a completed citation list as well as abbreviated findings, see Table 2.

Table 2 An abbreviated description of modeling and laboratory experimentation manuscripts from within the systematic review.

Author	Concern	Experiment Type	Result
Cao et al, 2014	Cabin air movement	Aircraft Mockup	Air more turbulent when cooling vents closed.
Coleman et al , 2007	Ozone Interaction with surfaces +VOCs	Laboratory Analysis	Ozone decreases with reactions on surfaces; Surface reactions form volatile products.
Isukapalli et al, 2013	Pesticide deposition	Aircraft Mockup	Aisle and center seat areas of the aircraft demonstrated elevated conc.
Ke et al, 2014	Airworthiness guidelines and compliance	Mathematical Modeling	Potentially useful model requiring experimental validation
Lushchekina et al, 2013	Reaction Kinetics- CBDP-BChE	Molecular Modeling	Describes bonding mechanisms and energy requirements for enantiomers
Megson et al, 2016	Chemical changes- new and used aircraft oil	Laboratory Analysis	No ortho-TCP was detected, xylenyl cresyl phosphates present in used oil
Megson et al, 2018	Transisomerisation via catalysis	Laboratory Analysis	TCP is probably not being altered by the palladium catalyst found onboard aircraft
Nicholas et al, 1998	Estimation of radiation dose	Mathematical Modeling	Estimated annual dose between 0.2 and 5.3 mSv
Pan et al, 2019	Particle deposition- Multislot diffuser	Laboratory Analysis	Lagrangian model suitable for prediction of particle deposition velocity.
Pan et al, 2020	Particle deposition- Multislot diffuser improvement	Aircraft Mockup	Surface roughness impacts deposition, nozzles currently fairly smooth, inconclusive
Pan...Dong et al, 2020	Particle deposition- Multislot diffuser improvement	Aircraft Mockup	New nozzle design lessens proximal particle deposition
Rai and Chen, 2009	Ozone Interactions with surfaces	Aircraft Mockup	Increase in surface area/reactive surfaces increases ozone removal efficiency
Sun et al, 2008	Photocatalytic air treatment	Aircraft Mockup	Complete decomposition of some VOCs (Toluene, Ethanol, Isoprene); Intermediate products of other compounds increase significantly
Tamas et al, 2006	Ozone Interactions with surfaces	Aircraft Mockup	Humans responsible for the majority of ozone removal; Used HEPA filters remove more ozone than new
Wu and Ahmed, 2012	Aircraft ventilation method	Mathematical Modeling	Periodic as opposed to constant fresh air supply may improve mean cabin air age. Potentially useful model requiring experimental validation
Zhou et al, 2020	Catalysis of TCP	Laboratory Analysis	Iron (II) Hydroxide or Ruthenium (II) Hydroxide catalysts effective in solution
Zhu et al, 2016	Engine oil toxicity improvement	Laboratory Analysis	Bisphenol AF bis(diphenyl phosphate) (BAFDP) may be an effective replacement for TCP

Modeling airflow and efficiency throughout the cabin and the soiling of air nozzles (multi-slot diffusers) and contaminant deposition and reactions were common themes within this category (Table 2). The soiling of air nozzles discussed by Pan et al., 2019, Pan et al., 2020a and Pan et al. (2020b) were more concerned with the appearance of contamination as compared to concentration, in that preventing deposition on air nozzles will result in suspension or deposition of the contaminants elsewhere in the cabin. Therefore, the problem they are attempting to solve demonstrates CAQ issues. Ozone reactions and removal experimentation were also common (Coleman et al., 2008; Rai and Chen, 2012; Tamás et al., 2006). In general, the findings indicate that increased surface area, especially the presence of passengers, increases ozone removal from the air and contributes to volatile ozone reaction products within the cabin. Catalysis of contaminants within the cabin was also explored; Sun et al. (2008) installed photocatalysts in a mock aircraft cabin. Results indicate that ethanol, isoprene, and toluene were fully photo-catalytically decomposed, but intermediate products of photocatalytic ethanol oxidation such as formaldehyde and acetaldehyde were elevated. Zhou et al. (2020) attempted to develop a catalyst for the degradation of TCP. Iron (II) hydroxide or ruthenium (II) hydroxide catalysts in solution were determined to be capable of limited degradation of the compounds over a relatively short time, perhaps leading to a future in pathway catalyst for bleed air. Additionally, research is seeking to find alternatives to the use of TCP in engine oil. Zhu et al. (2016) sought to determine the effectiveness of bisphenol AF bis(diphenyl phosphate) (BAFDP) as an anti-wear additive. The thermal decomposition of BAFDP begins at approximately 359.8 °C.

The total decomposition occurs at over 800C. TCP begins to decompose at 273.5 °C. The authors determined that a 2% by weight inclusion of BAFDP created the best lubrication properties in pentaerythritol oleate (PETO) as the lubricant mixture. It surpassed TCP in the same concentration in reducing wear experienced during testing. For a summary of other Laboratory/Aircraft Mockup resources included within this review, see Table 2.

1.4.2.1 Summary and future work

Although there has been more of a focus on direct characterization of the aircraft cabin environment (Section 1.4.1), laboratory experimentation and modeling have yielded promising methods for improving safety within the aircraft cabin. While many potential mitigating factors are early in their development, continued work should allow for their implementation on aircraft. Catalysis, filtration, and the exchange of compounds for less harmful alternatives all seem to be promising avenues for reducing occupational risk.

1.4.3 Biological sampling: evidence of chemical exposure

The following subsections examine the potential health consequences to the individual worker should they be exposed to identified onboard contaminants or contaminant mixtures. For a complete timeline outlining progress and the gradual change of experimental focus of biomonitoring manuscripts found within this review, see Figure 2. For more detailed information, see SI-Table 3.

1.4.3.1 Animal exposure studies

Eight studies were identified as animal studies, implying the use of whole animals or tissues; mice or rats or rabbits were the subjects of all studies (Baker et al., 2013; Hausherr et al., 2017; Duarte et al., 2017; Naughton et al., 2018; Naughton et al., 2020; Sibomana et al., 2019; Sibomana and Mattie, 2020; Sibomana et al., 2021). Multiple manuscripts sought to determine the impacts of ToCP exposure (SI-Table 3). ToCP forms the metabolite cresyl saligen phosphate (CBDP) within the body through activation of the Cytochrome P450 enzyme superfamily. CBDP is a potent neurotoxicant demonstrated by resources in this review to be capable of inhibiting BChE, Neuropathy Target Esterase (NTE), impacting the microstructure of neurons, and reducing voltage-gated calcium channels reaction to KCl, on a dose-dependent basis. This is not altogether surprising as ToCP has been known to cause neurological damage for over 100 years (Petroianu, 2016). However, ToCP has mostly been removed from aircraft engine oils and is rarely detected within the aircraft cabin (Table 1; SI-Table 2). Examining the more commonly identified tri-meta isomer has led to the conclusion that while not as potent as the ortho isomers, it also possesses the capability to cause neurological illness despite differing chemical pathways in the animal subjects (Figure 2; SI-Table 3).

In general, the literature suggests that it is not BChE inhibition by ToCP or the other TCP isomers responsible for the complaints/symptoms associated with “Aerotoxic Syndrome” and that another action is more likely responsible. Damage to myelinated axons and decreased axonal transport, which persisted at least 30 days following exposure, was determined in rats following DFP exposure (Naughton et al., 2018; Figure 2). Organ mass has also been demonstrated to be impacted in rats following dermal exposure to engine oil (Sibomana and Mattie, 2020). AChE inhibition arising from dermal exposure of whole oil products on rats has also been demonstrated with a possible sex-linked correlation (Sibomana et al., 2021; SI-Table 3).

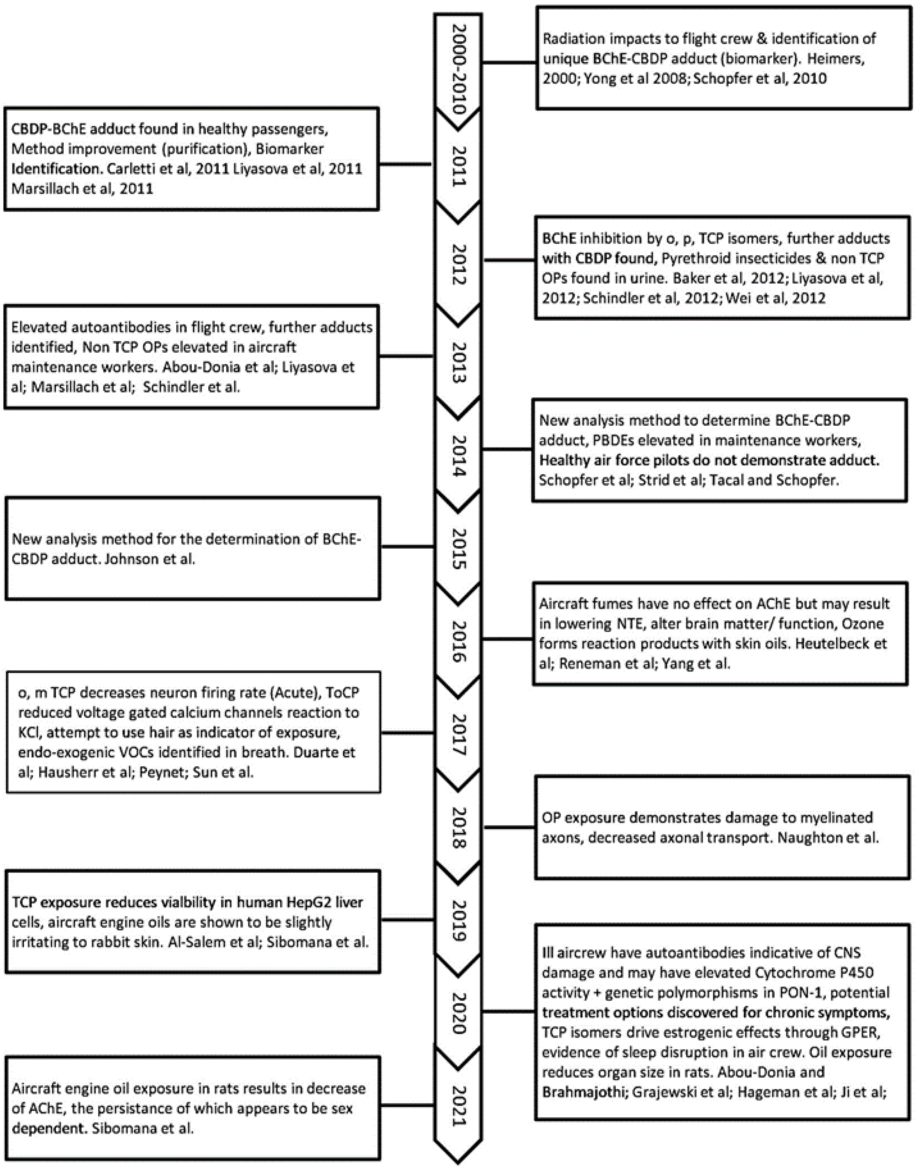
1.4.3.2 Human exposure-organophosphates

Several manuscripts employed human tissues/fluids, either unaltered or with contaminant additions, to identify exposure incidences or health consequences (Figure 2; SI-Table 3). Biological sampling, especially in those cases where the participants have been subject to aircraft environments, provides further context into the potential effects of exposure and reveals possible genetic precursors that may relate to the more seriously impacted. Polimanti et al. (2012) describe genetic variation in the cytochrome P450 superfamily. Hageman et al., 2020a, Hageman et al., 2020b acknowledged this variability, as well as genetic differences in paraoxonase enzyme (PON-1); when tested, those believed to be symptomatic as a result of exposure were found to possess mutations on one or both. Hageman et al., 2020a, Hageman et al., 2020b suggest that if an individual were to have a low PON 1 activity and high cytochrome P450 action, they might be up to 4000× more susceptible to OP exposure. Additionally, elevated autoantibodies indicative of CNS damage have been identified in the blood of ill flight crew by Abou-Donia et al. (2013) and Abou-Donia and Brahmajothi (2020). Healthy participants may also display indications of exposure; Carletti et al. (2011) describe an adduct of CBDP and BChE, which may be unique to ToCP exposure; this adduct was identified in half of a group of airline passengers who displayed no health effects (Liyasova et al., 2011). Tacal and Schopfer (2014), searching for the same adduct in healthy Airforce pilots, could not find it. Schindler et al., 2013, Schindler et al., 2014 sought TCP and other OP metabolites in healthy aircrew and maintenance workers' urine, finding no ortho TCP and very little evidence of other TCP isomer exposure. This claim is disputed by Schopfer et al. (2014) as they argue Schindler et al., 2013, Schindler et al., 2014) were not looking for the expected human urinary metabolites. Additionally, blood, tissue, and imaging experiments in those exposed have reported damage to liver cells, identification of other CBDP adducts, and changes to brain blood flow and white matter (Al-Salem et al., 2019; Liyasova et al., 2012, Liyasova et al., 2013; Reneman et al., 2016). Treatment options to mitigate exposure risk were also sought; Baker et al. (2013) note that a compound in grapefruit (naringenin) may be an effective treatment post OP dose. It inhibits the breakdown of triaryl phosphates to their more toxic metabolites. Naughton et al. (2020) describe that previously approved drugs, when used outside their current purpose, may prevent axonal damage and long-term neurological problems arising from OP exposure, namely lithium chloride and methylene blue.

1.4.3.3 Other biological studies

ToCP and other TCP compounds provide the simplest explanation for the neurological symptoms expressed by a small percentage of aircrew. However, they do not wholly describe what could be considered an occupational risk. The measurement of melatonin levels as an indicator of sleep disruption found that flight attendants have a significant disruption in circadian rhythm (Grajewski et al., 2003). Chromosomal abnormalities were noted in Heimers (2000) and related to cosmic radiation exposure; the abnormalities were eight times higher in Concord pilots than the control group but insignificantly increased compared to subsonic pilots. Yang et al. (2016) attempted to identify which compounds found in skin oils form reaction products with ozone. Reaction products of ozone included acetone, 6-MHO, nonanal, and decanal, each with several potential precursors in the skin oil extract. Both pyrethroid pesticides and certain PBDEs were found to be elevated in exposed flight crew and, in the cases of PBDEs, maintenance workers (Wei et al., 2012; Strid et al., 2014). Pesticides and insecticides may be related to health concerns, but they also serve critical purposes. In these and all incidences of exposure, it is essential to weigh the pros of the compound's presence with the health risks associated with their absence. Pang et al. (2020) described chemical exposure and symptoms in flight crew via pyrethroid insecticides but described the effects as limited due to very high exposure and low symptomatic response. Wei et al.

(2012) also found evidence of this exposure. This must be weighed against the potential transmission of insect vectored diseases.



Radiation impacts to flight crew & identification of unique BChE-CBDP adduct (biomarker). Heimers, 2000; Yong et al 2008; Schopfer et al, 2010

CBDP-BChE adduct found in healthy passengers, Method improvement (purification), Biomarker Identification. Carletti et al, 2011 Liyasova et al, 2011 Marsillach et al, 2011

BChE inhibition by o, p, TCP isomers, further adducts with CBDP found, Pyrethroid insecticides & non TCP OPs found in urine. Baker et al, 2012; Liyasova et al, 2012; Schindler et al, 2012; Wei et al, 2012

Elevated autoantibodies in flight crew, further adducts identified, Non TCP OPs elevated in aircraft maintenance workers. Abou-Donia et al; Liyasova et al; Marsillach et al; Schindler et al.

New analysis method to determine BChE-CBDP adduct, PBDEs elevated in maintenance workers, Healthy air force pilots do not demonstrate adduct. Schopfer et al; Strid et al; Tacal and Schopfer.

New analysis method for the determination of BChE-CBDP adduct. Johnson et al.

Aircraft fumes have no effect on AChE but may result in lowering NTE, alter brain matter/ function, Ozone forms reaction products with skin oils. Heutelbeck et al; Reneman et al; Yang et al.

o, m TCP decreases neuron firing rate (Acute), ToCP reduced voltage gated calcium channels reaction to KCl, attempt to use hair as indicator of exposure, endo-exogenic VOCs identified in breath. Duarte et al; Hausherr et al; Peynet; Sun et al.

OP exposure demonstrates damage to myelinated axons, decreased axonal transport. Naughton et al.

TCP exposure reduces vialbility in human HepG2 liver cells, aircraft engine oils are shown to be slightly irritating to rabbit skin. Al-Salem et al; Sibomana et al.

Ill aircrew have autoantibodies indicative of CNS damage and may have elevated Cytochrome P450 activity + genetic polymorphisms in PON-1, potential treatment options discovered for chronic symptoms, TCP isomers drive estrogenic effects through GPER, evidence of sleep disruption in air crew. Oil exposure reduces organ size in rats. Abou-Donia and Brahmajothi; Grajewski et al; Hageman et al; Ji et al;

Aircraft engine oil exposure in rats results in decrease of AChE, the persistance of which appears to be sex dependent. Sibomana et al.

Figure 2 Timeline of biomonitoring and principal conclusions within the systematic review. Additional citations listed under table 3, supplementary information. Line to center indicates year of publication of manuscripts included in text box.

Increasingly, the field has become more focused on low-dose chronic exposure. To quote Nicholson (2009), “Sub-clinical long-term effects cannot be completely ruled out, and whether contaminants in the air supply could be a factor in the malaise experienced by passengers remains uncertain.” Howard (2017) describes axonal transport damage caused by repeated low-dose exposure to OPs. Axonal damage is also noted in Naughton et al. (2018) and Naughton and Terry (2018). Howard (2017) suggests that multiple proteins may be “irreversibly modified by OPs” based on the findings that adducts have been found with tyrosine and lysine and suggests that the axonal damage and protein damage may be the potential cause of the symptoms experienced in flight crews. The sensitivity of individuals is also a significant concern. Variations in cytochrome p450 superfamily, PON-1, or BChE enzyme reactivity could play an important role in some passengers' sensitivity (Carletti et al., 2011; Chen et al., 2011; Hageman et al., 2020a, Hageman et al., 2020b).

1.4.3.4 Summary and future work

The examination of animal models and workers occupationally exposed to identified contaminants demonstrate undesirable effects and therefore occupational risk. Evidence is mounting; however, efforts should be made to ensure that exposure is related completely or synergistically with the aircraft environment. Additionally, if sensitivity to compounds of concern can form from exposure, as theorized by Watterson and Michaelis (2017), historical exposure to the contaminants must be considered. Schopfer et al. (2010) describe TCP as being a lead scavenger from leaded gasoline. The product is still used in the leaded fuel for piston-engine aircraft (AVGAS) and is sanctioned by the FAA (Alcor, 2012). This implies that commercial pilots in training, or any flight crew that fly recreationally and are exposed to fuels, may interact with significant TCP concentrations before/outside of occupational exposure.

1.4.4 Health effects: survey, cohort, and records studies

The following subsections describe the examination of the aggregated health consequences of occupational exposure of aviation workers. For a completed citation list of manuscripts as well as abbreviated findings, see Table 3.

Table 3 An abbreviated description of the survey, cohort, and record study manuscripts.

Author	Concern	Experiment Type	Participants	Result
Burdon et al, 2017	Health concerns- Aircraft fumes	Survey	Part A- Pilots- 274 Part B- Flights with oil leaks- 15	Part A- 142 reported symptoms and diagnosis, 30 AHE, 77 healthy; Part B- 14 impairment of flight crew, 11 AHE, 4 passenger AHE
Weislander et al, 2000	Subjective cabin air quality	Survey	Flight Crew- 51	Noted improvement in CAQ following smoking cessation on aircraft
dos Santos Silva et al, 2013	Cancer Prevalence	Cohort	Flight Crew- 16329 Air Traffic Controllers- 3165	Lower than average risk as compared to the general UK population, less skin cancer. Lower than average risk as compared to the general UK population, not inclusive skin cancer
Grajewski et al, 2011	Circadian rhythm disruption; Cosmic radiation exposure	Records Review	Pilots (male)- 83	Potential for chronic sleep disturbance; Est. 1.92mSv annual. Additional risk due to solar storms, avg exposure rate 1 : 3.7 years
Lee et al, 2000	Subjective cabin air quality; Health concerns- Cabin environment	Survey	Flight Crew- 185	37% Reported CAQ as acceptable. 21% considered it to be poor. Humidity was a concern; Health symptoms ranging from none-severe. Majority not related to dryness low on scale.
Lindgren et al, 2007	Subjective cabin air quality	Survey	Flight attendants-58 Pilots-22	CAQ reported as improved when humidification present.
Lindgren et al, 2002	Subjective cabin air quality; Health concerns- Cabin environment	Survey	Flight Crew- 19	Improvement in all categories save facial rash following smoking cessation on aircraft; CAQ reported as improved after smoking ban
McLain and Jarrell, 2007	Perception of Safety VS obligation	Survey	Hazardous Job Workers- 239	Did not significantly demonstrate a relationship between working safely and production pressure
McMurtrie and Molesworth, 2017	Risk Perception	Survey	Pilots- 270	Pilots over estimated risk across age and experience categories. More experienced/older pilots tend to identify the risks in lower categories.
McNeely et al, 2018	Health concerns- Cabin environment	Cohort	Flight Attendants-5366	Increased risk of reproductive cancer, all cancers, fatigue, sleep disorders, mental health concerns; Reduced risk of respiratory and cardiovascular disease
Nicholas...Dosemeci et al, 1998	Health concerns- Cabin environment	Cohort- Mortality	Pilots & Navigators- 1538	Cancer of the prostate, colon, mouth, throat, lips, and brain increased Lungs, trachea, stomach reduced. Significant increase in motor neuron disease; suggested increase in nervous system and sense organs disease; Reduced heart, respiratory, and digestive system diseases
Pinkerton et al, 2016	Health concerns- Cabin environment	Cohort- Mortality	Flight attendants- 11311	Possible increased risk of ALS, not clearly linked to exposure (Employment duration not correlated).
Polimanti et al, 2012	Genetic variation- Cytochrome P450	Records Review	Individuals- 1694	Significant genetic differences in P450 super family observed in terms of ethnicity (Single nucleotide polymorphisms)
Sagiraju et al, 2020	Health Concerns- Military Service	Cohort	Service People- 1149620	Significant increase in ALS within the Airforce as compared to other disciplines. Significant increase in ALS people who routinely fly/ work with aircraft (Quantified tactical operations officers)
Schubauer-Berigan et al, 2015	Health concerns- Cabin environment	Cohort	Flight Attendants- 11324 (total)	Breast cancer rate 37% higher than general US population, could not be linked to workplace exposure, Link may reflect differing reproductive habits of flight attendants VS the US general population
Stravola et al, 2012	Health concerns- Cabin environment	Cohort	Flight Crew- 16327 Air Traffic Controllers-3162	Mortality (all cause) of flight crew was about 1/3 of the general population (largely male study group); Only category that exceeded the UK general population or the control group was death due to aircraft accidents
van Drongelen et al, 2015	Health concerns- Flight duration	Records Review	Flight Crew- 8228	Short haul flights linked to reduced sickness absenteeism.

1.4.4.1 Comparative health and cohorts

In general, pilots and flight attendants are considered to be more physically fit as compared to the general populations within their respective countries of residence, typically demonstrating reduced risk of cardiovascular and respiratory disease (De Stavola et al., 2012; Dos Santos Silva et al., 2013; McNeely et al., 2018). They do seem to have an elevated risk of certain cancers (sex-dependent), mental health, and neurological issues, with tenuous links to the workplace (Table 3). Of particular concern is the potential risk of working with/around aircraft and the incidence of amyotrophic lateral sclerosis (ALS). Two studies identified a potential link between this disease and work in the field; Pinkerton et al. (2016) conducted a mortality study of flight attendants collected from airline records (PAN AM) that indicated ALS occurrence in the cohort is 2.21× that of the general population. This finding was based on a small number (nine) of deaths and could not be correlated to flight hours. More significantly, a second study including more than a million post 9–11 servicemen and women in the United States demonstrated a significant increase in the disease within the Airforce branch of the armed forces and elevated within tactical operations officers (pilots, aircraft crews, and missile combat operations staff officers) as compared to other officers within this service

(Sagiraju et al., 2020). The authors suggest that due to the difference between the people who routinely work with aircraft and other officers, environmental concerns should be explored.

1.4.4.2 Self-reported symptoms and air quality

Generally, cabin humidity and the cessation of smoking on flights weigh heavily on perceived CAQ (Table 3). Lee et al. (2000), Lindgren et al. (2007) and Lindgren and Norback (2002) describe many symptoms noted by aircrew, the majority of which can be explained by cabin dryness with only a minority that could be neurologically related. Burdon et al. (2017) conducted surveys more directly related to chemical exposure (Table 2). More than half of the pilots surveyed reported specific symptoms and diagnoses. Approximately 10% reported adverse health effects (AHE), with 28% reporting no health effects. The majority of participants were aware of the risk of exposure. In a second study, 15 CAQ incidents on various aircraft were examined, in which leaking oil could be detected in 13 of the cases. Degrees of incapacitation/impairment of flight crew were reported in 14 of these cases, and adverse health effects were experienced in 11 cases. Adverse effects in passengers (four) were also noted. The authors use Bradford Hill causation criteria, and according to their interpretation, eight of nine factors were in agreement with only dose-response not met.

1.4.4.3 Safety and risk

McLain and Jarrell (2007) issued questionnaires to workers in hazardous positions, weighing safety versus production, to better understand how safety is treated when several conflicting demands are made upon the worker. The survey result failed to “find a significant relationship between pressure to produce and safe work behavior.” McMurtrie and Molesworth (2017) questioned how different pilots experience risk and assessment of said risk. They attempted to determine if the accuracy of risk assessment changes with many factors, including experience (i.e., rank, flight hours, license type, recency) and age. In general, the pilots overestimated risk across age and experience categories. However, more experienced/older pilots did trend to identify the risks in lower categories.

1.4.4.4 Summary and future work

The nature of these studies results in data that may lag years or decades behind changes made within the workplace. Continued cohort studies should be conducted to determine if any modifications, such as the introduction of bleed-free aircraft, have the desired effect on occupational risk. Additionally, surveys should be conducted, including those who fly recreationally or for training purposes and use products which contain TCP or similar products as lead scavengers for their fuel. This may help with the determination of historical exposure and sensitization. Finally, further work is needed to clear conflicting information that is provided by these manuscripts. Health effects range from several types of cancers and neurological concerns elevated in aircrew, to the only cause of elevated mortality in the group relating to aircraft accidents (Table 3; Nicholas et al., 1998a, Nicholas et al., 1998b; De Stavola et al., 2012). A clearer picture, addressing confounding variables, would be very beneficial for the determination of occupational risk.

1.5 State of the science: knowledge gaps and future recommendations

Numerous recognized incidences of illness, at least tangentially, are related to chemical exposure onboard aircraft, likely due to technological /design flaws. It is in everyone's best interest to ensure aircraft safety, yet the changes in technology/design are difficult to make due to financial, logistical, and technological reasons. Pilots, other aircrew, airline management, and manufacturers are aware, or should be made aware, of the potential for contaminated air to enter the aircraft (Burdon et al., 2017; Michaelis, 2017) and yet a potential hazard remains. Even if this applies only to severe fume events and not the low dose chronic exposure theorized by many, a mitigatable concern is not resolved; this is bound to create dissonance. Occupational risk reduction may be beneficial for all stakeholders involved. There may be financial benefits to freeing or limiting contaminant intrusion into the cabin. Shehadi et al. (2015) estimated the overall losses in 2012 by airlines in the USA due to fume events between $4.5 M to $7 M, with each incident ranging from S32K–$47 K.

It is evident within the literature that there are opposing viewpoints in determining occupational exposure risk to flight crew. Of the experimental manuscripts reviewed, 38% made declarative statements in favor of, or opposed to, the occupational risk of chemical exposure within the cabin and 62% did not. Within the declarative subset, those papers which were determined to be in favor of occupational risk acknowledged stakeholders in 33% of the manuscripts. Those manuscripts which were opposed to occupational risk acknowledged stakeholders in 67% of the cases. Stakeholders included pilot and flight attendant unions, advocacy groups, aircraft manufacturers, and operation firms. When pilot and flight attendant unions or advocacy groups were acknowledged, 80% found in favor of occupational risk, none were opposed, and the remaining 20% undeclared. When airline manufactures and operator stakeholders were acknowledged, 5% were in favor of occupational risk, 42% were opposed, and 53% were undeclared.

1.5.1 Summary of identified gaps requiring further research

This systematic review identified two key areas where there were significant knowledge gaps and need for further research. These included a better characterization of the on-aircraft environment and understanding the cause of adverse health effects. There is also an imperative to combine these two approaches as research appeared to be aimed at achieving one of these two objectives, however they should be considered in unison.

1.5.1.1 Characterization of the on-aircraft environment

From reviewing manuscripts that have measured the on-aircraft environment it is clear that the full exposome onboard aircraft has not been fully characterized. Modeling and laboratory-based experiments have provided useful insights to help develop mitigation technologies, however many of these are still in the proof of concept stage. To help address these knowledge gaps the following research would prove useful;

- A determination of the contamination contribution of the expansion turbines within the air conditioning systems on aircraft needs to be completed. These turbines use similar oil to that found in aircraft engines and contain the same principal contaminants of concern. This may apply not only to bleed-air equipped aircraft but also to bleed-free designs should they use this cooling method.
- The potable water on aircraft should be explored as it is currently an undefined potential source of exposure.

- Non-targeted screening of active air and passive samples taken on aircraft should be conducted to supplement targeted studies to identify potential contaminants that have not yet been described.
- Scaling up of proof of concept laboratory studies to involve more on flight testing of technologies to reduce contaminant levels in the cabin environment

Understanding the cause of adverse health effects

- Historical exposure to TCP and other OPs should be examined, especially in the cases of those who fly/flew recreationally or in training in piston engine aircraft using lead scavenging products in the fuel.
- Further evidence of genetic mutations responsible for OP exposure susceptibility is required as the claim is currently supported by small sample sizes.
- Continued monitoring of health, especially of those who work on bleed-free aircraft should be conducted. This will allow more complete source delineation should the symptoms of exposure remain or decrease on this type of aircraft.

1.6 Conclusions

Flying, in general, is safer than it has ever been. Improvements to technology and ruggedness of aircraft components and improved pilot training have led to fewer accidents (Oster et al., 2013). However, the manuscripts identified within this systematic review provide evidence of occupational risk. There is evidence that those who work in the aircraft cabin are at an increased risk of neurological injury or disease due to their profession. When examined the majority of biological sampling and cohort studies, most importantly those sampling individuals exposed to fume events on aircraft demonstrate physiological changes as compared to controls. To qualify, many of the individual manuscripts reviewed suffer due to small sample sizes, experimental design flaws, or perceived potential bias. However, when examining the totality of manuscripts, the potential for occupational risk cannot be ruled out. However, the sampling of aircraft has not yet identified a contaminant or mixture of contaminants in sufficient concentration proven to be capable of the symptomology. Further research is required to determine this contaminant or mixture should it exist, and further evidence of the impacts of chronic low dose exposure and susceptibility studies are required for the known contaminants. Additionally, fume events continue to create a significant risk for those flying; this applies to both flight crew and the passengers onboard. Despite the relatively low incidence of occurrence, in a return to normal flight frequency, several of these events would be estimated to occur daily.

1.6.1 Critical review of this systematic review

The strength of this manuscript is the large number of resources reviewed and the multiple lines of evidence used to explore the subject matter. Typically, a paper related to chemical exposure in the aircraft cabin would examine the concentrations of contaminants of concern, or the symptomologies of an unknown exposure but not both. This manuscript looks at both, resulting in the discovery of gaps in the knowledge of the field that may not have been otherwise. The main weakness of this manuscript is the inability to determine a causal pathway from exposure on aircraft to symptomology. There are several incidences in which individuals claim to be exposed and demonstrate illness, however, no manuscript reviewed measured contaminants of concern onboard an aircraft that can be directly linked to illness. There is little question that individuals are ill, and there is strong evidence that it is related occupational exposure but no one class of, or specified contaminant, has been demonstrably harmful; it seems likely that the unknown multiplicative or synergistic effects of the exposure mixture and the cumulative effects of extended exposure, are resulting in the described illness.

1.7 Declaration of competing interest

The authors declare that they have no known competing financial interests that could have influenced this paper's writing.

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1.10 Supplementary information

SI Table 1. Manuscripts not identified within the systematic review process added for completeness and justification for addition.

Author	Justification for Addition to Systematic Review
Abou Donia et al, 2013	First autoantibody paper by author, required background.
Allen et al, 2013	Important flame retardant exposure paper.
Chen et al, 2011	Background related to Cytochrome P450 Superfamily.
Costa, 2018	Background on OPs
Crump et al, 2011	One of the largest on aircraft sampling studies. Routinely cited in other papers.
Denola et al, 2011	Study combining active and passive sampling methods, military aircraft.
Goenechea and Raab, 1995	Earliest TCP biomonitoring paper identified in literature.
Harrison and Mackenzie Ross, 2015	Background of cabin contamination.
McKinlay et al, 2008	Background-pesticides.
Michaelis, 2018	Background- aircraft systems, oil use.
Moir and Seabridge, 2008	Background-aircraft systems- Identification of potable water pressurization.
NRC, 2002	Background- State of field in 2000's
Petrolanu, 2016	Background- TCP historical uses.
Pinkerton et al, 2016	Survey- Directly relating to search terms.
Polimanti et al, 2012	Study on variability of Cytochrome P450 Superfamily.
Ramsden, 2013	Illustrates unexplained incidence of ToCP in cabin.
Schindler et al, 2012	Important biomonitoring paper, commonly cited
Schindler et al, 2013	Important biomonitoring paper, commonly cited
Solbu et al, 2011	One of the largest on aircraft sampling studies. Routinely cited in other papers.
Tacal and Schopfer, 2014	Demonstration of contrast in findings, military aircraft
Winder and Balouet, 2000	Fundamental paper, routinely cited.
Winder and Balouet, 2002	Fundamental paper, routinely cited.

SI Table 2. Summary of on aircraft sampling methods and results.

					Measure of Center - Concentration			
Author	Sampling Method	Sampling Events	Sampling / Analysis Method	*Contaminant of Concern	Mean	Median	Max Measured Concentration	Detect by Sample
Allen et al, 2013	Air sampling -Active	59 Flights	Polyurethane foam and XAD-2 sorbent	PBDE 47		1.3 ng/m3	20 ng/m3	63%
			GCMS	PBDE 99		<1.3 ng/m3	41 ng/m3	22%
				PBDE 100		<0.4 ng/m3	9.4 ng/m3	29%
				PBDE 183		<1.3 ng/m3	98 ng/m3	32%
				PBDE 209		<1.2 ng/m3	2100ng/m3	42%
Allen and Stapleton et al, 2013	Dust sampling- Active	19 Flights**	Cellulose extraction thimble- Vacuum	PBDEs(ng/g)- BDE28, 33		54, 33 (floor, vent)	270	98%
			GCMS, LCMSMS	BDE47		950, 3500	19000	100%
				BDE49		40, 65	6300	76%
				BDE66		45, 230	1500	98%
				BDE75		370,310	47000	100%
				BDE85, 155		76, 200	2900	98%
				BDE99		950, 4200	37000	100%
				BDE100		180, 630	8900	98%
				BDE138		20, 62	680	84%
				BDE153		230, 630	5300	100%
				BDE154		120, 280	4700	98%
				BDE183		620, 390	9100	100%
				BDE209		495000, 473000	2600000	100%
				BIBPE		330, 1300	48000	100%
				anti-Dechlorane plus		330, 300	9600	100%
				Syn-Dechlorane plus		110, 160	9500	100%
				HBB		100, 45	540	74%
				alpha-HBCD		2300, 1600	290000	84%
				beta-HBCD		310, 230	75000	69%
				y-HBCD		4500, 7600	700000	98%
				Total HBCD		7600, 10000	1100000	100%
				TBB		350, 740	5000	100%
				TBPH		640, 1200	3600	100%
				TDCPP		2100, 5600	22000	100%
Crump et al, 2011	Air sampling- Active	100 Flights	Ion Science FirstCheck+5000 PID + Sorbent Tubes	(T)VOCs		2-4ppm	>10ppm	NA
			Electrochemical Sensor	CO		1-ppm	>5ppm	NA
			P-Trak ultrafine particle counter Model 8525	UFP	1000-100000 counts/cm3		>500,000 counts/cm3	NA
			Quartz wool+ TENAX TA Sorbent Tubes	ToCP	0.07ug/m3		22.8ug/m3	<5%
				TCPs	0.14ug/m3		28.5ug/m3	<6%
				TBP	1.07ug/m3		21.8ug/m3	NA
				Toluene	13.93ug/m3		170.2ug/m3	NA
				m+p Xylene	1.78ug/m3		52.3ug/m3	NA
				Limonene	11.85ug/m3		540.3ug/m3	NA
				TCE	0.43ug/m3		20.1ug/m3	NA
				Undecane	2.74ug/m3		87.3ug/m3	NA
Denola et al, 2011	Air sampling- Active	32 Flights**	Porapak Q and cellulose filters Sorbant tubes	TCP	2.90ug/m3		51.3ug/m3	NA
	Air sampling- Passive			ToCP	<LOD		<LOD	NA
			Heat exchanger and Coalescer bags from aircraft	TCP	Detected- NQ		NQ	NA
de Ree et al, 2014 (Referencing Dutch TNO Study)	Air sampling -Active	20 Flights	GC-MS	Total TCPs- Climb	25 ng/m3	5.9 ng/m3	155 ng/m3	40%
				Total TCPS- Cruise	4.7 ng/m3	2.9 ng/m3	17 ng/m3	45%
				Total TCPs- Decent	15 ng/m3	6.0 ng/m3	66 ng/m3	50%
				Total TCPs- Full Flight	6.9 ng/m3	2.9 ng/m3	32 ng/m3	45%
				ToCP	<LOD		NA	NA
				TCPs (wipe sampling)	<0.1ng/cm2		NA	NA
Giaconia et al, 2013	Air sampling -Active	14 Flights	DS1923 iButton	Relative Humidity	17.9 - 27.0%		59.20%	NA
			LSI-Lastem BSO 103	CO2	925 -1449 ppm		2252 ppm	NA
Guan et al, 2014 Qualification Study	Air sampling- Active	107 Flights	TD- GC-MS, Custom Syringe -Tenax-TA tube	6-Methyl, 5-Hepten-2-one ***				53%
				345 other VOCs detected		59 VOCs/Flight		41 VOCs >50%
Guan, Wang et al, 2014 Quantification Study	Air sampling -Active	51 Flights	TD- GC-MS, Custom Syringe -Tenax-TA tube	Limonene ***		15.1 ug/m3	1048.2 ug/m3	>50%
				Nonanal ***		12.1	70.9	>50%
				Acetone ***		8.2	384.4	>50%
				Tetrachloroethene ***		2.8	303.9	>50%
				Octanal ***				>50%
				6-Methyl, 5-Hepten-2-one ***		<LOD	23.2	>50%
Guan et al, 2019	Air sampling- Active	14 Flights	Grimm Mini-WRAS 1.371	UFP	417 +/- 1089 Counts/cm3		>10,000 counts/cm3	NA
			TELAIRE 7001	CO2	924- 2012 ppm		3374 ppm	NA
Guan et al, 2015	Air sampling- Active	6 Flights	ppbRAE 3000	TVOC	0.244- 0.433 mg/m3		0.618 mg/m3	NA
			TELAIRE 7001	CO2	848-1102 ppmv		2061 ppmv	NA
He et al, 2018	Dust Sampling	5 Flights**	GC-MS/MS	ΣOPFR	190ug/g***	NA	NA	NA
				ΣPBDEs	8.1ug/g***	NA	NA	NA
Lindgren and Norback, 1991	Air sampling- Active	26 Flights (7 nonsmoking)	Rieken R1-411	CO2	734ppm		1488ppm	NA
	Air sampling- Passive		Diffusion Sampler	Ozone	19.2 ug/m3		66ug/m3	NA
			Sibata P-5H2	Resp. Particulate	3ug/m3		7ug/m3	NA
			Glass fiber filter	Formaldehyde	<LOD	<LOD	15ug/m3	23%
			Diffusion Sampler	NO2	14.1ug/m3		37ug/m3	NA
Lee et al, 2000	Air sampling- Active	16 Flights	TSI Q Trak	CO2	NA		2500ppm	NA
			TSI Q Trak	CO	1.9-3 ppm		NA	NA
			Passive O3 badges	Ozone	<25% FAA Regs.		NA	NA
			Pulse Fluorescence	SO2	NA	NA	NA	NA
			Chemiluminescence	NO2	NA	NA	NA	NA
			TSI Q Trak	Resp. Particulate	7.6ug/m3		17ug/m3	NA
Lewis et al, 1999	Radiation sampling- Passive	5 Flights	Coventional radiation detector suite	Cosmic Radiation	3.12 mSv Annual		4.69mSv Annual	NA
		98+ Flights	Neutron Sensitive Bubble Detectors					
Li et al, 2014	Air sampling-Active	9 Flights	FLUKE Airborne Particle Counter	Particulate 0.3-0.5um	13257 counts/L		102473 counts/L	NA
				0.5-1um	1949 counts/L		20668 counts/L	NA
				1-2um	452 counts/L		6288 counts/L	NA
				2-5um	240 counts/L		2874 counts/L	NA
				5-10um	31 counts/L		321 counts/L	NA
				>10um	14 counts/L		110 counts/L	NA
			TELAIRE 7001	CO2	1079ppm (recirculated air)		2253ppm (recirculated air)	NA
Lindgren et al, 2007	Air sampling- Active	16 Flights	Q-TrakTM IAQ Monitor	CO2	1100-1200ppm Cabin		1320ppm	NA
					800ppm flight deck		1000ppm	NA
			P-TrakTM8525 UFP Counter	Particulate 0.02-1um	475 counts/cm2		300,000 counts/cm3	NA
			Dust-TrakTM Model 8520	1-10um	6ug/m3		7ug/m3	NA
			Glass fiber filters	Formaldehyde	<LOD	<LOD	NA	NA
Nagda et al, 1992	Air sampling- Active	92 Flights (23 Non smoking)	Optical sensor + Gravimetric	Resp. Particlulate	10.45, 64.35ug/m3		NA	NA
			NA	CO2	1756ul/L		NA	NA
			NA	CO	0.55ul/L		1.3ul/L	NA
			NA	Ozone	0.02ul/L		NA	NA
Nicholas et al, 1998	Magnetic fields	37 Flights	Emdex Lite personal dosimeter.	Electromagnetic Fields	16.8mG (Cockpit)		160.4mG	NA

Rosenberger, 2018	Air sampling- Active	17 Flights	GrayWolf Advanced Sense IQ-610	CO2	1883ppm, 1026ppm (cabin,cockpit)		5177ppm (cabin)	NA
				CO	1.0ppm, <LOD		2.2ppm (cabin)	NA
				Ozone	<LOD, 28ppb		205ppb (cabin)	NA
			Addition of TENAX TA tubes	(T)VOCs	228ug/m3, 7ug/m3		7200ug/m3 (cockpit)	NA
			Quartz filter and polyurethane foam	Organophosphates- TBP	0.09ug/m3, 0.05ug/m3 (cabin,cockpit)	0.09ug/m3, 0.03ug/m3	0.22ug/m3, 0.16ug/m3	NA
				TiBP	0.55ug/m3, 0.72ug/m3	0.43ug/m3, 0.63ug/m3	1.99ug/m3, 1.73ug/m3	NA
				TCEP	0.007ug/m3, 0.12ug/m3	0.005ug/m3, 0.04ug/m3	0.07ug/m3, 4.44ug/m3	NA
				TCPP	0.16ug/m3, 0.62ug/m3	0.17ug/m3, 0.39ug/m3	0.40ug/m3, 3.73ug/m3	NA
				TDCPP	0.003ug/m3, 0.011ug/m3	0.003ug/m3, 0.008ug/m3	0.010ug/m3, 0.035ug/m3	NA
				TPP	0.028ug/m3, 0.013ug/m3	0.028ug/m3, 0.011ug/m3	0.056ug/m3, 0.043ug/m3	NA
				TEBP	1.2ug/m3, 0.27ug/m3	0.11ug/m3, 0.10ug/m3	2.37ug/m3, 1.43ug/m3	NA
				DPEHP	0.03ug/m3, 0.03ug/m3	0.02ug/m3, 0.02ug/m3	0.155ug/m3, 0.151ug/m3	NA
				TEHP	0.01ug/m3, 0.01ug/m3	0.009ug/m3, 0.009ug/m3	0.025ug/m3, 0.022ug/m3	NA
				TXP	<LOD	<LOD	<LOD	NA
				Summed OPs	0.916ug/m3, 1.633ug/m3	0.65ug/m3, 1.13ug/m3	4.65ug/m3, 8.50ug/m3	NA
				Total TCP	0.024ug/m3, 0.065ug/m3	0.019ug/m3, 0.020ug/m3	0.11ug/m3, 0.981ug/m3	NA
			DNPH cartridges	Aldehydes- Formaldehyde		5.7ug/m3	NA	NA
				Acetaldehyde		6.3ug/m3	NA	NA
				Benzaldehyde		2.2ug/m3	NA	NA
				Hexaldehyde		1.4ug/m3	NA	NA
Rosenberger et al, 2016	Air Sampling- Active	110 flights	GrayWolf Advanced Sense IQ-610	Ozone	<LOD	<LOD	0.04ppm	NA
			DNPH cartridges	Aldehydes (ug/m3) -Formaldehyde	5.1, 6.3 (A380,A321)	4.5, 5.3	24, 44	100%
				Acetaldehyde	7.7, 5.3	5.1, 4.1	52, 90	100%
				Acrolein	0.2, 0.7	0.2, 0.6	0.4, 6.1	14%
				Propionaldehyde	2.8,1.9	0.9, 1.1	94, 34	92%
				Crotonaldehyde	0.4, <LOD	0.3, <LOD	1.5, <LOD	NA
				n-Butyraldehyde	1.3, 0.8	0.7, 0.8	15, 3	79%
				Benzaldehyde	1.8, 2.0	1.3, 1.7	6.9, 8.0	82%
				Isovaleraldehyde	2.1, 2.2	1.4, 0.9	9.6, 12.4	56%
				Valeraldehyde	1.2, 1.4	0.8, 1.1	5.9, 4.5	62%
				o-Tolualdehyde	0.3, 2.2	0.3, 1.8	0.6, 8.8	NA
				m-Tolualdehyde	1.1, 2.5	1.1, 2.1	3.1, 8.0	26%
				p-Tolualdehyde	0.9, 1.6	0.8, 0.5	2.6, 6.0	NA
				Capronaldehyde	1.7, 1.9	1.3, 1.7	7.6, 7.0	79%
				2,5-Dimethylbenzaldehyde	0.7, 0.5	0.6, 0.5	2.1, 1.1	7%
Schuchardt et al, 2019	Air sampling -Active	177 Flights	TENAX TA tubes+DNPH cartrages	Aldehydes	13-35ug/m3		NA	NA
			TENAX TA tubes	(T)VOCs	122-370ug/m3		NA	NA
			Quartz filter + PUR foam	OPs- Triisobytyl phosphate (ug/m3)	0.086, 0.016 (Bleed, Non-Bleed)		NA	NA
				TnBP	0.58, 0.24		NA	NA
				Tris(chloroethyl) phosphate	0.028, 0.007		NA	NA
				TCPP	0.4, 0.50		NA	NA
				Tris(1,3-dichloroisopropyl) phosphate	0.0095, 0.005		NA	NA
				TPP	0.012, 0.006		NA	NA
				Tris(butoxyethyl) phosphate	0.14, 0.035		NA	NA
				Diphenyl-2-ethylhexylphosphate	0.018, 0.013		NA	NA
				Tris(ethylhexyl)phosphate	0.0093, <LOD		NA	NA
				ToCP	<LOD, <LOD		NA	NA
				TompCP	<LOD, <LOD		NA	NA
				ToomCP	<LOD, <LOD		NA	NA
				Toop/ommCP	<LOD, <LOD		NA	NA
				ToppCP	<LOD, <LOD		NA	NA
				TmCP	0.0072, 0.007		NA	NA
				TmmpCP	0.0093, 0.010		NA	NA
				TmppCP	0.0068, 0.006		NA	NA
				TpCP	0.003, 0.003		NA	NA
				Trixylyl phosphate	0.0115, <LOD		NA	NA
				Total TCP	0.027, 0.026		NA	NA
				Total OPs	1.24, 0.820		NA	NA
Solbu et al, 2011	Air sampling- Active	47 Flights	Millex HV filter	TCP -Air sampling		<LOD	5.9ug/m3	4%
				TPP Air		0.11ug/m3	0.11ug/m3	2%
				TnBP Air		0.47ug/m3	4.1ug/m3	100%
				DBPP Air		0.12ug/m3	0.77ug/m3	92%
	Air sampling- Passive		Klinion 5x5cm sterile non-woven compresses	TCP -Wipe sampling		<LOD, 1.15 ng/dm/day (Jet, Prop)	8.3ng/dm/day	39%
				TiBP wipe		<LOD, <LOD	0.42ng/dm/day	3%
				TnBP wipe		<LOD, 0.10ng/dm/day	19.0ng/dm/day	37%
				DBPP wipe		<LOD, 0.13ng/dm/day	20ng/dm/day	42%
				TPP wipe		0.37, 0.76ng/dm/day	15ng/dm/day	74%
			Thermal desorption tubes	TVOCs		0.73mg/m3	1.3mg/m3	NA
				TCP HEPA		2.6ng/g/hr	42ng/g/hr	100%
Spengler et al, 2004	Air sampling-passive	106 flights	Ogawa Badge	Ozone	80ppb		208ppb	NA
van Netten, 2009	Air sampling- Active	2 Flights	Novel Aircraft Air Monitor	Total TCP	72ng/m3 (adjusted)		108ng/m3	100%
van Netten, 1998	Air sampling- Active	5 Flights	Q-Trak Indoor Air Quality Monitor	CO2	800-2700ppm		2700ppm	NA
				CO	<LOD		3ppm	NA
			Activated Carbon Tubes	VOCs	NQ		NA	NA
			Fliter Cassette	SVOCS+Heavier Oils	<LOD		NA	NA
				Total TCP	<LOD		NA	NA
			Odyssey 2001 gas monitor	NOx	<LOD		ppb range (NQ)	NA
Verhaegen and Poffijn, 2000	Radiation sampling-Passive	88 Flights	Thermoluminescent TLD-700H detectors	Low-LET radiation	1-4mSv Annual		4mSv Annual	NA
			Neutron Sensitive Bubble Detectors	High- LET radiation				
Wang et al, 2014	Air sampling- Active	14 Flights	Tenax-TA tubes	VOCs (ug/m3)- Benzene	18.24	10.01	NA	NA
				Toluene	30.66	13.41	NA	NA
				Ethylbenzene	6.35	3.76	NA	NA
				p-xylene	4.53	2.55	NA	NA
				o-Xylene	4.33	3.7	NA	NA
				Decanal	25.75	24.43	NA	NA
				Nonanal	18.35	17.78	NA	NA
				Dodecane	5.94	4.73	NA	NA
				Undecane	2.55	2.21	NA	NA
				Octanal	6.55	6.79	NA	NA
				2-ethyl-1-Hexanol	6.91	6.13	NA	NA
				Tetrachloroethylene	2.79	2.57	NA	NA
				Benzaldehyde	5.91	6.26	NA	NA
				D-Limonene	62.86	31.21	NA	NA
				Acetic acid	9.89	11.43	NA	NA
				6-methyl-5-Hepten-2-one	8.79	8.71	NA	NA
				Styrene	2.38	1.23	NA	NA
				Menthol	3.47	2.5	NA	NA
				Acetone	4.29	0.46	NA	NA
				Sum VOCs	236.48	205.03	NA	NA
Wieslander et al, 2000	Air sampling- Active	4 Flights	SWEMA Logger 15	Relative Humidity	3.8%, 6.4% (Fore, Aft)		8.7%, 18.9%	NA
			Sibata P-5H2	Resp. Particulate	4 , 3 ug/m3		12, 7 ug/m3	NA
			Rieken RI-411A	CO2	520, 570 ppm		1190, 1160 ppm	NA
Zhai et al, 2014	Air sampling- Active	9 Flights	Fluke 983 Airborne Particle Counter	Particulate (counts)- 0.3-0.5um	7642		81162	NA
				0.5-1.0um	1130		17278	NA
				1.0-2.0um	281		4809	NA
				2.0-5.0um	186		2727	NA
				5.0-10um	32		536	NA

SI Table 3 Summary of biological studies identified.

Author	Compound of Concern	Receptor	Effect
Abou-Donia et al, 2013	Aircraft Fumes	Human- Blood and Blood Products+ CNS Protiens	Elevated autoantibodies in flightcrew as compared to control
Abou-Donia and Brahmajothi, 2020	Aircraft Fumes/Conditions	Human- Blood and Blood Products+ CNS Protiens	Elevated autoantibodies indicative of CNS damage in active group.
Al-Salem et al, 2019	TCP	Human- Liver Cells (HepG2)	Reduced Viability
Baker et al, 2012	TCP- Tri o, m, and p isomers.	Mice- Blood+Tissues	BChE Inhibition, Less p isomer - Require Bioactivation
	Other Triaryl Phosphates		BChE Inhibition-Require Bioactivation
Carletti et al, 2011	CBDP	Human- BChE	BChE-CBDP adduct
			BChE inhibition
Duarte et al, 2017	TCP	Rat- Neural Networks	o, m isomers- Decrease in neuron firing rate (Acute)
			o,m,p isomers-Decrease in neuron firing rate (48hr)
Grajewski et al, 2020	Melatonin	Human- Urine	Circadian rhythm disruption
Hageman et al, 2020	Aircraft Fumes	Human- Blood and Blood Products+ Genetic testing	Elevated Cytochrome P450 activity, genetic polymorphisms in PON-1
Hausherr et al, 2017	TCP CBDP	Mice- Neuron Cells	Reduced voltage gated calcium channels reaction to KCl
			Cytotoxicity
Heimers, 2000	Radiation Exposure	Human-Blood or Blood Products	Chromosomal abnormalities- Cells with dicentrics/ translocations
Heutelbeck et al, 2016	Aircraft Fumes	Human-AChE	No Impact
		Human-neuropathy target esterase (NTE)	Suggests a lowering in NTE
Ji et al, 2020	TCP	Human Blood and Blood Products- Hormonal	Antagonistic effects on estrogen receptor alpha
			TCP isomers drive estrogenic effects through G Protein-Coupled Estrogen Receptor
Johnson et al, 2015	ToCP	Human- Blood or Blood Products	BChE-CBDP adduct
Liyasova et al, 2013	CBDP	Human- BChE+ Amino Acids (Histidine-438)	No impact
		Human-BChE+ Amino Acids (Free Histidine)	Adducts formed- suggesting involvement of other protiens
Liyasova et al, 2012	CBDP	Human- Blood or Blood Products	Lysine, Tyrosine, and two Histidines form adducts.
Liyasova et al, 2011	Aircraft Fumes	Human- Blood or Blood Products	CBDP-BChE adduct found in healthy passengers
Marsillach et al, 2013	Organophosphates	Human- Blood or Blood Products	Method development for Biomarker Identification
Marsillach et al, 2011	CBDP	Human- Blood or Blood Products	Method improvement (purification), Biomarker Identification
Naughton et al, 2018	Diisopropyl- fluorophosphate (DFP)	Rat- Blood and Brain Matter	Damage to myelinated axons.
			Decreased axonal transport.
Naughton et al 2020	DFP	Rat- Embryos	Potential treatment of long term neurological deficits due to OP exposure
	LiCl + Methylene Blue		
Peynet, 2017	TCP	Human-Hair	No significant difference between active and control
			Suggestion that above threshold values indicate exposure in aircrew.
Reneman et al, 2016	Aircraft Fumes	Human- Brain Imaging (MRI)	White matter impacted. Increased blood flow to left occipital cortex.
			Hypoactivation in the precuneus and right prefrontal cortex.
Schindler et al, 2012	TCP	Human- Urine	No ToCP metabolites found. Other TCP isomers found in one case
	Other OPs		Five OP metabolies found to be enhanced as compared to controls
Schindler et al, 2013	TCP	Human- Urine	No TCP metabolites found.
	Other OPs		Tributyl and Triphenylphosphate metabolites elevated compared to controls
Schopfer et al, 2010	CBDP	Human- Blood or Blood Products	BChE-CBDP adduct- Possibly unique to ToCP
Schopfer et al, 2014	BChE-CBDP Adduct	Human- Blood or Blood Products	New analysis method.
Sibomana et al, 2019	Aircraft Engine Oil	Rabbit- Dermal	Slightly irritating to the skin
Sibomana et al, 2021	Aicraft engine oils	Rat- Dermal+ Blood and Blood Products	AChE decrease, persistance sex dependant.
Sibomana and Mattie, 2020	Aircraft Engine Oil	Rat- Dermal, Organ, Blood and Blood Products	Decrease in mass of sexual organs (Testies, Uterus)
			Changes in liver, spleen, and adrenal weights (sex dependant)
			Changes in blood parameters.
Strid et al, 2014	PBDEs	Human- Blood and Blood Products	Median concentrations elevated in maintanence workers.
			Max concentration found in aircrew. High value also found in control.
Sun et al, 2017	VOCs	Human- Breath	Identified 10 VOCs with greater than 50% detection rate.
			Additinally identified VOCs as exo/endogenic
Tacal and Schopfer, 2014	ToCP	Human- Blood and Blood Products	No evidence of BChE-CBDP adduct.
			Elevated autoantibodies indicative of CNS damage
Wei et al, 2012	Pyrethroid insecticides	Human- Urine	Elevated metabolite concentrations in active group
Yang et al, 2016	VOCs- Reaction products with ozone	Human- Skin Oils	Reaction products included acetone, 6-MHO, nonanal, and decanal
Yong et al 2008	Radiation Exposure	Human-Chromosome translocations	Average translocation was 25% higher in pilots than the control group

SI Table 4 Summary and generalized findings of review papers identified.

Author	Focus of Review	Generalization
Bagshaw and Illig, 2019	Cabin Air Quality	Acknowledges cabin contamination, health effects not proven to be linked.
Bendak and Rashid, 2020	Fatigue	Describe scenarios in which fatigue becomes an issue for aviators.
Bull, 2007	Cabin Filtration	Description of filtration and effectiveness onboard aircraft.
Burdon, 2015	Rebuttal	Letter to editor regarding de Ree of same title.
Chaturvedi, 2009	Cabin Air Quality	Describes fatal air quality events, and carbon like buildup inside ECS.
Chen et al, 2011	Cytochrome P450	Provides information about the Cytochrome P450 superfamily, discusses variability, opportunity for personalized medication.
Chorley et al, 2011	UV light Exposure	Describe tentative link between pilots and cataract frequency.
Co and Kwong, 2020	Breast Cancer	Describe levels of breast cancer in flight crew similar to that of the general population.
Costa, 2018	OPs	Describes historical use and health implications of OP exposure
de Boer et al, 2014	Aerotoxic Syndrome	Knowledge gap identification.
de Ree et al, 2015	Rebuttal	Reply to Burdon, 2015 letter to editor regarding de Ree of same title.
Farrauto et al, 2016	Catalysis	Fouling of palladium catalysts onboard aircraft.
Furlong et al, 2017	Cabin Air Quality	Estimation of incidence frequency and discuss sensitivity to exposure.
Grout and Leggat, 2021	Fitness to Fly Requirements	Discusses Fitness to Fly disparities across the industry- Focus on Covid 19
Hageman et al, 2020	Aerotoxic Syndrome	Discusses potential diagnostic criteria for the determination of Aerotoxic Syndrome.
Harrison and Mackenzie Ross, 2015	Cabin Air Quality	Discuss gaps in literature and issues that prevent forming firm conclusions about the health implications of contaminated cabin air.
Howard, 2017	OP Exposure	Informs on neurobiological processes that allow for illness related to low dose chronic exposure to Ops
Jong, 2017	Cabin Air Quality	Air movement within the cabin is discussed.
Ke et al, 2014	Airworthiness Guidelines	Discusses current legislation and how to better allow manufacturers to reach compliance.
McKinlay et al, 2008	Pesticides	Discuss pesticides as a potential health risk to aircraft crew and passengers.
Michaelis, 2017	Cabin Air Quality	Discusses historical findings of aviation regulatory bodies and stakeholders.
Michaelis, 2018	Cabin Air Quality	Discusses the lack of compatibility between clean air requirements and the operation of bleed air systems.
Moir and Seabridge, 2008	Aircraft Pneumatics	Describes aircraft pneumatic systems and their operation.
Naughton and Terry, 2018	OP Exposure	Discusses neurobiological consequences of OP exposure and identifies non-conventional receptors.
Nicholson, 2009	Cabin Air Quality, Sleep Disruption	Discusses the inability to rule out chronic low dose exposure. Discusses jet lag.
Nicholson et al. 2003	Risks of Flight	Describes cabin air quality concerns, mild hypoxia, and blood clotting issues.
NRC, 2002	Cabin Air Quality	Describes sources of air contamination on aircraft and potential for risk.
Oster et al 2013	Aviation Safety	Describes state of aircraft safety and proactive approach outlined by regulators.
Pang et al, 2020	Pyrethroid Insecticides	Reviews the evidence of health consequences related to insecticide application on aircraft.
Ramsden, 2013	Cabin Air Quality	Describes the disparity between theoretical ToCP concentration and actual found on aircraft.
Rim, 2017	Reproductive Health	Discusses the reproductive implications of microwaves on aircraft.
Rudikoff, 1998	Relative Humidity	Describes the health effects of dry skin as a result of low humidity environments.
Shehadi et al, 2015	Bleed Air Cost Benefit	Discusses the financial cost of fume events to the airline industry.
Silverman and Gendreau, 2009	Risks of Flight	Describes pre-existing medical conditions that may be complicated by flight, discusses other flight risks.
Wang et al, 2007	VOCs and Catalysis	Compares VOC loadings within aircraft as compared to other forms of transportation. Low Humidity may be a problem for photocatalytic degradation of compounds.
Watterson and Michaelis	Threshold Limits	Discuss the premise that conventional TLVs should not be applied to the aircraft environment.
Winder and Balouet, 2000	Aerotoxic Syndrome	Discusses the symptomology of flight crew linked to chemical exposure.
Winder and Balouet, 2002	Aerotoxic Syndrome	Discusses the chemical composition of aircraft engine oil and the associated health risks of the components.
Wolkoff et al, 2016	Cabin Air Quality - Comparison	Discuss the similarities between symptoms of aircrew and office workers.
Wright et al, 2018	ECS Fouling	Describes function of aircraft air conditioning system and potential reasons for pack failure.

2.0 INORGANIC ANALYSIS OF FRESH AND USED AIRCRAFT OIL

Author Contributions Statement

- Conceptualization: Kevin Hayes
- Methodology: Kevin Hayes, David McKendry
- Investigation: Kevin Hayes, Gwen O'Sullivan, David McKendry
- Formal analysis: Kevin Hayes
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2.1 Abstract

An elemental analysis was conducted on matched pairs of new and used aircraft engine oils for various aircraft engine types (piston, turboprop, and jet). The analysis aimed to determine what, if any, accumulation or loss of oil and fuel additives may occur with engine use. Losses in elemental loadings from new to used oils imply that there may be a possibility for the element-containing compounds to enter the pneumatic system of bleed air pressurized aircraft at a higher rate than that of oil attrition and potentially impact cabin air quality. Accumulations of wear metals/elements or fuel additives in the used oil can alter the product, creating a more hazardous substance than the new product, and create an occupational risk that is not present otherwise. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES) was employed to complete the elemental analysis. This study details a greater than twenty percent loss of phosphorus from new to used jet oils. Phosphorus-containing additives appear to be lost from jet engine oil with use, potentially becoming available to the bleed air systems of the aircraft in excess of the intact oil that may enter the cabin in certain conditions. A significant accumulation of lead is noted in the used oil of piston aircraft (Range: <LOD to 6821 ± 83 mg kg^{-1}; n=2). The concentration of lead in the used oil product is many times greater than that of leaded aviation fuel and warrants additional precautions when handling the product.

2.2 Introduction

Additives within the lubricating oil of aircraft engines are suspected of being a source of contamination responsible for increased occupational risk within the aircraft industry

(Winder and Balouet 2002; Harrison and Mckenzie Ross 2016; Megson et al. 2016; Michaelis et al. 2017; Hayes et al. 2021). The premise of the exposure to intact and pyrolyzed oil in pressurized aircraft is justified by a viable unfiltered pathway from the engines to the cabin and cockpit via the aircraft bleed air system (Moir and Seabridge 2008). The ultimate transfer of this lubricating oil along with other potential contaminants like hydraulic fluid to the receptors (principally flight attendants and pilots) has been described as having both acute and chronic health effects (Winder and Balouet 2000; Abou-Donia et al. 2013; Hageman et al. 2020). The residues of oils in the aircraft cabin are usually sought by employing organic analytical methods (Solbu et al. 2011; Wang et al. 2014; Rosenberger 2018; Schuchardt et al. 2019). This has proven to be a useful practice with contaminants of concern (tricresyl phosphate isomers) being located in jet aircraft cockpit/cabins and demonstrating a strong correlation to the relative isomer profile within the aircraft oil, albeit at below ng cm^{-2} levels in wipe samples (de Ree et al. 2014; Crump et al. 2011; Solbu et al. 2011). Phosphorus-containing compounds are added to jet and turboprop oils to act as boundary wear additives, and are typically included in jet and turboprop oil at percentage levels (Johnson 2016; Imperial 2021).

Dermal and oral exposure to oil and oil additives may be a significant pathway for aircraft mechanics, or those who operate smaller, privately owned, or privately maintained aircraft, due to aircraft pilots/owners being permitted to complete preventative maintenance on their own aircraft (FAA 1964). Blood and wipe sampling for lead on aircraft hangar surfaces based upon the metals concentrations in AVGAS have been completed (Chen and Eisenberg 2013). Inorganic lead dust (primarily lead oxide) concentrations were determined; lead oxide is approximated to account for 95% of the lead resultant from the combustion of AVGAS containing tetraethyl lead (TEL), and is emitted with the exhaust (Chen and Eisenberg 2013; Kumar et al. 2018). Results of wipe analysis indicated that lead was present on many surfaces within and outside of the aircraft hangar, and noted that lead was prominently present on workers hands (colorimetric wipes) prior to hand washing (Chen and Eisenberg 2013). Exhausted lead oxide describes only one pathway to the human receptors however, a significant quantity (5%) of the lead is expected to remain in the engine and engine oil (EPA 2008).

The multi-elemental analysis of engine oils is routinely performed in many industries including aviation; monitoring for wear metals from various engine components can be a very useful tool for preventative maintenance (Dellis 2019; Kim et al. 2013; Grimmig et al. 2021). It may also be a tool for assessing the occupational risk of personnel working with or on aircraft. Multi-elemental analysis of the oil may provide valuable insight describing what oil and fuel additives are potentially accumulating, being degraded, or being lost from aircraft oil. The presence or lack of potentially toxic elements is important for describing additional potential exposure pathways (van Netten 1999). Inhalation exposure within the cabin is largely a concern in bleed air pressurized aircraft.

Elemental analysis of unused aircraft lubricating oil and hydraulic fluid has been conducted in the past with the aim of determining if potential toxic elements were present in the new products and if they may contribute to air quality concerns (van Netten 1999). This seminal analysis demonstrated that the concentration of toxic inorganic elements in the new aircraft fluids could not be associated with ill health. However, the absence of used oil samples in van Netten (1999) does not address the potential for wear metals from the engine, nor, elements from the fuel or fuel additives accumulating in the oil. When considering pressurized aircraft, the presence of potential elements of concern within the new oil, does not intrinsically describe whether or not a potential pathway to the aircraft cabin exists. This study aims to address these knowledge gaps. Additionally, higher lead concentration on workers hands, as compared with other surfaces within the hangar identified the Chen and Eisenberg (2013) study, would suggest that deposition of inorganic lead from piston engine exhaust does not adequately explain that exposure pathway. The multi-elemental analysis of new and used oils, in multiple aircraft engine types, when coupled with the accumulation or depletion of elements within used oils as compared to new, provides more holistic data as to what contributions these oils may have to the occupational or personal risk of maintaining and operating aircraft.

2.3 Methods

2.3.1 Sample collection of used and new aircraft engine oil

Used engine oil samples from seven piston engine and eight turboprop engine aircraft were collected with the assistance of maintenance employees of the Mount Royal University Aviation program. Sample collection was opportunistic, with collection occurring during routine service of the aircraft. All non-jet aircraft were based out of the Springbank Airport (Calgary, Alberta, Canada). The aircraft models, engine, and lubricating oil brand are displayed in Table 4. Used jet aircraft oil samples were sourced from Falcon Business Jets in Europe (Table 4).

New engine oils (FJ1; FT1; FP1) were purchased from Skygeek.com. Respectfully, the sample abbreviations refer to Mobil Jet Oil II (ExxonMobil, USA); Eastman Turbo Oil 2380 (Eastman Aviation Solutions, Eastman Chemical Company, USA); and XC Aviation Oil 20W50 (Phillips 66, USA). These oils provide matched pairs for each of the used aircraft oils sampled.

Additional sample information that could be potentially beneficial to this research was not available. Engine hours were provided only for turboprop aircraft between oil changes and do not describe volume of top up oil added between services. Engine hours are not available for piston or jet engine aircraft oil samples. Additives to the oils or fuels of all aircraft, with the exception of tetraethyl lead in AVGAS for piston engine types, are also unknown. These can be derived experimentally by comparing against the new oil product, but the brand, amount, or frequency of addition is unknown. In most cases, however, it is sufficient to demonstrate the presence and concentration of an element of concern, as this will adequately describe the risk of the used oil product; additional information would provide information as to how quickly, or why it is accumulating, but not change the occupational risk associated with the concentration.

Table 4 Sampled Aircraft, Oil, and Engine Type. The used lubricating oil for each aircraft type (indicated in the table) was matched with the same unused oil. The new oil of each type is indicated by the letter F proceeding the sample: FP1 is new XC- Aviation 20W50; FT1 is new Eastman Turbo Oil 2380; FJ1 Is new Mobil Jet Oil II. Jet and Beechcraft 1900D aircraft have pressurized cabins, all other aircraft are unpressurized. * Indicates the typical configuration(s) for the aircraft when actual engine type is unknown.

Sample ID	Aircraft Type	Lubricating Oil	Engine #	Engine Type
	Piston			
P1	Cessna 172R	XC- Aviation 20W50	Single	Lycoming IO-360*
P2	Cessna 172R	XC- Aviation 20W50	Single	Lycoming IO-360*
P3	Cessna 172R	XC- Aviation 20W50	Single	Lycoming IO-360*
P4	Cessna 172R	XC- Aviation 20W50	Single	Lycoming IO-360*
P5	Cessna 172R	XC-Aviation 20W50	Single	Lycoming IO-360*
P6	Piper PA-34-220T	XC-Aviation 20W50	Dual	Continental TSIO-360*
P7	Piper PA-34-220T	XC-Aviation 20W50	Dual	Continental TSIO-360*
	Turboprop			
T1	DHC-6-300	Eastman Turbo Oil 2380	Dual	PT6A-27
T2	Beechcraft 1900D	Eastman Turbo Oil 2380	Dual	PT6A-67D
T3	Beechcraft 1900D	Eastman Turbo Oil 2380	Dual	PT6A-67D
T4	DHC-6-300	Eastman Turbo Oil 2380	Dual	PT6A-27
T5	DHC-6-300	Eastman Turbo Oil 2380	Dual	PT6A-27
T6	DHC-6-200	Eastman Turbo Oil 2380	Dual	PT6A-27
T7	DHC-6-200	Eastman Turbo Oil 2380	Dual	PT6A-27
T8	DHC-6-300	Eastman Turbo Oil 2380	Dual	PT6A-27
	Jet			
J1	Falcon (model unknown)	Mobil Jet Oil II	Dual or Tri*	Rolls-Royce Pearl 10X, PW307D, PW307A, PW812D, Honeywell TFE731-60, PW308C*
J2	Falcon (model unknown)	Mobil Jet Oil II	Dual or Tri*	

2.3.2 Sample and calibration solution preparation

Used and unused oil samples were homogenized then diluted using PremiSOLV ICP Solvent (SCP Science, Canada) to ten percent by weight (standard deviation of DF = 0.07) using an analytical balance (Sartorius MC1 Analytical AC 210S, Germany). Piston oil samples were further diluted (400:1 diluent: oil) following initial analysis to ensure Pb concentrations fell within the dynamic range of the instrument calibration. The dilute samples were introduced directly to the instrument without further treatment. Procedural blanks consisted of neat PremiSOLV.

Calibration curves for sulfur and a multi-element standard (Conostan, SCP Science, Canada; SI Table 4) were prepared as percent by weight dilutions of the stock standard solutions with PremiSolv. A five-step calibration was established for sulfur; range: 0.05 mg kg^{-1} - 25.0 mg kg^{-1}. A six-step calibration was completed for the multi-element solution; range 0.025 mg kg^{-1} - 25.0 mg kg^{-1}. The R^2 value of the curves for all elements exceeded 0.997. Calibration was completed before sample analysis daily, as well as, following torch changes necessitated by carbon build up. Calibration and drift check samples were composed of 2.5 mg kg^{-1} and 0.5 mg kg^{-1} multi-element calibration standard in PremiSOLV. One of these check samples was analyzed following every five samples, proceeded and trailed by procedural blanks. Each calibrant and unknown sample completed four runs for the purposes of calculating mean and relative standard deviation (RSD). Data presented in this manuscript is a representation of the mean data of the four runs.

2.3.3 ICP-OES parameters

An ICP-OES (ICAP 6300 Duo, Thermo Scientific), operated with an RF power of 1150 W and a nebulizer gas flow of 0.35 L min^{-1}, was equipped with a baffled cyclonic spray chamber, 1 mm center tube, and a V-groove nebulizer (Duo Organics Kit, Thermo Scientific). Samples were introduced manually with the instrument standard sample probe and short highly inert tubing (SolFlx, SCP Science) via peristaltic pump (30 RPM). Uptake and wash cycles were completed at 40 RPM, with the wash process taking place without solvent to limit carbon loading on torch/optical path. Element wavelengths were chosen according to software suggestion followed by manual selection based upon peak shape and minimization of interfering spectra (SI Table 1). All wavelengths chosen agree with those suggested in ASTM D5185 (2018).

2.3.4 Drift correction and statistical models

Carbon buildup on the torch and optical path resulted in a slow downward drift in analytical sensitivity. This drift was monitored closely during the run and if it exceeded 5% between checks for monitored elements, the run was stopped, the torch replaced, and the data discarded to the previous check that fell within tolerance. The instrument was then restarted and recalibrated prior to resuming analysis. The allowable drift that occurred was corrected via a geometric drift correction method (McLelland and Fleck 1978). Limit of detection (LOD) and Limit of Quantification (LOQ) were calculated by determinization of 3x and 10x the standard deviation of all procedural blanks, respectively (SI Table 2). The elements K, Ag, Co, Na, and V were present in the multielement standard but below the LOD in all samples and were therefore not included in the data analysis.

2.4 Results

The mode of power for the aircraft (engine type), and if the aircraft is pressurized by the engines plays a large role in the potential exposure pathways that may be present. The piston engine aircraft in this manuscript are not pressurized and therefore, the risk associated with the oil is primarily dermal exposure of maintenance workers or pilots who do their own routine maintenance. The turboprop aircraft used in this study were a mix of non-pressurized and pressurized aircraft (Table 4). The pressurization of the cabin via the engines adds a potential exposure pathway in which the oil (possibly pyrolyzed) may reach the aircraft cabin occupants (Winder and Balouet 2001). The same is true of jet aircraft, in which the cockpit and passenger cabin are almost exclusively pressurized by bleed air from the engines (Moir and Seabridge 2008; Hayes et al. 2021).

2.4.1 Piston engine oil- Wear elements

FP1 was determined to have no measurable concentration (>LOQ) of any element sought, save for sulfur (mean 878.4 mg kg^{-1}). All used samples collected (n=7) were from aircraft which use exclusively FP1 oil. Figure 3, demonstrates the loading of wear metals determined to be in the oil by individual aircraft oil sample. Many wear metals that exist below LOD/LOQ in the new oil samples were routinely found above LOQ in the used oil samples, especially Cr, Cu, Mo, Zn, Al, Cd, Fe and Ni. Each of these wear metals is associated with engine components as described in an established method (ASTM 2019).

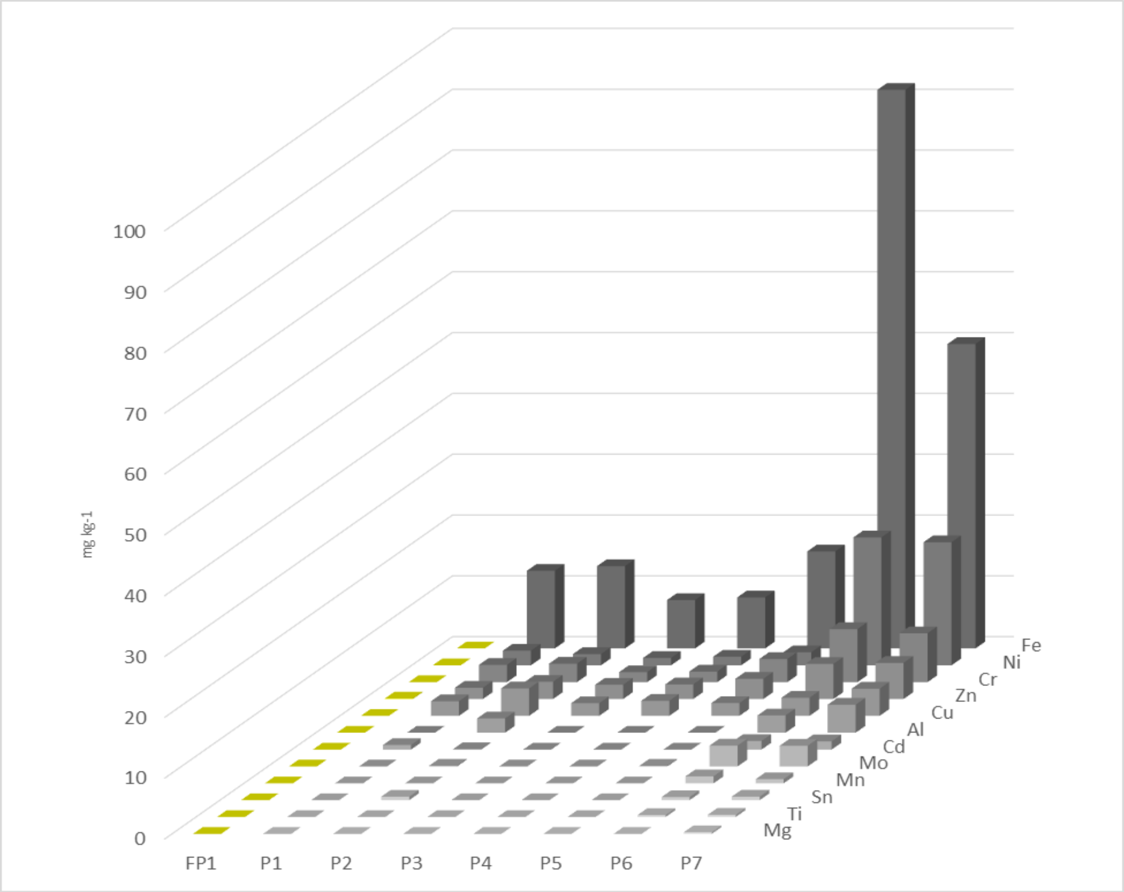

Figure 3 Accumulation of wear elements (mg kg^{-1}) in used oil as compared to new in piston aircraft. FP1 describes the average of three replicate samples of new XC-Aviation oil. P1-5 describe used oil samples taken from Cessna aircraft. P6-7 describe used oil samples taken from Piper aircraft.

2.4.2 Piston engine oil – Oil and fuel additives

Elements are also introduced to the engine oil due to natural loadings or additives in the fuel. The piston engine aircraft sampled all use 100 octane low lead (100LL) fuel. This fuel has tetraethyl lead added to it to prevent engine knocking at high RPM (FAA, 2022). Lead was not present above LOD (2.603 mg kg^{-1}) in new oil and increased to several thousand mg kg^{-1} in the used oil samples (min-max 5114 mg kg^{-1}- 6821 mg kg^{-1}) (Figure 4). 100LL AVGAS contains up to 0.56 g of TEL per liter (EPA 2008; Kumar et al. 2018). At 15 degrees Celsius the density of 100LL is reported as 710 kg m^{-3} (Government of Canada 2016). This results in a concentration of TEL in the fuel of approximately 0.79 g kg^{-1}. Comparing this with the concentrations of lead found in the used aircraft oil samples (acknowledging that speciation of the lead is not possible with this dataset), the lead concentration in the used oil, on average ($\bar{x}$ = 5.77 g kg^{-1}), exceeds that of the AVGAS by a factor of 7.3. Comparative lead values in used piston engine aircraft oil (0.3-3.1 g/L) were identified in Turgut et al. (2020). Turgut et al. found lead in the exhausted emissions of aircraft, as well as the used oil product, while finding low comparative amounts (334 ug/L) in the new oil. They similarly attribute this concentration to the oil scavenging the lead from surfaces within the engine.

Sulfur is present in the new piston aircraft oil and within 100LL fuel, which is exempted from sulfur-reducing measures in Canada (Government of Canada 1999). Used oil has accumulated on average 1.64x the sulfur of new oil for the Cessna aircraft and 3.9x on average of the two Piper aircraft (Figure 4). Phosphorus-containing compounds are not typically present in 100LL fuel, nor are they found in FP1 oil above LOD. However, aircraft oil samples P6 and P7 both exhibited an accumulation of this element (Figure 4).

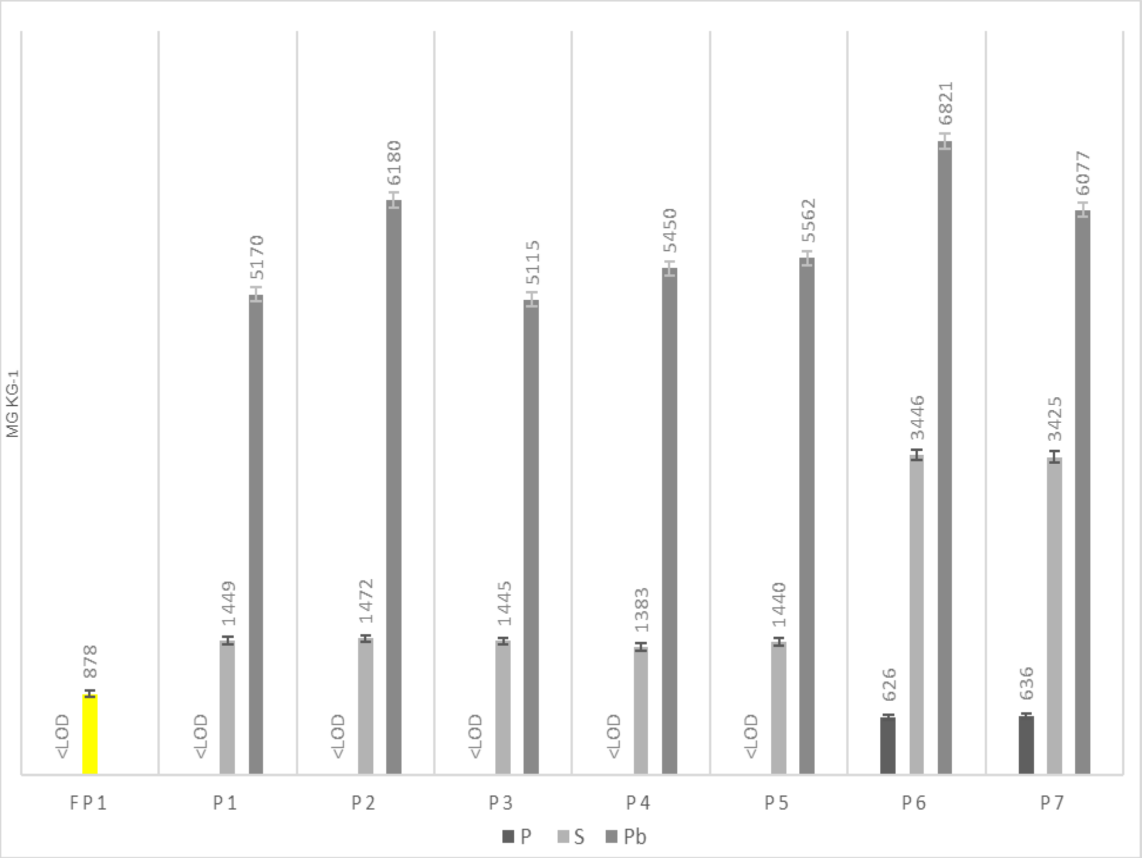

Figure 4 Accumulation of phosphorus, sulfur, and lead (mg kg^{-1}) in piston engine oil due to the addition of additives or fuel. FP1 describes the average of three replicate samples of new XC-Aviation oil. P1-5 describes used oil samples taken from Cessna aircraft. P6-7 describe used oil samples taken from Piper aircraft. Uncertainty is expressed as the Poisson Error of the discrete measurements.

2.4.3 Turboprop oil analysis

FT1 is a matched oil type to that used in all turboprop aircraft within this study. The only element detected in the new turboprop oils present in concentrations above the LOQ was phosphorus. New oils contained more phosphorus than used oils (Figure 5); this is indicative of phosphorus additives degrading or otherwise being lost from the oil during engine operation. Sub mg kg^{-1} levels of Mo, Mg, Zn and Fe exceeded respective LOQ values for used oil sample T6; Zn was also detected above LOQ in T7 and T8; no other elements met LOQ for any sample. This data describes only conditions at the oil change and does not account for any makeup oil that may have been added to the engines.

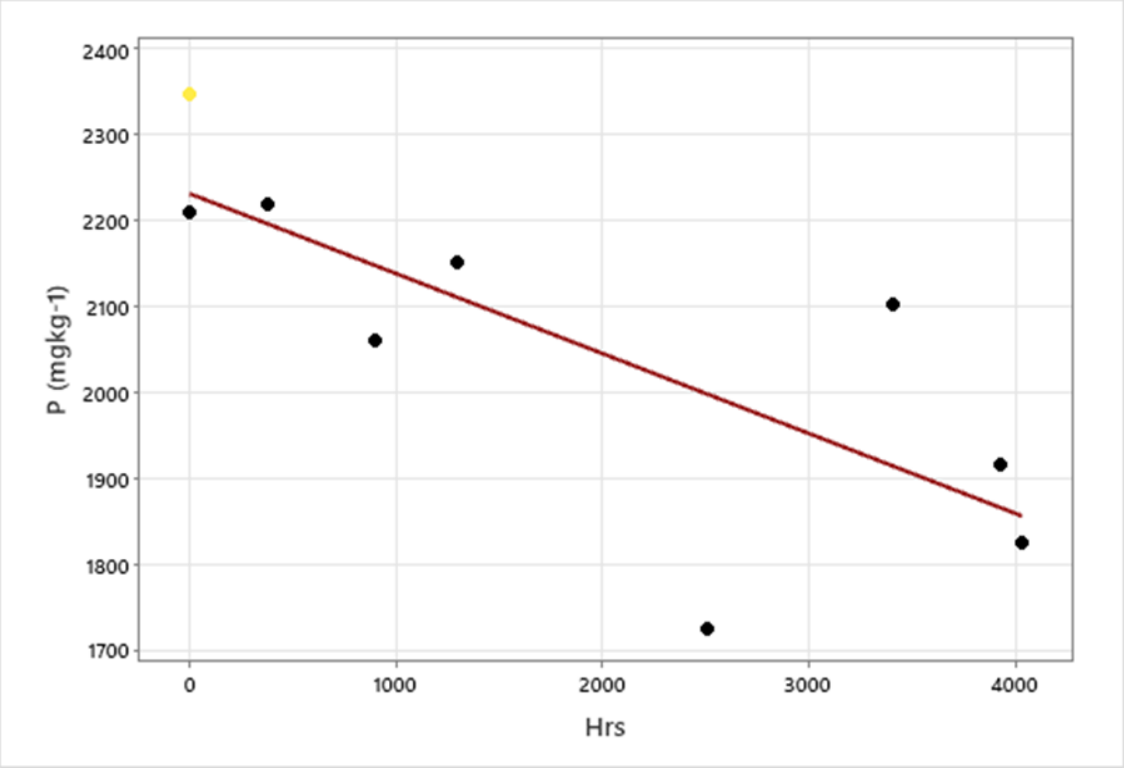

Figure 5 Phosphorus concentration in new and used turboprop engine oil. The yellow dot represents the average of phosphorus concentration of four replicates of new Eastman Turbo Oil 2380. Black dots represent the average of replicates (T3: 1825 mg kg^{-1} and T4: 1724 mg kg^{-1}) or individual phosphorus concentrations of used oils. There is a negative correlation (r = -0.76 p = 0.017) described between the phosphorus concentration in the oil and engine hours. The p-value may not accurately represent the confidence in the correlation, or the strength of the correlated relationship because the number of data points within the regression is limited (n = 9).

2.4.4 Jet oil analysis

The only element present in concentrations above LOQ in new jet oil was phosphorus. Upon analysis of four replicate samples of FJ1 it was determined to have an average P loading of 2291.4 mg kg^{-1} (Std-dev 40.15mg kg^{-1}).

Two samples of used jet oil J1 and J2 were analyzed; sub mg kg^{-1} levels of Cu, Zn, Fe, and Sn (indicative of the operational wear of various engine components) were detected in both used oil samples (Figure 6). Elements present in jet fuel, namely the Jet 1A, used almost exclusively in locations outside of the USA, also appear to have accumulated in the oil; barium was not detected in new oils and but was present in both of the used oil samples J1 and J2 (Table 5). Barium is known to exist in sub mg kg^{-1} levels in Jet 1A fuel (Abegglen et al. 2016); the concentration detected in the used jet oils is approximately 20 mg kg^{-1}, suggesting significant enrichment in the oil (Table 5). Sulfur content in jet fuel by regulation must be lower than 3000 mg kg^{-1}; this limit is significantly higher than the concentration regularly reported in fuel which is typically less than 1500 mg kg^{-1} (EASA 2010). The results indicate that sulfur from the fuel also appears to be accumulating in the oil during engine operation (Table 5).

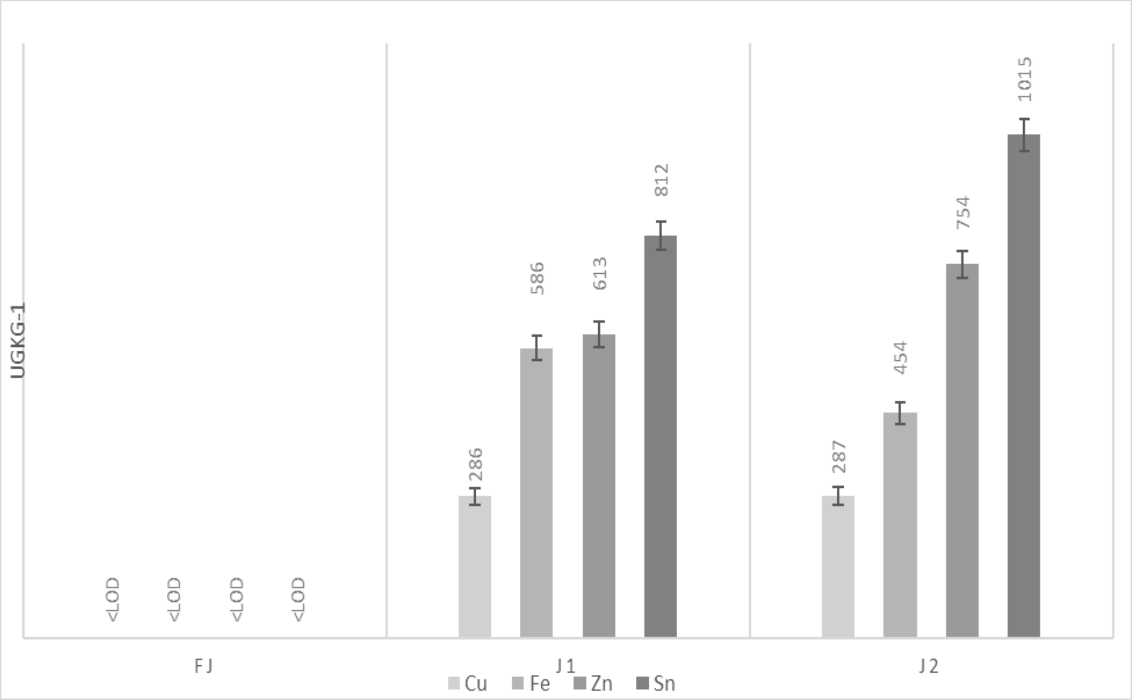

Figure 6 Accumulation of wear elements (µg kg^{-1}) in used oil as compared to new in Jet aircraft. FJ describes the average of four replicate samples of new Mobil Jet Oil II. J1 describes the average of two replicate samples of used Mobil Jet Oil II. J2 describes two replicates of used Mobil Jet Oil II. Uncertainty is expressed as the Poisson Error of the discrete measurements.

Table 5 Elements (mg kg^{-1}) found in jet engine oil due to the addition of additives or fuel. FP1 describes the average of four replicate samples of new Mobil Jet Oil II. J1 and J2 represent two replicate samples of each used Mobil Jet Oil II respectively. Uncertainty is expressed as the Poisson Error of the discrete measurements.

mg kg^{-1}	FJ1	J1	J2
P	2291 (± 48)	1424 (± 38)	1829 (± 43)
S	<LOD	158 (± 13)	244 (± 16)
Ba	<LOD	20 (± 5)	23 (± 5)

2.5 Discussion

2.5.1 Piston engine aircraft – Exhaust

To gain a commercial pilots license, individuals are required to complete hundreds of flight hours and gain the possession of a personal pilot’s license, much of which takes place utilizing small piston engine aircraft (Government of Canada 2019; AOPA 2022; EASA 2022). The non-pressurized cabins of piston engine aircraft limits but does not eliminate the potential interaction with the aircraft engine oil. Inhalation of exhaust fumes, ranging from generalized minor exposure at the airfield to potentially very serious exposure if a leak has occurred in the exhaust system is possible (Ells 2015). The oil present in this exhaust is limited; the operator’s manual for the Lycoming engine Cessna aircraft (Table 4) suggest that at performance cruise (2450 RPM) the engine will consume between 8.8 and 12.3 gallons (33.3-46.6 L) of fuel per hour, with a maximum allowable oil consumption of between 0.39 and 0.5 quarts (0.37-0.47 L) per hour, depending on engine series (Lycoming 2005). This implies that at maximum allowable oil consumption, approximately one percent of what is being burned and emitted could be attributed to the oil.

2.5.2 Piston engine aircraft oil – Lead

Lead is listed as a hazardous component of 100LL AVGAS in the safety data sheet; but as noted, is not mentioned in the SDSs of the oil despite the increased concentration in the used product (Phillips 66 2018; Phillips 66 2017). Lead (IV) oxide has an estimated oral acute toxicity estimate of 500 mg kg^{-1} (Sigma-Aldrich 2023). TEL has a noted, lowest observed adverse effect level for repeated oral dose toxicity of 0.2 mg/kg in rats; the acute toxicity is noted as 14.18 mg kg^{-1} (Sigma-Aldrich 2023b). There are few studies that describe dermal exposure to tetraethyl lead; the data suggests that TEL is absorbed through the skin, and in doing so becomes systemically available (NIOSH 2017). Inorganic lead is also known to be absorbed dermally (Lippmann and Leikauf 2020).

Dermal contact with used aircraft oil is a possibility, with oil changes necessitating direct handling of the product. Modern Cessna service manuals note that skin contact with engine oil is to be avoided and to remove any oil that gets on your skin immediately, this is not true of the older Cessna manual, nor the manual version of the Piper aircraft in this study (Cessna 2007; Cessna 1977; Piper 1997). The SDS for the new piston engine oil used in this study warn against skin contact with used oil based upon the carcinogenic properties, noting that the oil can accumulate impurities that may be harmful; the new product does not meet classification criteria that would designate it as a hazardous substance, and does not warn of lead accumulation in the used product (Phillips 66 2017). Inhalation exposure of the metal is also a possibility. Prescribed procedure for engine oil changes of the piston engine aircraft described in this study requires that the oil be drained when the engine is at operating temperatures (Cessna 2007; Cessna 1977; Piper 1997) This coupled with the volatility of TEL could increase inhalation risk should the contaminant be present in the oil during service; TEL's initial boiling point is approximately 85 degrees Celsius and time-weighted average exposure thresholds Canada and the United States range from 0.05mg/m3 to 0.1mg/m3 (Sigma-Aldrich 2023b). Individuals servicing the aircraft do not appear to be informed of the elevated concentrations of lead in the oil and may not be taking appropriate steps to mitigate exposure.

Exposure to lead is demonstrably damaging to the body; systems negatively impacted include neurological, renal, cardiovascular, hematological, reproductive, and immune at concentrations of 5-10 ug/dL; Kumar et al (2018) reports these effects at blood levels as low as 5-10 ug/L (Kumar 2018; Klemick et al. 2022; Rubens et al. 2001; Wani et al. 2015; WHO, 2021) The environmental emission of lead from the combustion of aviation fuel has been identified as a cardiovascular risk to senior citizens living in close proximity of airports that service primarily piston engined aircraft (Klemick et al. 2022). At higher and/or chronic exposure, but still often subclinical dosing, negative neurophysiological effects have been identified (Araki et al. 2000; Rubens et al. 2001; Thomson and Parry 2005; Gidlow 2015). The higher concentration on workers hands, as compared with other surfaces within the hangar in the Chen and Eisenberg (2013) study, would suggest that deposition of inorganic lead from piston engine exhaust does not adequately explain the exposure. Given the results of this study (average concentration of lead in used aircraft oil = 5.77 g/kg), workers' contact with engine oil can account for this discrepancy. Analyzed lead blood levels in the workers present in the Chen and Eisenberg (2013) study were below 10 µl dL^{-1} and therefore urinalysis was not completed. This does not satisfactorily explain the true occupational exposure or personal risk, especially if TEL is present in the used oil. TEL, unlike inorganic lead, is metabolized by the liver and largely excreted through urine, resulting in blood levels that are moderately increased, but substantial increases in urinary levels (Gidlow 2015). Regardless of the mechanism, the WHO suggests that no level of lead exposure is safe, and that any continued exposure will result in the body absorbing more lead and furthering the elements harmful effects (WHO 2021).

2.5.3 Piston engine aircraft oil – Wear Elements and Phosphorus

Multiple wear metals were found to be accumulating in used piston engine aircraft oil. Elements, such as cadmium and nickel, which are demonstrably damaging to human health were found in only the used oil product (Gates et al. 2023; Koons and Rajasurya, 2023). These potentially toxic elements bioaccumulate, so there is a potential for concern if routinely exposed, even to non-acutely toxic quantities (Witkowska et al. 2021). The presence of these elements makes the used oil product of piston engine aircraft more hazardous to handle than the new product.

Phosphorus was not found in the new piston engine aircraft oils but was present in used oils of piper aircraft (Figure 4). One possible explanation for the presence of this element in significant concentrations (mean 630.9 mg kg^{-1}) could be the addition of an after-market fuel additive product like ‘TCP’ (Alcor USA). This product is FAA approved and is used to prevent lead buildup on sparkplugs and valves; the product contains tricresyl phosphate at 10-20% by weight and is prescriptively added to the fuel at 0.03L ‘TCP’ per 37.85L of fuel (Alcor 2014). Another potential source of phosphorus in piston engine oil is the addition of oil additives; aircraft with a variety of Lycoming engines are subject to an airworthiness bulletin from the FAA which requires the addition of LW-16702 (tricresyl phosphate) at approximately one ounce per quart of engine oil as an anti-wear agent, or requires the use of a commercially available oil that already meets this requirement (Lycoming 2009; Aircraft Spruce 2017). Tricresyl phosphate, with emphasis on its ortho-substituted isomers, is neurotoxic, and has been identified as a contaminant of concern regarding the occupational risk of chemical exposure in aviation (Winder and Balouet 2002; Harrison and Mckenzie Ross 2016; Hayes et al. 2021).

2.5.4 Jet and turboprop aircraft – Phosphorus

The phosphorus content in the used jet oil as compared to new demonstrates that during aircraft operation additive phosphorus-containing compounds are lost from the oil (Table 5). It is important to note that this is a small sample size (two used jet oils), and that other important information such as make-up oil volume, engine hours, engine type and aircraft model are not known. However, this finding demonstrates a loss of phosphorus from jet oil due to use, and this loss implies that phosphorus-containing compounds may be available to the bleed air system in excess of the intact oil that may enter the environmental control system. This is amplified when considering oil changing practices for most commercial jet aircraft; The aircraft burn enough oil that fresh oil is required to be added regularly, therefore there is no need for oil to be drained from the engines under normal circumstances (ExxonMobil 2016).

2.6 Conclusions

Used aircraft oils demonstrate elemental compositions that differ from their new counterparts. As expected, and by design of the lubricant system, concentrations of elements present in components of the engines of the aircraft appear in the used engine oil. Wear metals from these components were detected in all used engine oil types, but were most prevalent in piston engine aircraft.

The accumulation of toxic elements in the oil with aircraft operation creates a used oil that is more hazardous to handle than the new product. When considering the human health implications of the oils, fuel and oil additives are also significant. Of particular interest was the increased lead loading in the used oil of piston engine aircraft (<LOD new oil to 5768 mg kg^{-1} mean) and the decrease in phosphorus found in both turboprop and jet engine oil resultant from engine operations (Figure 5; Table 5). This provides evidence indicating that contaminants of concern (namely phosphate esters and organometallic complexes of lead), as proxied by their non-organic components, are both accumulating and depleting from aircraft engine oils with use.

In pressurized aircraft the evidence suggests that phosphorus-containing compounds, intact or degraded, are available to the bleed air system and possibly entering the cabin or other pressurized systems. In smaller, non-pressurized aircraft, the presence of lead and phosphorus in used piston engine oil indicates the potential for toxic exposures via dermal pathways or exposure to vapor during maintenance. The risk associated with hazardous accumulations in the used oil is not adequately communicated, and therefore is likely often unmitigated. Additional research should be undertaken to speciate the compounds that are linked to elemental loadings described in this manuscript.

2.7 Declaration of competing interests

The authors have no known competing financial, or non-financial, interests.

2.8 Acknowledgements

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2.9.1 Supplementary Information

SI Table 1. Wavelength and measurement mode of elements selected for analysis.

Element	Wavelength (nm)	Order	Measurement Mode
Cr	267.716	126	Axial
Mn	257.610	131	Axial
Cu	324.754	104	Axial
P	178.284	489	Axial
Pb	220.353	453	Axial
Mo	202.030	467	Axial
Zn	213.856	458	Axial
Mg	285.213	118	Axial
Ti	334.941	101	Axial
Ag	328.068	103	Axial
Al	308.215	109	Axial
Ba	233.527	445	Axial
Cd	214.438	457	Axial
Fe	259.940	130	Axial
Co	237.862	142	Axial
S	180.731	487	Axial
Si	212.412	459	Axial
Sn	189.989	477	Axial
V	292.402	115	Axial
Ni	221.647	452	Axial

SI Table 2. Limit of detection and quantification for the various elements examined via ICP-OES. Limit of Detection (LOD) was calculated as the mean plus three times the standard deviation of all (21) procedural blanks. Limit of Quantification (LOQ) was calculated as the mean plus ten times the standard deviation of the same blanks.

mg/kg	STDDEV	LOD	LOQ
Cr	0.002	0.006	0.020
Mn	0.003	0.008	0.026
Cu	0.002	0.006	0.020
P	0.135	0.515	1.457
Pb	0.781	2.603	8.070
Mo	0.001	0.003	0.010
Zn	0.002	0.005	0.016
Mg	0.002	0.007	0.022
Ti	0.002	0.006	0.021
Ag	0.003	0.009	0.030
Al	0.021	0.062	0.207
Ba	0.005	0.014	0.047
Cd	0.002	0.006	0.021
Fe	0.003	0.010	0.033
Co	0.003	0.009	0.029
Na	0.441	1.425	4.511
S	0.249	0.939	2.678
Si	0.186	0.748	2.050
Sn	0.004	0.012	0.041
V	0.003	0.008	0.027
Ni	0.002	0.006	0.021

3.0 HYDRAULIC OIL INFILTRATION INTO POTABLE WATER THROUGH AIRCRAFT PNEUMATIC SYSTEMS: A QUALITATIVE ASSESSMENT OF CHEMICAL CONTAMINATION

Author Contributions Statement

- Conceptualization: Kevin Hayes
- Methodology: Kevin Hayes, Gwen O’Sullivan, Eric Fries, Roxana Sühring
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3.1 Abstract

Potable water on aircraft is currently monitored for microbiological contaminants of water quality such as E. coli, but because the source water for aircraft is pre-treated water from municipalities, chemical contaminants are not assessed. This neglects the possibility of aircraft pneumatic systems, interconnected with other systems such as the engines and hydraulic oil reservoirs, from becoming fouled and contaminating the potable water onboard with organophosphate esters and other contaminants of concern. In this novel initial qualitative study potable water samples were taken on twenty domestic and international flights on various commercial aircraft. The samples were analyzed with high-resolution liquid chromatography mass spectrometry and compared against tap water blanks drawn from departing airports. Suspect compounds were identified using safety data sheets for commonly used aircraft oils and compounds previously identified in aircraft cabin contamination research. Tributyl phosphate, the primary component in aircraft hydraulic oil, was confirmed to be present in the potable water of the majority of flights sampled (11 of 21 flights). Other organophosphates were also identified in the water on a high percentage of flights (TCPP: 20%; TPhP: 10%; TBEP: 10%). The qualification of the compounds is supported by mass accuracy, fragment, isotope abundance, and adduct data. This work suggests that as there is currently an unknown and unaddressed occupational and public health risk. Detailed quantitative

chemical monitoring of aircraft potable water is therefore recommended to fully establish the magnitude of this risk.

3.2 Introduction

By its definition, for water to be considered potable, it must be safe for human consumption. The World Health Organization strengthens this definition, adding that the water must "not represent any significant risk to health over a lifetime of consumption" (WHO 2017). Drinking water guidelines/regulations/directives require that this water meet various microbiological, chemical, radiological, and aesthetic requirements to meet this definition (WHO 2017; EU 2020; USEPA 2023; GC, 2022). Adulteration of the water by contaminants may make the water hazardous to consume/use for other purposes, such as bathing or cleaning surfaces (Moody and Chu 1995; Shah et al. 2023). Water treatment and monitoring take place to prevent or limit contaminated water from reaching human receptors, but further complexity exists when water systems are utilized by the public but are operated outside of those managed and regulated by local authorities; an example is potable water systems found in various modes of transportation, such as ocean-going vessels, rail systems, and airplanes. The potable water systems on aircraft, to a greater extent than municipal water systems or those on other forms of mass transport, are vulnerable to chemical contamination (Croddy and Akerman 2019). The aircraft systems contain relatively small volumes of water, limiting the effects of dilution, and have no water treatment capabilities (Croddy and Akerman 2019; WHO 2009).

Water is loaded onto aircraft at the departing airport using locally available water supplied by the municipality (ICAO 2021). The aircraft operators, the airport, and the relevant local health authorities are responsible for ensuring that the water is of sufficient quality for its transfer into an aircraft potable water system. However, once on board, the water's quality and safety become the airline's sole responsibility (WHO 2009). The onboard system services both employees and the public; therefore, the safety and enforcement guidance fall under the purview of the various occupational health and safety agencies and government entities responsible for public health (GC 2022b; EPA 2023). The aircraft potable water system is composed of an external filling port, a pressurized tank (typically made of stainless steel), and a variety of plumbing and fixtures used to transport the potable water from the tank(s) to the onboard galleys and lavatories (WHO 2009). The water is then used for a variety of purposes: Directly consuming chilled water is now less common, as bottled water is often available on flights, but hot beverages (coffee, tea, baby formula, etc.) are prepared with the aircraft potable water; the water is also used for oral hygiene, cleaning hands, and cleaning surfaces (Handschuh et al. 2015).

Historically, concerns about the microbiological aspects of water quality have dominated both the research and regulatory guidance regarding aircraft potable water (Handschuh et al. 2015; Treglia et al. 2022; WHO 2009). However, the pressurization of the potable water tank and other aircraft systems by engine bleed air, accompanied by the interconnectedness of the pneumatic system on most aircraft, may result in chemical contamination of the potable water with engine oil, hydraulic oil, or other potential contaminants such as deicing fluids, aircraft exhaust, oil or fuel additives, etc. (Hayes et al. 2021; Scholz 2022). That the water systems are rarely fully drained and are not cleaned with this type of contamination in mind, means that the pressurized water tanks of aircraft may act as a reservoir for these unwanted chemical contaminants (USEPA 2009; Handschuh et al. 2015; Hayes et al. 2021; Scholz 2022; GC 2022b). While heating the water (i.e., for tea or coffee preparation) may remedy much of the microbiological concern, it will not prevent chemical exposure should the water be ingested or otherwise introduced to the body (Treglia 2022; Tong et al. 2022).

Certain organophosphate esters (OPEs), which constitute or are additives present in aircraft hydraulic and engine oil, have the potential to contaminate potable water on aircraft. Tricresyl phosphate (TCP), an engine oil anti-wear additive, is suspected of contributing to increased neurological illness and injury experienced by pilots and flight attendants (Winder and Balouet 2000; 2002; Liyasova et al., 2012; Reneman et al., 2016; Al-Salem et al., 2019; Hayes et al., 2021). Tributyl phosphate (TBP), another organophosphate ester, is used as a plasticizer, solvent, and metal ion extractant and is also the primary component of aircraft hydraulic oil (55 – 100% w/w) (Eastman 2016; Eastman 2019; Imperial 2023; Imperial 2021). It is commonly found within the aircraft cabin (Crump et al. 2011; Rosenberger 2018; Schuchardt et al. 2019; Solbu et al. 2011). The hydraulic oil reservoirs on commercial jet aircraft are pressurized via the bleed air/pneumatic system, and a known problem on certain aircraft is the contamination of the pneumatic system with hydraulic oil and fumes from this source (Lombardo 1993;

Wild 1996; Brady 2022). Once in the pneumatic system, there are no barriers to the hydraulic oil/ fumes from being transported to the potable water tanks. TBP is listed as a chemical contaminant on the US EPA Contaminant Candidate List 5; it is currently unregulated but has been identified as a potential risk to drinking water (ATSDR 2012; USEPA 2022). Ingestion is a known prevalent pathway for exposure to organophosphate esters (Gbadamosi, Abdallah, and Harrad, 2021). For the public who fly regularly, the estimated daily intakes for the OPEs present in aircraft potable water, may need to be adjusted substantially (Gbadamosi, Abdallah, and Harrad, 2021).

It has already been established that engine and hydraulic oil fumes and additives can enter the cabin via bleed air transport through the pneumatic system (Crump et al. 2011; Solbu et al. 2011; de Ree et al. 2014). What has yet to be definitively established is if the same is true of the potable water systems onboard aircraft. This study is the first of its kind and aims to determine if the water systems are becoming contaminated by engine oil, engine oil additives, or hydraulic oils. The qualified presence of suspect compounds could warrant an examination the chemical safety and the potability of the water used by passengers and crew.

3.3 Methods

3.3.1 Sample collection and preparation

Potable water samples were collected from domestic and international flights within North America, the United Kingdom, and Europe. Water samples were collected from aircraft lavatory sinks (n = 25) and hot water via the rear galley (n = 1). Collection occurred on 20 unique flights, including nine aircraft types and/or airframe configurations, with duplicate samples collected from 7 flights (Table 6). Nitrile gloves were worn, and the water was dispensed into certified clean 250 mL wide-mouth amber glass bottles (VWR, Pennsylvania, USA), rinsed completely with the sampled water, and filled and capped. Collection occurred during the cruise phase of each flight. Blanks of airport tap water (potable water from source reservoirs that had not been on a plane) were collected from water fountains prior to two individual flight departures (samples 10 and 10b and 21 and 21b); each blank was collected in duplicate. Sample collection was opportunistic. The collection procedures, along with the overall project, were submitted and reviewed by the Manchester Metropolitan University Science and Engineering Research Ethics and Governance Committee and given a favorable ethical opinion (Reference Number: 16308). Samples were collected from December 2019 until March 2022 and stored at 4°C upon arrival at the final destination (maximum storage duration= 2.25 years). Samples remained in the sealed amber glass storage vessels in an effort to minimize photodegradation until analysis.

In preparation for instrumental analysis, aliquots were drawn from just below the water's surface, with care taken not to displace potential sediment at the bottom of the sample vessel nor draw in any film that may have formed at the sample's surface. A new, solvent cleaned (18 Megaohm milli-Q water/MeOH) glass Pasteur pipette was used to transfer the water from each amber sample vial to 1.5 ml amber LC vials. The uncapped LC Vial was zeroed on an analytical balance (Accuris Instruments Analytical Series W3100A-120). Following this, 0.9500 g (Mean: 0.9517 g; min-max: 0.9368 g - 0.9676 g; Standard Deviation: 0.0076g) of aircraft water was pipetted into the vial on the scale. The transfer was completed unfiltered, as the glass fiber/nylon filters were determined to be a source of coeluting contamination (679.5129 & 396.8020 m/z). The scale was then zeroed, and 50ul / 0.0500 g of 1 ng μl^{-1} 13C pentaerythritol was dosed to each sample via a 50 µl Hamilton pipette (Mean: 0.0495 g; min-max: 0.0481 g – 0.0507 g; Standard Deviation: 0.0006 g; mean RT= 3.02; RSD% of RT = 1.02%; mean peak area = 179116; RSD% peak area = 11.22)). This method was completed for all analytical samples and airport tap water and Milli-Q water blanks. Milli-Q water blanks consisting of 18Mohm deionized water (Milli-Q) and 13C pentaerythritol in the same concentrations as the analytical samples were run in duplicate before and following each 10 sample injections (maximum) to monitor for carry-over.

Table 6 Sampled aircraft and location. Samples taken in duplicate from the same aircraft are indicated by the letter b. Samples 3 and 4 are from the same flight but differing sample locations resulting in unique sample numbering. Note that sample number 1 was collected but is not included in subsequent analysis due to instrument error.

Sample Number	Aircraft	Sample Location	City of Departure
1	Boeing 737-700	Lavatory Sink	Calgary, Canada
1b	Boeing 737-700	Lavatory Sink	Calgary, Canada
2	Boeing 737-700	Lavatory Sink	Phoenix, USA
2b	Boeing 737-700	Lavatory Sink	Phoenix, USA
3	Boeing 737 Max	Hot water from galley	Toronto, Canada
4	Boeing 737 Max	Lavatory Sink	Toronto, Canada
5	Boeing 737 Max	Lavatory Sink	Charlottetown, Canada
6	Airbus A330-300	Lavatory Sink	Amsterdam, Netherlands
7	Boeing 737 Max	Lavatory Sink	Calgary, Canada
8	Boeing 737-800	Lavatory Sink	Los Angeles, USA
9	Airbus A330-300	Lavatory Sink	Chicago, USA
9b	Airbus A330-300	Lavatory Sink	Chicago USA
10	Airbus A320-211	Lavatory Sink	Toronto, Canada
10b	Airbus A320-211	Lavatory Sink	Toronto, Canada
11	Boeing 737-800	Lavatory Sink	Manchester, England
11b	Boeing 737-800	Lavatory Sink	Manchester, England
12	Boeing 787-900	Lavatory Sink	Amsterdam, Netherlands
12b	Boeing 787-900	Lavatory Sink	Amsterdam, Netherlands
13	Boeing 737-800	Lavatory Sink	Charlottetown, Canada
14	Avro RJ100	Lavatory Sink	Ekati, Canada
15	Embraer E190	Lavatory Sink	Cork, Ireland
16	Airbus A330-300	Lavatory Sink	Amsterdam, Netherlands
17	Boeing 737-800	Lavatory Sink	Amsterdam, Netherlands
18	Boeing 737-700	Lavatory Sink	Calgary, Canada
19	Boeing 737 Max	Lavatory Sink	Toronto, Canada
20	Boeing 737 Max	Lavatory Sink	Toronto, Canada
21	Boeing 787-900	Lavatory Sink	Calgary, Canada
21b	Boeing 787-900	Lavatory Sink	Calgary, Canada
BLK1	Airport Water Fountain	Toronto, Canada	Toronto, Canada
BLK1b	Airport Water Fountain	Toronto, Canada	Toronto, Canada
BLK2	Airport Water Fountain	Calgary, Canada	Calgary, Canada
BLK2b	Airport Water Fountain	Calgary, Canada	Calgary, Canada

3.3.2 Instrumental analysis

An Agilent 1260 Infinity II high-performance liquid chromatography (HPLC) system was coupled to an Agilent 6546 quantitative time-of-flight mass spectrometer (QToF-MS)(Santa Clara, California, USA) and used for all instrumental analysis. The column used was an Agilent InfinityLab Poroshell 120 EC-C18 (3.0 × 100 mm, 2.7 µm), and ionization was performed using a Dual Agilent Jet Stream (AJS) ESI ion source.

The separation method was previously presented in Fries and Sühring (2023), with minor modifications (SI Table 1). Briefly, an initial composition of 90% water (with 0.1% formic acid) and 10% acetonitrile (ACN) was used at a constant flow rate of 0.2 $mL\cdot min^{-1}$. This was increased to 40% ACN over five minutes, then to 100% ACN over seven minutes, and held for an additional eight minutes. Finally, the mobile phase composition was returned to starting conditions over 0.1 minutes, with a seven-minute post-run used to equilibrate the column.

The auto-MS/MS feature of the QToF-MS was used to ionize and automatically fragment possible contaminant ions measured in the potable water samples. Two collision energies (15 eV and 30 eV) were used to fragment ions of different labilities. Detailed parameters and thresholds used in the auto-MS/MS method are presented in SI Table 2.

3.3.3 Statistical methods and Identification Confidence

Initial screening of compounds within samples was completed using MassHunter Qualitative Analysis software (Agilent Technologies) utilizing a Water Contaminants spectral library (Personal Compound Database and Library-PCDL) (Agilent Technologies). Compounds pre-identified as potential suspects were sought in the list of spectral library matches. A minimum match score of 90.00 was selected to qualify a compound for further analysis. Each suspect compound that met this criterion was then subject to further screening: Detection (LOD) and quantitation (LOQ) limits were established as the average of all Milli-Q water blanks plus three and ten times the standard deviation of the blank measurements of that compound, respectively. Airport tap water was found to contain a slightly greater concentration of analytes of interest (e.g. TBP) and as such additional LOD and LOQ thresholds were established with airport tap water blanks. The blank type (Milli-Q or Airport tap water) with the higher and therefore more stringent LOD and LOQ were used to determine detection of each compound assessed. Mass accuracy, fragment, adduct, and isotope abundance testing was then employed to improve confidence in identifying the various compounds. Summary statistics and data handling were completed using Microsoft Excel and Access.

Confidence in compound identification utilizes the scale developed for non-targeted assessments in Schymanski et al. (2015). The highest confidence level (1) is reserved for compounds that have been matched with reference standards. This confidence level indicates that the chemical structure present in the sample is certain and defined. Level 2 is the next most robust, indicating a confidence level of probable structure. This requires previous knowledge of the compound’s presence in related samples (suspect screening) along with structural information, or if completing non-targeted analysis, the exact mass, isotope, adduct, and fragment information to match the compound identification. Level three indicates a tentative candidate, in which all evidence to promote the compound to a probable structure is not present but fragment data is consistent with the compound in question. Level 4 lacks sufficient structural evidence (i.e. the sample is run in only MS mode), but has an exact mass match some other form of complementary information (adduct, isotope, etc.). Finally, level 5, or mass of interest, describes a compound for which the only information is that it is detected multiple times in samples and an exact mass match. This scale is established and commonly used within the non-targeted analysis field when utilizing high resolution mass spectrometry.

3.4 Results and Discussion

3.4.1 Blank and analytical sample comparison

When comparing potable water from aircraft against the Milli-Q and airport tap water blanks, 117 compounds unique to the aircraft samples were identified. 41 of the 117 compounds were found in multiple samples, 32 of which were found on multiple flights. Several classes of water contaminants were identified within this group, including herbicides (organosulfonic; chloroacetanilide; triazine), fungicides, insecticides (organophosphate, pyrethroid, thiadiazole; inorganic fumigant), pharmaceuticals (antibiotic; heart; hormone; etc.), illicit narcotics, and corrosion inhibitors (SI Table 3). No further examination beyond identification via spectral library match for these compounds was completed in this study; however, their presence in multiple flights/aircraft samples and absence from blanks allow for the contaminants listed to be considered Masses of Interest (level 5), and to be added to suspect screening lists for further study of aircraft potable water (Schymanski et al. 2015).

3.4.2 Suspect screening: Hydraulic oil in the potable water

The primary constituent (55-100%) of aircraft hydraulic oil, TBP, exceeded the match score for all samples (mean score: 99.03; Standard Deviation: 0.51) and blanks (mean score: 99.28; Standard Deviation: 0.52). The peak area counts for TBP present in Milli-Q and airport water blanks were more substantial than expected. As such, 3σ and 10σ method LOD and LOQ values were applied to ensure that declarations of detection were made with greater than 99% confidence. When applying the LOD and LOQ values to the samples collected demonstrated that on ten flights TBP was detectable above airport tap water blanks (Figure 7). One flight (Sample 13) demonstrated a peak area that greatly exceeded LOQ (2.90 times Milli-Q LOQ; 2.87 times airport tap water LOQ) (Figure 7).

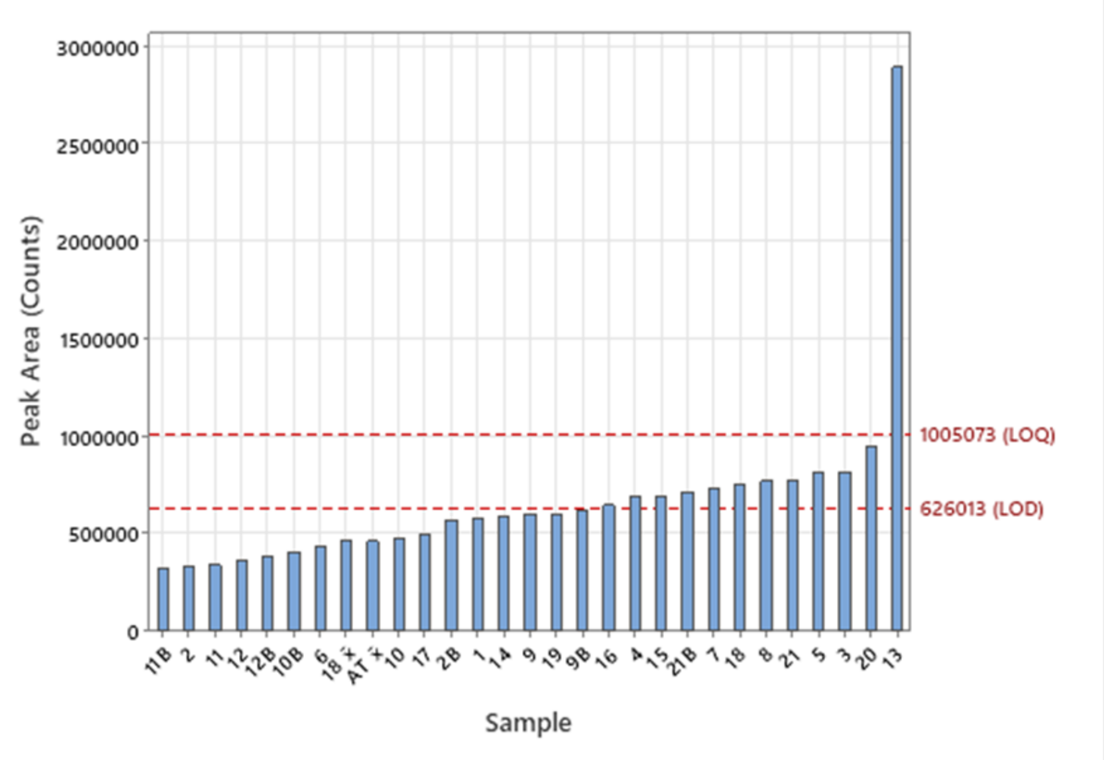

Figure 7. Relative peak areas of TBP in aircraft potable water as compared to airport tap water blanks (AT x̄). Samples are ordered by increasing peak area counts. LOD (3 times the standard deviation of airport tap water blank measurements added to the mean of blank measurements) and LOQ (10 times the standard deviation of aircraft tap water blank measurements added to the mean of blank measurements) of TBP are displayed in the figure as dashed lines. (18 x̄) refers to the average peak area of TBP within Milli-Q water blanks. Sample 1 as represented in this figure is sample 1B.

The presence of TBP in the samples was supported with an exact mass match with the predicted (M+H)+ mass associated with the compound (m/z 267.1719) with sub-ppm mass accuracy in all samples (x̄ = - 0.26) and blanks (x̄ = -0.17) (Table 7). However, potential isobaric compounds, with differing molecular formulas but masses that fell within instrumental accuracy tolerances, were found: Atenolol and the sodium adduct of ADBI (Celestolide). These compounds were assigned the same retention time and mass-to-charge ratio as TBP. Atenolol was identified by the software alongside TBP in 16 of the analytical samples; the Na+ adduct of ADBI was identified in 26 samples, and TBP was found in all samples (n = 27) (SI 1.).

The HPLC QTof-MS was operated in (MSMS) mode in an effort to generate identifiable fragments which could further support or assist in rejecting the suspected presence of TBP in the potable water. In all aircraft water samples, with the exception of sample 11B, a prominent fragment (m/z 98.984~; Max m/z 98.9847, Min m/z 98.9837) was detected (Table 7). This mass appears to represent the fully protonated phosphate fragment from TBP ([H4 O4 P]+: Predicted m/z: 98.9847). The Atenolol molecule could not explain this fragment; The sodium adduct of ADBI could make a fragment of similar mass [C5 Na O]+, although it is unlikely to be prominent given the molecular structure of the compound and the requirement for the sodium adduct to be included with the fragment.

The abundance of ^{13}C in the samples was used to estimate the carbon number to further distinguish between the mass-matched compounds (Table 8). The [M+]+1 abundance most closely resembles TBP with an average ^{13}C: ^{12}C of 15.28% (Table 8). Isotopes of other elements present in the suspect compound were considered to be negligible in the calculation as their natural abundances are much lower than that of ^{13}C or they are monoisotopic; however, their omission, accompanied by mass accuracy discrepancies may account for the difference between predicted and measured isotope ratio values (Table 8). Sodium adducts of TBP (m/z = 289.154~) were found within all aircraft water samples with concentrations above the LOD except for sample 18 (Table 7; Table 8). Sample 18 also demonstrated a lower ^{13}C: ^{12}C percentage than predicted and cannot be tentatively qualified.

Based on these findings, it was probable that TBP was the compound found in aircraft water in excess of laboratory and airport tap water blanks. The retention time, exact mass, and isotope data were compared against a TBP standard (Wellington Laboratories) and its presence in the aircraft water was confirmed. The existence of this compound as the primary component of hydraulic oil on commercial jet aircraft and the co-pressurization of hydraulic oil reservoirs and the potable water system by the pneumatic system of the aircraft provides a tenable contamination source and pathway.

Table 7. Detection and mass spectral data indicative of compound qualification. Percentage detected refers to suspect compound detection on a flight basis. Mass accuracy represents the mass accuracy of all suspect compounds with >90.00 match factor (MassHunter Qualitative Analysis; Common Water Contaminants PCDL). Confidence level is derived from "Matrix of Identification V. Identification Confidence" (Schymanski et al. 2015)

	Percentage Detected	Known on Aircraft	Molecular Ion (+H) m/z	Mass Accuracy (ppm)		Diagnostic Fragments m/z	^{37}Cl Isotope Match	Sodium Adduct	Confidence Level
TBP	55	Yes	267.1719	Mean	-0.26	Present 98.984~, 155.047~	NA	Yes: <1ppm mass accuracy	1
				Min	-0.63				
				Max	0.07				
TPhP	15	Yes	327.0781	Mean	0.20	Absent NA	NA	Yes: >1ppm mass accuracy	3
				Min	-0.88				
				Max	0.43				
TEP	10	No	183.0781	Mean	-0.21	Present 98.984~	NA	Yes: >1ppm mass accuracy	3
				Min	-0.53				
				Max	0.14				
TBEP	10	Yes	399.2505	Mean	-0.36	Present 98.984~, 199.073~, 299.165~	NA	Yes: <1ppm mass accuracy	3
				Min	-1.05				
				Max	0.24				
TCPP	20	Yes	327.0079	Mean	-0.52	Absent NA	Yes	Yes: >1ppm mass accuracy	3
				Min	-0.83				
				Max	-0.30				
TCP	0	Yes	369.12~	Mean	NA	Absent NA	NA	NA	5
				Min	NA				
				Max	NA				

3.4.3 SDS suspect screening: Engine and hydraulic oil

A suspect screening for organophosphate compounds was completed, searching for compounds known to exist in aircraft engine oil and hydraulic oil, as well as those OPEs commonly found in the aircraft cabin in previous research (Solbu et al. 2011; Rosenberger 2018; Schuchardt et al. 2019; Hayes et al. 2021). Suspect compounds were selected for analysis by their listed presence at percent levels within Material Safety Data Sheets of oils approved for, and commonly used on commercial turbojet aircraft (Eastman 2016; Eastman 2019; Imperial 2023; Imperial 2021).

Of the other compounds listed as contributing to the composition of aircraft engine and hydraulic oil on the SDS sheets, only triphenyl phosphate (TPhP) and tricresyl phosphate (TCP) were identified with sufficient match factors to complete further analysis in aircraft potable water (Table 7). In previous studies, TPhP has been found in the aircraft cabin via air and wipe sampling (Solbu et al. 2011; Rosenberger 2018; Schuchardt et al. 2019). Of the samples, TPhP was identified in four of the Milli-Q blanks and three of the airport tap water blanks, and fifteen of the aircraft water samples. TPhP is listed as contributing 1-5% of a commonly used hydraulic fluid (Eastman 2019); it is also found in the same concentration range within new fluid formulations (Eastman 2023). TPhP is suspected to have been found above LOD in five samples representing three flights (Table 9). Three samples: 3, 9, and 9B, met LOQ for the compound (Table 9).

It should be noted that TPhP was found in other flights with peak areas above the LOD and, in one case, above LOQ but were not included due to the match score threshold (example: Flight 13 met LOQ for TPhP; Score 89.07; as such it was omitted from further analysis). Sub-ppm mass accuracy for the compound was determined for all samples (Table 7). No isobaric compounds were identified in the suspect screening. When analyzing the MSMS data, no identifiable fragments were generated for the compound. However, for most samples in which TPhP was detected, the 13C: 12C ratio closely resembled the predicted value for samples that meet LOQ (Table 8.)

An exception was sample 4, which met LOQ for TPhP but had an isotope ratio that suggested a much lower carbon number (Table 8). Sodium adducts of TPhP were found in each of the detected samples, but the mass accuracy in four of the five samples for the adduct exceeded one ppm ($|\bar{x}| = 2.45$ ppm). TCP had a sufficient match factor in only one aircraft water sample (3), and this sample did not meet LOD or LOQ requirements.

3.4.4 Other organophosphates

Several other organophosphates have been identified in previous research in the aircraft cabin (Hayes et al., 2021). Included amongst these compounds are tris(chloroethyl) phosphate (TCEP), tris(chloropropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), tris(butoxy ethyl) phosphate (TBEP), 2-ethylhexyl diphenyl phosphate (DPEHP), dibutyl phenyl phosphate (DBPP), tris(ethylhexyl) phosphate (TEHP), and trixylenyl phosphate (TXP).

When inspecting aircraft potable water for the compounds listed, TBEP and TCPP were identified (Table 7; Table 9). Additionally, Triethyl phosphate (TEP), while not previously described on aircraft, was also identified. TEP and TCPP met the 90.00 match score for all samples and blanks; TBEP met the match score for all samples and blanks, excluding Airport Water Blank 1 and Aircraft Water Sample 8. The compounds were found and met LOD or LOQ thresholds on multiple flights, albeit less frequently than TBP (Table 7; Table 9)

TEP displayed the same confirmatory phosphate fragment as TBP (m/z= 98.984~) and a mass accuracy below 1ppm. Of the two TBEP detects, one (sample 9b) had the same fragment (m/z`= 98.984~), as well as fragments present at m/z 299.165~ and 199.073~ describing the molecular ion less one and two butoxyethyl groups respectively (Table 7). No confirmatory fragments were determined for TCPP, but mass accuracy for the molecular ion remained below one ppm for the samples that met LOD ($|\bar{x}|$= 0.45 ppm). The 13C: 12C percentages for each compound suggest that the carbon number matches the suspected molecule (Table 8). Additionally, the M+2 isotope percentages of TCPP detections suggest the presence of three chlorine atoms in the molecule (Table 7; Table 8). Sodium adducts of TEP, TCPP, and TBEP were found in all samples in which they were detected. The mass accuracy of the sodium adducts was below one ppm for TBEP but above one for TEP and TCPP (Table 7).

Table 8. Predicted and measured isotopic abundances of suspect compounds. Predicted values calculated with a ^{13}C abundance of 1.1%. Isotopic abundance is calculated only for samples that exceeded the LOD for the respective compound.

Compound	Carbon Number	Predicted 13C:12C
TBP	12	15.2
ADBI	17	23.0
Atenolol	14	18.2

Sample	3	4	5	7	8	13	15	16	18	20	21	21B
M+ Abundance	60307.0	53456.6	68063.1	50865.9	56329.1	284690.1	54049.8	45101.0	60769.6	69527.8	62549.3	61207.8
M+1 Abundance	9280.7	8242.0	10652.7	7750.2	9083.0	43417.5	8455.8	6888.2	8125.8	10387.5	9614.8	9577.1
M+2 Abundance	1184.2	1099.9	1496.6	1132.9	1058.6	5311.2	634.5	818.0	1225.2	952.2	1131.5	1459.4
M+ Na Abundance	950.1	407.4	844.9	610.3	699.5	3686.6	800.7	573.0	Absent	1142.0	743.0	392.3
%M+1	15.4	15.4	15.7	15.2	16.1	15.3	15.6	15.3	13.4	14.9	15.4	15.6
%M+2	2.0	2.1	2.2	2.2	1.9	1.9	1.2	1.8	2.0	1.4	1.8	2.4
%M+Na	1.6	0.8	1.2	1.2	1.2	1.3	1.5	1.3	Absent	1.6	1.2	0.6

Compound	Carbon Number	Predicted 13C:12C
TEP	6	7.1
TCPP	9	11.0
TBEP	18	24.7
TPPA	18	24.7

Compound	TEP	TEP	TCPP	TCPP	TCPP	TCPP	TCPP	TBEP	TBEP	TBEP	TPPA	TPPA	TPPA	TPPA	TPPA
Sample	1B	9	3	6	9	9B	10	9	9B	19	3	4	5	9	9B
M+ Abundance	38476.3	57530.3	18689.9	12844.8	16919.9	13482.5	13497.1	42664.4	31192.9	24305.3	3424.7	5045.6	5494.5	19139.7	12102.1
M+1 Abundance	2814.3	4713.6	2223.2	1654.1	2008.9	1822.2	1392.4	9630.9	6713.6	5471.6	862.6	761.5	1258.8	4210.9	3054.9
M+2 Abundance	339.9	439.5	18071.1	13683.1	16692.4	13147.0	12449.6	2224.3	1398.3	873.2	108.6	193.1	160.4	809.0	315.2
M+Na Abundance	100.7	61.8	275.8	223.0	158.0	181.7	143.7	1401.7	885.4	1043.7	98.2	95.2	53.3	593.5	315.7
%M+1	7.3	8.2	11.9	12.9	11.9	13.5	10.3	22.6	21.5	22.5	25.2	15.1	22.9	22.0	25.2
%M+2	0.9	0.8	96.7	106.5	98.7	97.5	92.2	5.2	4.5	3.6	3.2	3.8	2.9	4.2	2.6
%M+Na	0.3	0.1	1.5	1.7	0.9	1.3	1.1	3.3	2.8	4.3	2.9	1.9	1.0	3.1	2.6

3.4.5 Confidence of qualification

The presence of TBP on aircraft, coupled with the exact mass match, fragment, isotope, and adduct data, and a standard match, confirms that TBP is in the potable water of a large percentage of jet aircraft. Reference standards for the remaining compounds identified in this study were not available and this precludes them from the application of the same confidence level under the identification confidence scale as outlined in the seminal work of Schymanski et al. (2015). Of the other organophosphates screened for, TPhP can be considered a tentative candidate (Level 3) (Table 7). This is supported by its known presence in aircraft fluids and an identical pathway to the potable water system of aircraft. TEP, TBEP, and TCPP can also be considered tentative candidates as aircraft water contaminants, as each was identified with a high degree of mass accuracy. TBEP and TCPP are known to exist on aircraft, but a contaminant pathway for the compounds to enter the potable water system onboard the aircraft is not established (Table 7). TBEP is supported by fragment and sodium adduct data, strengthening the confidence in its identification. TCPP lacks conclusive fragment data but has a robust isotopic match and was detected in 20% of all flights. TEP is not previously known to exist on aircraft, and the mechanism by which it could be concentrated in or otherwise enter aircraft potable water systems is not established; the identification is supported by fragment, adduct, and isotopic data (Table 7).

13C pentaerythritol, a potential polymeric fragment of the C5 to C10 fatty acid esters of pentaerythritol and dipentaerythritol, was selected as an internal standard. The intact esters were identified as the primary component in Mobil Jet oil II [19]. The selection of the internal standard proved to be a misstep, as fragments of the intact esters of the aircraft oil were not identified in any sample. Additionally, the selection does not allow for comparison or quantification with compounds of concern that were identified, (namely organophosphates) because of molecular and retention time dissimilarity. Increased peak area of the IS in the Milli-Q water blanks (First Milli-Q blank preparation: Mean peak area = 721574, RSD% peak area = 1.13) suggests that the analytical sample matrix may be interacting with or suppressing the instrumental response for the IS compound. While not useful for analyte identification or quantification the internal standard, prepared as described above, demonstrates instrument operational parameters for the analytical samples. Future quantitative work will require the selection of more appropriate internal standards, however, as the primary purpose of this research is the qualitative suspect screening for compounds of concern, this does not impact our findings.

3.4.6 Discussion of significance and aircraft implications

The suspect organophosphates were detected across aircraft manufacturers and airframes (Table 9). Contaminant accumulation in potable water seemed commonplace regardless of aircraft type (Table 9). Of importance was the detection of TBP in the potable water of samples 21 and 21b: This aircraft type, unlike all other commercial jet aircraft, does not use bleed air to pressurize the cabin; but, like other commercial jet aircraft, does utilize the pneumatic system and bleed air for a variety of other functions on the aircraft, including, pressurizing the hydraulic system reservoirs and the potable water storage tank in flight (Boeing 2008; OAT 2023). The detection of TBP in these samples indicates that an alternative route of exposure to organophosphates of concern is present for this aircraft type, regardless of removing bleed air from use in cabin pressurization. Additionally, the comparatively high concentration of TBP found in sample 13 may be indicative of a leak of hydraulic fluid into the pneumatic system on that aircraft. This study, largely due to the challenges of opportunistic sampling on aircraft, has a relatively small sample size. To determine the scale of aircraft water contamination, further samples are required and quantitative methods should be employed. This manuscript demonstrates the proof of contaminant pathway and necessity for this further research.

Table 9. Identification, detection, and quantification key for suspected compounds. ND indicates that the compound was not found above 90.00 in a spectral library match. ID indicates that the suspect compound was identified via spectral library match with a score above 90.00. >LOD indicates that the level of detection threshold was reached. >LOQ indicates that the level of quantification over laboratory and airport tap water blanks was reached.

Sample	Aircraft	TBP	TCPP	TEP	TBEP	TPP	TCP
1b	Boeing 737-700	ID	ID	>LOD	ID	ID	ND
2	Boeing 737-700	ID	ID	ID	ID	ND	ND
2b	Boeing 737-700	ID	ID	ID	ID	ND	ND
3	Boeing 737 Max	>LOD	>LOQ	ID	ID	>LOD	ID
4	Boeing 737 Max	>LOD	ID	ID	ID	>LOQ	ND
5	Boeing 737 Max	>LOD	ID	ID	ID	>LOD	ND
6	Airbus A330-300	ID	>LOD	ID	ID	ND	ND
7	Boeing 737 Max	>LOD	ID	ID	ID	ID	ND
8	Boeing 737-800	>LOD	ID	ID	ND	ND	ND
9	Airbus A330-300	ID	>LOQ	>LOD	>LOD	>LOQ	ND
9b	Airbus A330-300	>LOD	>LOD	ID	>LOD	>LOQ	ND
10	Airbus A320-211	ID	>LOD	ID	ID	ND	ND
10b	Airbus A320-211	ID	ID	ID	ID	ND	ND
11	Boeing 737-800	ID	ID	ID	ID	ID	ND
11b	Boeing 737-800	ID	ID	ID	ID	ID	ND
12	Boeing 787-900	ID	ID	ID	ID	ID	ND
12b	Boeing 787-900	ID	ID	ID	ID	ID	ND
13	Boeing 737-800	>LOQ	ID	ID	ID	ID	ND
14	Avro RJ100	ID	ID	ID	ID	ID	ND
15	Embraer E190	>LOD	ID	ID	ID	ID	ND
16	Airbus A330-300	>LOD	ID	ID	ID	ID	ND
17	Boeing 737-800	ID	ID	ID	ID	ID	ND
18	Boeing 737-700	>LOD	ID	ID	ID	ND	ND
19	Boeing 737 Max	ID	ID	ID	>LOD	ID	ND
20	Boeing 737 Max	>LOD	ID	ID	ID	ID	ND
21	Boeing 787-900	>LOD	ID	ID	ID	ID	ND
21b	Boeing 787-900	>LOD	ID	ID	ID	ND	ND

3.5 Conclusions

This study provides evidence that TBP, the primary aircraft hydraulic oil constituent, is present in aircraft potable water, detected regularly when compared against relative peak areas of the compound in municipal tap water. Similarly, there is a high likelihood that other organophosphate esters, both previously known and unknown to exist within the aircraft cabin, are present in the potable water of a large percentage of aircraft. These findings are not exclusive to an individual aircraft, airframe, or manufacturer, and appear to impact both bleed-air cabin pressurized and non-bleed air cabin pressurization aircraft. With this conclusion, the contaminant pathway from the pneumatic system to the potable water system is validated and implies that any contaminant from the engines, hydraulic system, or other aircraft system connected to the pneumatic system may contribute to water fouling.

Airport tap water was not collected from all municipalities, and as such, the municipal source water for each flight cannot be directly compared with each flight. This limits direct comparison to two flights (samples 10, 21 and respective duplicates) and slightly more broadly to other flights departing from Calgary or Toronto with the assumption that the municipal water chemical composition remains relatively consistent over time. This is further limited by the fact that aircraft do not drain to empty and fill their potable water tanks as a practice at each departing airport. This means that there is a high likelihood that the potable water found in every aircraft is not solely from one airport municipality, and may be a collection of many municipalities water. Aircraft receiving water from multiple municipal sources, both domestic and international, may seem to add variability; however, this study has multiple samples taken from aircraft with potable water topped up in the same municipal water districts. This repeat sampling limits the likelihood of contamination being solely attributed to water sources and strengthens the argument that the contamination is originating on the aircraft; namely, that because water was taken from multiple aircraft, that were topped up at the same municipal source, and then demonstrated different chemical compositions, that the aircraft themselves were the likely contributors of the chemical loadings. The OPEs (TEP, TCPP, TBEP) suspected to be present in the water but not yet attributed to a source linked to the potable water system on aircraft, should be further researched to establish the contamination pathway. Additionally, further research should seek to establish thresholds that may establish oil leak conditions on aircraft, examine additional chemical classes, confirm the tentative and probable contaminants with standards, and quantify the contaminants' concentrations.

The results of this study indicate that aircraft potable water systems should be monitored for a variety of chemical contaminants. The currently monitored and regulated microbiological water quality standards on aircraft are insufficient, as they do not accurately describe the potential chemical risk of consumption or use of the product. Quantification of this exposure risk to those flying and flying on aircraft should be completed and steps taken to mitigate the contaminants identified from entering the potable water system.

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3.7 Competing Interests

The authors have no known competing financial, or non-financial, interests.

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3.9 Supplementary Information

Table 1. Analytical separation method used for potable water analysis.

Time (min)	A (H_2O + 0.1% formic acid)(%)	B (ACN)(%)
0	90	10
5	60	40
12	0	100
20	0	100
20.01	90	10

Table 2. ESI ion source and auto-MS/MS parameters used for potable water analysis.

Source Parameters	Value
Gas Temp.	300 ºC
Drying Gas Flow	10 L·min^{-1}
Nebulizer Pressure	35 psi
Sheath Gas Temp.	275 ºC
Sheath Gas Flow	12 L·min^{-1}
Capillary Voltage	3500 V
Nozzle Voltage	1500 V
Fragmentor Voltage	75 V
Skimmer Voltage	65 V
Oct 1 RF Vpp Voltage	750 V

Spectral Parameters	Value
MS Min Range	100 m/z
MS Max Range	930 m/z
MS Acquisition Rate	4 spectra·s^{-1}
MS Acquisition Time	250 ms·spectrum^{-1}
MS/MS Min Range	50 m/z
MS/MS Max Range	500 m/z
MS/MS Acquisition Rate	4 spectra·s^{-1}
MS/MS Acquisition Time	250 ms·spectrum^{-1}
Isolation Width	Narrow (~1.3 m/z)

Collision Energy	Value
Use Fixed Collision Energies	15 eV, 30 eV

Precursor Selection I	Value
Max Precursor Per Cycle	6
Absorbance Threshold	25000 counts
Rel. Threshold	0.01%
Active Exclusion	Enabled
Active Exclusion -Excluded After	2 spectra
Active Exclusion -Released After	0.1 min
Static Exclusion Range	120-122 m/z; 920-922 m/z
Mass Error Tolerance	+/- 20 ppm
RT Exclusion Tolerance	+/- 0.2 min

Precursor Selection II	Value
Isotope Model	Common Organic Molecules
Precursor Charge State Selection - Active	1
Sort Precursors by Abundance only	Enabled
Scan speed varied based on precuros abundance	Enabled
Target	25000 counts/spectrum
Use MS/MS accumulation time limit	Enabled
Purity Stringency	100%
Purity Cutoff	30%

Table 3. Substances identified in this study with match factors above 90 that are exclusive to aircraft potable water and not found in airport tap water or Milli-Q water blanks.

When comparing potable water from aircraft against the laboratory and airport tap water blanks, 117 compounds unique to the aircraft samples were identified (the full list of 117 samples is included below). 41 of the 117 compounds were found in multiple samples, 32 of which were found on multiple flights. Several classes of water contaminants were identified within this group, including herbicides (organosulfonic; chloroacetanilide; triazine), Fungicides, insecticides (organophosphate, pyrethroid, thiadiazole; inorganic fumigant), medications (antibiotic; heart; hormone; etc.), illicit narcotics, and corrosion inhibitors (SI Table 3). No further examination beyond identification via spectral library match for these compounds was completed in this study; however, their presence in multiple flights/aircraft samples and absence from laboratory and tap water blanks allows for the contaminants listed to be considered Masses of Interest (level 5), and to be added to suspect screening lists for further study of aircraft potable water (Schymanski et al. 2015).

Several of these substances identified in this "aircraft exclusive" list are not believed to exist in the aircraft potable water samples (deuterated and isotopically labeled substances etc.). The identified compounds may have been close to instrument detection limits, and no isotopic, adduct, or fragment data was analyzed for any of the compounds listed, save for TCP.

Compound Name
17beta-Estradiol (E2)
2-(4-Morpholinyl)benzothiazole
2,4-DP / Dichloroprop
2-Morpholinothiobenzothiazole
2-Naphthalenesulfonic acid
4-Aminoantipyrine (Ampyrone)

4-Androstenedione

4-Hydroxyantipyrine

4-Nitroaniline

ABT / 2-Aminobenzothiazole

Acesulfame (Acesulfame-K)

Acetochlor OXA (Acetochlor OA)

Allethrin I

Amitraz

Androsterone

Apramycin

BBIT / n-Butyl-1 ,2-benzisothiazolinone

Benzocaine

Benzophenone

Benzylhydroxybenzoate (Benzylparaben)

Bethoxazin

Bisoprolol

BP-2 / Benzophenone-2

Buprofezin

Butyl 4-hydroxybenzoate (Butylparaben)

Caffeine

Cefalexin

Copper(2+) bis(1-cyclohexyl-2-oxohydrazinolate)

Cotinine

Cyclamic acid (Cyclamate)

Cycluron

Cymoxanil (Curzate)

Cyproconazole(I)

Danazole

Desethylsebutylazine (Sebuthylazine-desethyl)

Diaveridin

Diazepam-d5

Dibutyl phenyl phosphate

Diclofenac

Dienestrol

Diethofencarb

Difenoxuron

Digoxigenin

Dioxacarb

Ecgonine methyl ester

Enalaprilat

Enoxacin

Enrofloxacin

Estriol

Ethoprop (Ethoprophos)

Ethyl N-acetyl-N-butyl-Î²-alaninate

Fenetylline

Flufenacet ESA

Fluoxetine-d5

Heroin

Hexyl cinnamaldehyde

Hydroxychloroquine

Imazapyr

Imazethapyr

Lisinopril

Lorazepam

Lovastatin

MBT / 2-Mercaptobenzothiazole

Medroxyprogesterone

Mephosfolan

Mesocarb

Metamitron

Metaxalone

Methadone

Methamidophos (Metamidophos)

Methyltestosterone

Metolachlor

Metolachlor ESA

Metolachlor OXA (Metolachlor OA)

Metyrapone

MIT / Methylisothiazolinone

Modafinil Acid

MTBT / 2-(Methylthio)benzothiazole

Musk Ketone

Myclobutanil

Naphazoline

N-cyclohexyldiazeniumdioxy-potassium (K-HDO)

Neospiramycin

n-Heptylphenol

Nifedipine

Nitrazepam

OD PABA / Octyldimethyl PABA (Padimate O)

Oseltamivir

Oseltamivir acid

PBS / Phenylbenzimidazole sulfonic acid

PCP / Phencyclidine

Pefloxacin

Pentoxifylline

Picaridin (Bayrepel) (Icaridin)

Propargite

Pyrimethanil

Simetryn

Simvastatin

Spectinomycin

Sulfamethoxazole-13C6

Sulfuryl fluoride

Tamoxifen

Tazobactam

TCMTB (Busan (30))

TCP / Tricresylphosphate

Tebutam

Terbutaline

Testosterone

Theobromine

Timolol

Triflusulfuron-methyl

Tri-iso-butyl phosphate

Trimethoprim

Tyrosine

Ursodeoxycholic acid

Valproic acid

Vildagliptin

4.0 A COMPARATIVE ASSESSMENT CABIN CONTAMINATION IN BLEED AND NON-BLEED AIR PRESSURIZED AIRCRAFT

Author Contributions Statement

- Conceptualization: Kevin Hayes
- Methodology: Kevin Hayes, James Walker, Emily Carrol, Caleb Marx
- Investigation: Kevin Hayes, trained citizen scientists, supervisory team members
- Formal analysis: Kevin Hayes
- Data curation: Kevin Hayes
- Writing – original draft: Kevin Hayes
- Writing – review & editing: Kevin Hayes, David Megson, Gwen O’Sullivan, Aidan Doyle

ADD NOTE HERE THAT AN ABBREVIATED VERSION OF THIS CHAPTER, PRESENTING THE PRELIMINARY RESULTS, HAS BEEN SUBMITTED AS A SHORT COMMUNICATION TO SCIENCE OF THE TOTAL ENVIRONMENT. PROVIDE THE BIBLIOGRAPHIC CITATION AND INDICATE STATUS IF NOT YET PUBLISHED. (e.g. submitted, in review, in revision). REMOVE THE COPY FROM THE SI.

4.1 Abstract

Contamination of the aircraft cabin environment has been associated with an increased occupational risk and incidences of chemical injury in pilots and flight attendants (Hayes et al., 2021). The aircraft environment is chemically complex; interior furnishings are impregnated with halogenated and organophosphate flame retardants, pesticides and herbicides are regularly employed to prevent unwanted transmission or transport of pests and disease vectors, and significantly, air is bled from the engines in the majority of commercial aircraft and used to pressurize the cabin. This bleed air from the engine can transport intact or pyrolyzed engine and hydraulic oil and the additives contained within these products. There is only one aircraft, commercially operated, that does not pressurize its cabin in this fashion, the Boeing 787. Within this chapter we directly compare this bleed-free aircraft with several bleed-air pressurized aircraft to determine qualitative chemical differences that may be associated with the pressurization pathway.

Twenty tray table wipe samples from on-board commercial aircraft were assessed. The samples and trip blanks were extracted and run with minimal cleanup on a flow-modulated GCxGC-TOFMS in tandem ionization mode (SepSolv- BenchTOF). To more completely qualify the chemical exposome present in the aircraft cabin, a quasi-non-targeted analysis was completed; hard and soft ionization coupled with the separatory power of multidimensional chromatography is used in this instance in the place of high-resolution mass spectrometry for tentative compound identification. The results of this analysis provide a suspect screening list that can be used, confirmed with standards, and quantified in future aircraft analysis. Of particular note are compounds found to be enhanced in and unique to bleed air pressurized aircraft in this assessment which have noted source descriptions linked to aircraft in the literature such as short chain organic acids and aldehydes.

4.2 Introduction

The majority of commercial aircraft compress atmospheric air in flight in forward sections of their engines; a percentage of this compressed air is bled into the aircraft, where it is conditioned, mixed with recirculated air, and used to pressurize the aircraft cabin (de Boer et al., 2014). Leaking seals, bearings, and hydraulic components may allow contaminants from the engines, namely engine oil, and oil- additives, to enter the cabin through the pneumatic system (Michaelis, 2018). One aircraft is a noted exception, the Boeing 787, the cabin of which is pressurized by electrical compressors (Boeing, 2008).

Previous environmental monitoring and risk assessment on aircraft has primarily focused on organophosphate additives (tricresyl phosphates) present in aircraft engine oils or the targeted analysis of various volatile and semi-volatile organic compounds; the lack of a non-targeted assessment of the exposome is a noted knowledge gap identified in Hayes et al. (2021). Additionally, while it is believed that the Boeing 787 should mitigate many contaminants of concern by eliminating bleed air pressurization of the cabin, a holistic comparative assessment between this aircraft and bleed air pressurized aircraft still needs to be completed. To further complicate this claim, tricresyl phosphate was found within the cabin of Boeing 787 aircraft by Schuchardt et al. (2019), an immensely improbable possibility if the aircraft engine oil is the only source.

Within this chapter, a quasi-non-targeted analytical approach is taken to assess what chemical contaminants, if any, can be identified as exclusive or enhanced in bleed air-pressurized aircraft compared to non-bleed air-pressurized aircraft.

Table 10. List of flight samples included for Bleed V.S. Bleed-Free comparison and associated aircraft. Aircraft type proceeded by A indicates the aircraft is manufactured by Airbus with the number indicating plane model. Aircraft type that begin with the number 7 are manufactured by Boeing, with the 787 aircraft type as the non-bleed air-pressurized aircraft. Samples collected on the same flight are indicated by a and b and a matching date of collection.

Sample	Aircraft Type	Date of Collection
WS1	A320	6/14/2019
WS13a	A321	6/13/2019
WS14b	A321	6/13/2019
WS21	A321	6/22/2019
WS24	A319	6/30/2019
WS39	737-600	6/9/2019
WS41a	737-600	6/9/2019
WS42b	737-600	6/9/2019
WS49	737-600	6/17/2019
WS54a	737-600	6/17/2019
WS56b	737-600	6/17/2019
WS65	737-600	6/15/2019
WS68a	787	8/16/2019
WS70b	787	8/16/2019
WS73a	787	8/24/2019
WS76b	787	8/24/2019
WS89	A330	12/14/2019
WS92	A321	1/4/2020
WS95	787	2/23/2020
WS98	787	2/29/2020

The data represented in this chapter is a portion of the total acquired (SI- Table 1); the selected samples were chosen to describe the comparison of bleed-pressurized and non-bleed pressurized aircraft, represent a variety of aircraft manufacturers, and to provide a proof of concept in regards to our non-targeted analytical methods.

4.4 Materials and methods

4.4.1 Sample collection

In order to efficiently and economically collect an appropriate n-number of wipe samples during flight, a citizen science initiative was developed. Participants (28) were recruited in two formats, and several considerations were taken to minimize variability associated with this sampling choice.

A presentation / workshop was led by the author at the International Network of Environmental Forensics Conference (2019). Environmental forensics practitioners were trained in the sample collection method and provided with a detailed sampling method and pre-constructed wipe sampling kits for use on their return flights from the conference. Additional kits were provided if additional flights were to be taken. This generated 39% (n = 38) of all samples taken.

The author or the supervisory team approached individuals who routinely flew or were opportunistically known to be flying shortly. These individuals were trained personally in the sample collection method and provided with the sampling method and sampling kits to complete the sample collection. This generated 15% (n = 15) of all samples taken.

The remaining 46% (n = 45) of samples were collected by the author, members of the O'Sullivan lab group, or the authors' supervisory team. All of whom were trained in or participated in developing the sampling method. The recruitment and training process resulted in 85% of our samples being collected by trained scientists, allowing us to have increased confidence in the reliability of sample collection.

Citizen scientist participants were provided with a sampling kit composed of a sample collection sheet and pen, a set of nitrile gloves, two commercially available, individually packaged, 70% isopropyl alcohol wipes (BD Medical), two small plastic sample collection bags (DispenserBag RELOC-Zippit; labeled B and S), and a 10cm x 10cm dust free card template (SKC). Each sampling kit was also provided with a prelabeled envelope and postage to send the sample by mail back to Mount Royal University (Calgary AB, Canada).

When the trained participants boarded their flight, they were asked to complete the following wipe sampling method:

Once the plane has reached cruising altitude and it is safe to do so;

1. Remove the sampling kit and put on the purple nitrile gloves.
2. Open one of the IPA Swabs by tearing the packet. Place and seal this swab in the packaging into the small bag labeled B. This swab acts as a trip blank.

3. Open the second IPA swab packet, take the wipe out. Hold the template provided in the center of the closed tray table in front of you.
4. Firmly wipe within the template using first four horizontal wipes, then four vertical wipes. Use the same side of the swab for all wiping and attempt to cover the entirety of the surface within the template.
5. Place the swab back into the packaging it came in. Place and seal the swab in its packaging inside the small bag labeled S.
6. Fill out the required information on flight number, aircraft type, etc. while taking note of any odors or irregularities that you have experienced on the flight.
7. Place the sample bags, as well as the sample information sheet, inside the postage paid envelope and after landing mail as soon as convenient.

In certain instances, sample collectors had final destinations in Calgary (23% of all samples), which allowed samples to be directly placed into cold storage (SI Table 1). All other samples were transported to Mount Royal University by mail or on return flights, where they were then placed in cold storage until extraction. Samples represent aircraft from two major manufacturers and four aircraft types. One electronically pressurized (i.e., non-bleed air pressurized aircraft), the Boeing 787 Dreamliner, is commercially flown and was sampled in this experiment; six samples over four individual flights. This is contrasted with seven samples on six flights of the Airbus A320 and A330 families of aircraft and seven samples from six flights on the Boeing 737-600 series aircraft (Table 10.).

4.4.2 Wipe extraction method testing

To determine wipe extraction efficacy and ease, wipes were spiked with 100 µL of 1 µg mL-1 Triamyl phosphate (TnAP) and tricresyl phosphate (TCP) in its tri-ortho, meta, and para isomer forms (ToCP, TmCP, TpCP) and extracted using three methods.

1. Accelerated Solvent extraction (ASE) was completed (Dionex ASE 150), following Dionex Application Note 319 (Dionex, n.d.). Following ASE, the sample was transferred from the collection 40mL VOA vial to a centrifuge tube, dried with 3.000 grams of sodium sulfate (99% Acros Organics), and placed in a centrifuge (10 minutes, 2000RPM). The supernatant was returned to the VOA vial for

solvent reduction/ blowdown with ultrapure nitrogen to near dryness and then redissolved into 100 µL of toluene (Labconco – RapidVap Vertex Evaporator, 5psi, 35°C).

2. Vortex microextraction was completed utilizing EPA method 3572 (EPA, 2014). Spike wipes were placed in a 50mL clean centrifuge tube. 5 mL of HPLC grade isopropyl alcohol/dichloromethane (1: 9) (Fisher Chemical) was added, and the sample was placed on a vortex mixer (VWR) for 5 minutes. The solvent was transferred to a clean VOA vial, and the process was repeated. The solvent volume was then reduced via nitrogen blowdown and redissolution.
3. Sonication microextraction methodology was based on previous success with the method by a supervisory team member (Dr. Megson). Spiked wipes were placed in 40mL VOA vials, and 15mL of HPLC grade acetone: dichloromethane: ethyl acetate (1:2:1), or 15mL of HPLC grade dichloromethane only, were added to the vials. The samples were then sonicated (Branson 3200) in a water bath for 15 minutes, the solvent was transferred to a centrifuge tube, and the process was repeated. The sample was then dried with ~3.000g of sodium sulfate (99% Acros Organics) and span, as described above, in a centrifuge before transferring to the original VOA vial and subsequent solvent reduction/ blowdown/ redissolution.

Ten wipes were spiked and extracted to test each extraction method. The extracts were then analyzed using Multidimensional Gas Chromatography-Time of Flight mass spectrometry (GCxGC-ToFMS. Each chromatogram was visually inspected, and the peak heights of the added samples were compared. Noting very little difference in extraction efficiency between the methods, sonication microextraction in dichloromethane (DCM) was selected as the extraction method of choice for the aircraft samples as it allowed multiple samples to be extracted simultaneously.

4.4.3 Wipe extraction- QA/QC

Wipe extraction of collected samples and trip blanks was complicated by the presence of small amounts of liquid (condensation) on the interior of the sample collection bag. To avoid the loss of analyte, the bag was fully opened, and a second, new, isopropyl alcohol wipe was used to wipe the interior of the sampling bag; 4 horizontal and five vertical wipe passes ensured complete coverage. The sample and second wipes used to collect the condensation were then placed in the VOA vial and extracted. This necessitated the inclusion of sample bag wipe blanks for analysis.

Additional Blanks:

- Wipe blanks; new isopropyl alcohol wipes mirroring sample and trip blank treatment and completed for every ten samples.
- Wipe packaging blanks; wipes were removed from commercial packaging, and the inside and outside of the package were wiped, followed by an extraction procedure.
- Drying agent (Na_2SO_4) blanks: 3 grams of the powder, no wipe addition, same blank rate. This work was completed while wearing Nitrile gloves, which were changed out for each sample and blank.

Before adding solvent, wipes were spiked with 100µL of a 1000ug L^{-1} Deuterated Kovats Lee Retention Index (DKLRI) compound mix to act as internal standards (Boegelsack et al., 2021).

4.4.4 Nitrogen blowdown

Solvent reduction of the extracted samples under an Ultrapure nitrogen stream was conducted inside the VOA vials to an approximate volume of 300 µL. Vial sides were rinsed three times during the blowdown procedure with 2 mL of new DCM using a sample/blank specific, clean glass Pasteur pipette. The volume-reduced analyte was pipetted from the VOA vial to a new fused insert (500 µL, conical) amber GC vial. The solvent was then further reduced to near dryness using a Thermo Scientific Reacti-Therm III heating block with an N2 block attachment. The dry samples were then reconstituted in the vials with 100 µL of 99% Nonane (Thermo Scientific/ Alfa Aesar), which had been spiked with triamyl phosphate (TCI America) to a concentration of 960 ug L^{-1} acting as a recovery standard. The samples were then sealed (GC lid and parafilm and stored at 4°C until instrumental analysis.

4.4.5 Instrument (GCxGC-ToFMS) justification

Instrumental analysis of all samples and blanks was completed with an Agilent 7890A Gas Chromatograph (GC) with an Insight flow modulator fitted to allow for comprehensive two-dimensional chromatography (Sepsolve). The GC was attached to a

BenchTOF-Select mass spectrometer (ToF-MS) (Markes) operated using concurrent tandem electron ionization at both 70 and 16 electron volts (eV).

This instrument allows for the separation of individual chemical constituents of complex organic mixtures over two dimensions as opposed to the one possible with conventional GC-MS analysis; this can increase the resolution of individual compounds by limiting overlapping peaks (Ramos, 2009; Boegelsack, 2021b). This separatory technique is necessitated in this case for two reasons: 1. Conventional GC MS analysis has been demonstrated to create challenges for identification in non-targeted analysis because of poor peak resolution (partially or entirely overlapping peaks). 2. When analyzed, the commercial wipe sample chosen to be our sampling medium demonstrated a large number of unwanted contamination peaks (Figure 8). If using conventional GC MS, it would be very challenging to distinguish peaks in the samples compared to the trip blanks; anything above or below the one second of secondary retention would likely be obscured by other peaks (Figure 8).

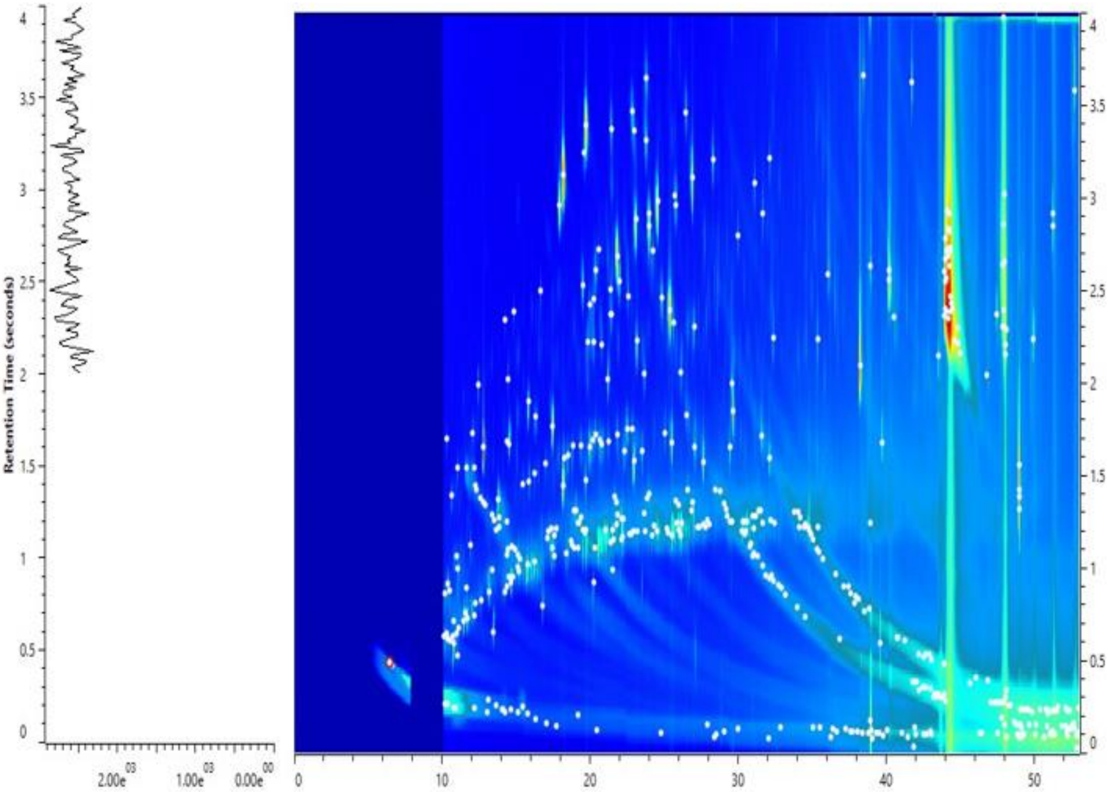


Figure 8. WS24TB. This figure represents a typical trip blank wipe sample analyzed via GCxGC ToFMS. Multiple peaks exist in the blank. Note that some peaks (light blue ovals) that are present in this blank sample are not identified by the software with a white circle. The presence of a white circle is indicative of the software recognizing a chromatographic peak. These unidentified peaks are not deconvoluted and will not be present in peak tables.

Concurrent tandem ionization is beneficial because it allows hard and soft ionization to be completed within the same analytical run (SepSolve Analytical, 2021). Hard ionization, for the purposes of this chapter, ionization that takes place with an electric potential difference of 70 eV, is very useful in that it ionizes and fragments molecules that pass through the electron source; these fragments are mostly reproducible and may be diagnostic of key features and/or the compound. However, this fragmentation is extensive, creating many fragments but often resulting in the absence of the complete molecular ion, an obviously useful diagnostic mass spectral feature. This has been overcome by 'soft' ionization methods, such as lower eV EI ionization, chemical ionization (CI), and atmospheric pressure chemical ionization (APCI) (Halloum et al., 2016; SepSolve Analytical, 2021). These soft ionization techniques reduce fragmentation and preserve more of the molecular ion and larger fragments but can reduce the sensitivity of the instrument. Tandem ionization allows both hard and soft ionization to take place on the same sample injection simultaneously. This results in the benefits of fragments and sensitivity of hard ionization, while preserving more of the molecular ion with soft, furthering the ability to more accurately qualitatively determine the chemical composition of the samples.

4.4.6 Instrument parameters

The findings of previous instrument optimization work (Boegelsack, 2021b) allowed for the selection of an appropriate separatory column combination. Column dimension, stationary phase, gas flow balance, and column orthogonality in chromatography were addressed. The first-dimension column was a non-polar 25m BPX5, with a 0.15mm internal diameter and 0.25 µm film thickness. The second-dimension column was a semi-polar 5m BPX50, with an internal diameter of 0.25mm and a film thickness of 0.15 µm. The remaining alterable instrument parameters can be found in Table 11.

Table 11. GCxGC- ToF operation parameters.

GCxGC-ToF Parameters	
Modulation Frequency	4 Seconds
Injection Volume	2 µL
Inlet Temperature	300°C

Split Ratio	5 to 1
Flow Rate	0.5000mL/min
Initial Oven Temperature	60°C
Ramp	10°C per minute to 150°C
Ramp 2	4°C per minute to 310°C
Ramp 3	Hold at 310°C for 5 minutes
Total Run Time	54 minutes
Transfer Line Temperature	280°C
Ion Source Temperature	280°C
Filament Voltage	1.70
Filament Delay	600 seconds
Mass Range	30 - 568.8
Hard Ionization (eV)	70
Soft Ionization (eV)	16

Each trip blank and sample was injected in triplicate sequentially. A solvent blank (99% nonane) was injected proceeding and following each trip blank and sample pair to monitor for sample carryover. A daily injection of a 1 µg mL^{-1} DKLRI mix in nonane was injected throughout the instrumental analysis to monitor for instrument drift over time and changes that may have arisen due to routine instrument maintenance or unexpected problems/ changes.

4.4.7 Data processing method

All data files were converted from a software-specific file type (.lsc) into a Common Data Format file type (.cdf) using ChromSpace software (SepSolve Analytical). The files were then processed and deconvoluted using AnalyzerPro XD (SpectralWorks). Alterable data processing method parameters can be found in Table 12.

Table 12. AnalyzerPro Data Processing Parameters.

AnalyzerPro Data Processing Parameters	
1D-2D Matching	80%
Mass Range	40-5000
Min Masses for Peak ID	4
Area Threshold	50
Height Threshold	0
Signal to Noise	5 to 1
Gaussian Smoothing	3
Minimum Peak Width	0.001 minutes
Library Searching	NIST-Main Library
Min Match Confidence	60%

Parameters were chosen to provide adequate peak identification while minimizing the identification of false or absent peaks and informed with the assistance of the software developer/director (Moncur, 2023). Many of the alterable choices made in the data processing and deconvolution are arbitrary; regardless of the stringency of the processing method, chromatographic peaks may be missed; likewise, increasing the method stringency increases the likelihood of identifying peaks that result from instrument noise or artifact. One important consideration is the minimum number of masses for peak ID. While identification confidence can be improved by increasing this number, a low number is necessary if the same method is used to process both 70eV and 16eV sample data due to decreased fragmentation occurring with soft ionization. Figure 8 demonstrates peaks present upon visual inspection but not identified by the software. Data processing method optimization prior to the future semi-quantification process will ideally minimize the number of unidentified peaks. However, for a qualitative comparison between bleed air and non-bleed air pressurized aircraft, strict first and second-dimension individual peak retention time windows coupled with a visual inspection of identified peaks in their respective chromatograms allow for peak comparison of software-identified peaks with minimal error.

4.5 Results and discussion

4.5.1 Bleed air and bleed-free aircraft comparison sample screening

Utilizing Analyzer Pro, a software-based multivariate screening of the bleed and non-bleed-air-pressurized aircraft samples was completed. This allowed for preliminary visualization of trends in terms of chemical component composition and loading between the aircraft classes. Figures 9. and 10. compare all non-bleed air pressurized aircraft (Boeing 787) with the other aircraft models in this data subset. Each triangle on either side of the centerline represents a component compound found in the respective aircraft. The x-axis represents a Log2 fold change of abundance (i.e., movement from the centerline to a '2' in either direction implies a 4x increase in abundance for that component as compared to the other aircraft type; movement to a '4' in either direction implies a 16x increase in abundance, etc. Movement up the Y axis indicates increased statistical significance, with component compounds exceeding p=0.05 being placed above the yellow dashed line. Green and red triangles, regardless of statistical significance, represent component compounds that were found only in their respective aircraft type or compounds that are unique to that aircraft type for the specific comparison. Volcano plots were generated using AnalyzerPro XD (SpectralWorks).

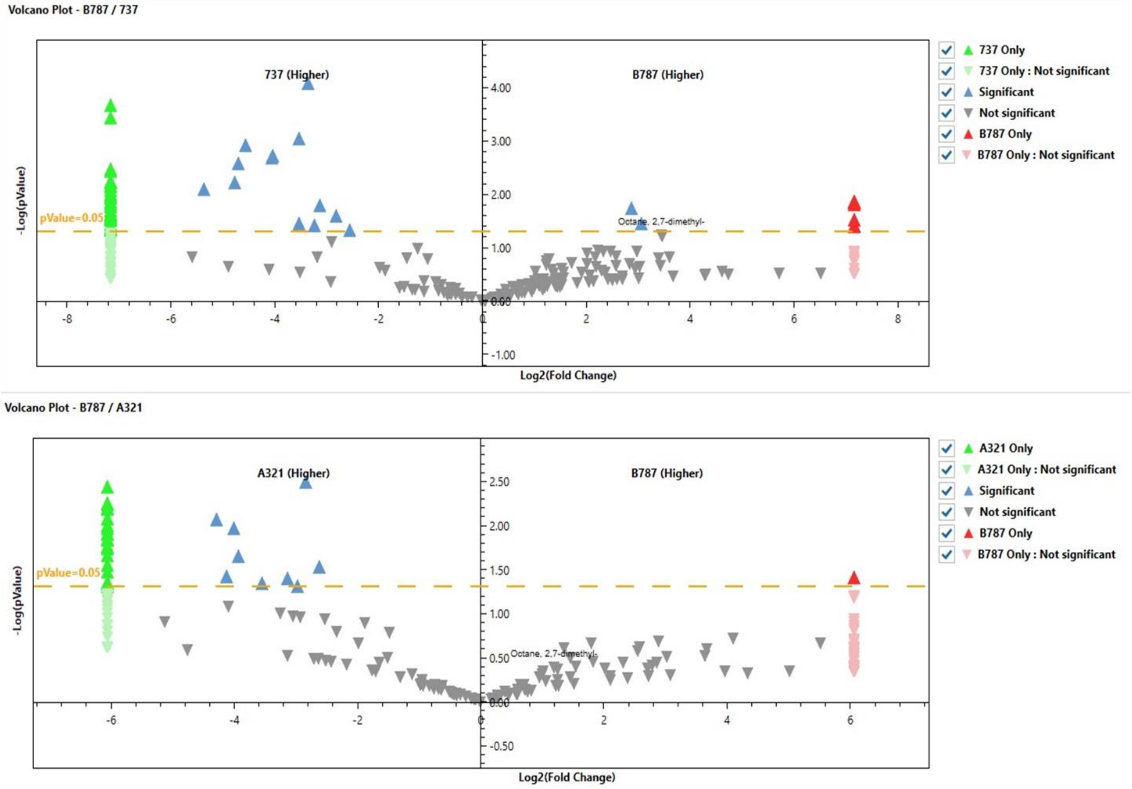


Figure 9. Volcano plots demonstrating compound loadings on Boeing 787 aircraft compared to Boeing 737-600 and Airbus A321 series aircraft. Left Top: Boeing 737-600 series aircraft – Bleed Air Pressurized. Right Top: Boeing 787 series aircraft – Non-Bleed Air Pressurized. Left Bottom: Airbus A321 series aircraft – Bleed Air Pressurized. Right Bottom: Boeing 787 series aircraft– Non-Bleed Air Pressurized.

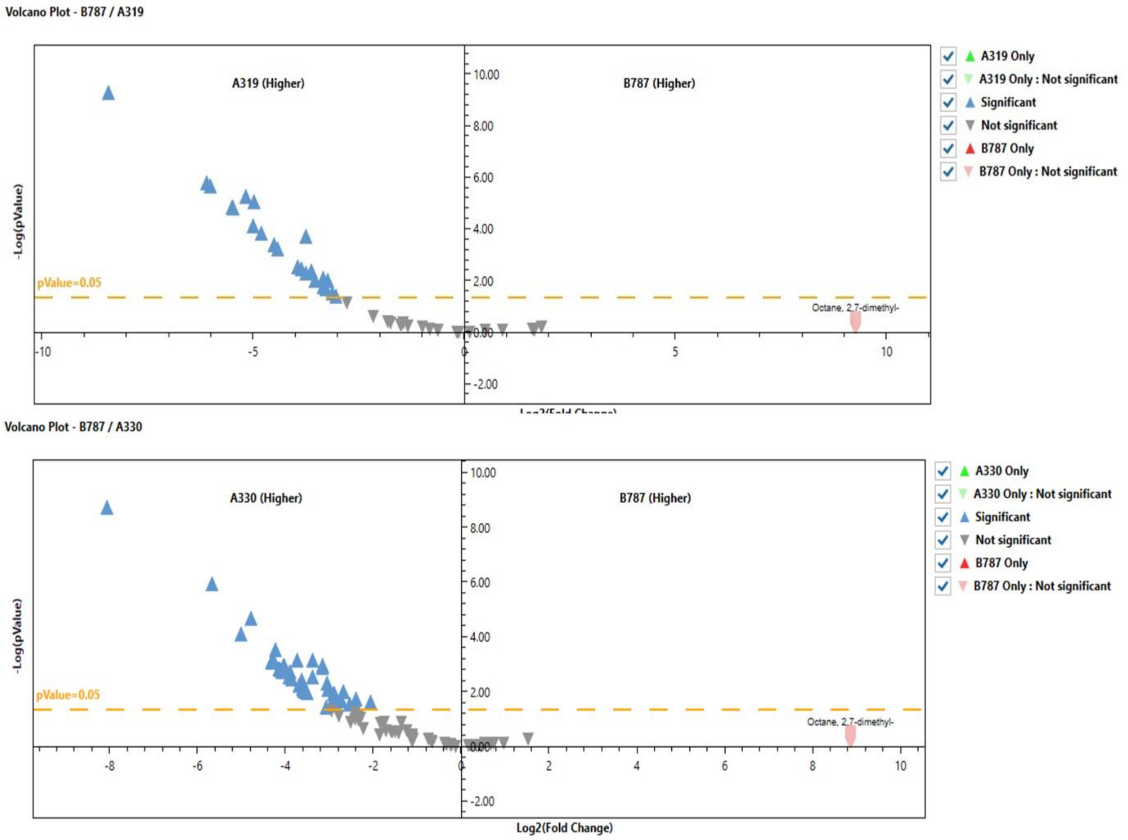


Figure 10. Volcano plots demonstrating compound loadings on Boeing 787 aircraft compared to Airbus A319 and Airbus A330 series aircraft. Left Top: Airbus A319 – Bleed Air Pressurized. Right Top: Boeing 787 series aircraft – Non-Bleed Air Pressurized. Left Bottom: Airbus A330 – Bleed Air Pressurized. Right Bottom: Boeing 787 series aircraft – Non-Bleed Air Pressurized.

When examining the volcano plots for each aircraft comparison with the Boeing 787, it is evident that bleed air-pressurized aircraft contain more statistically significant compounds with higher abundance than non-bleed air-pressurized aircraft. It is also apparent that bleed air pressurized aircraft have more statistically significant unique component compounds present. The number of statistically significant compound components found to be higher in, or unique to, an aircraft type in these comparisons is detailed in Table 13.

Table 13. Volcano plot summary indicating the number of statistically (non-zero p-Values less than 0.05) higher or unique component compounds when comparing bleed air pressurized aircraft to the Boeing 787.

Volcano Plot Summary				
Aircraft	Bleed Air Higher	Bleed Air Unique	Non-Bleed Air Higher	Non-Bleed Air Unique
Boeing 737-600	13	40	2	10
Airbus A321	9	36	0	19
Airbus A319	23	0	0	0
Airbus A330	42	0	0	0

Based on the number of compounds found in abundances higher or unique to bleed air-pressurized aircraft, it is plausible that engine-driven pressurization systems are contributing to contaminant loading onboard the aircraft. It is noteworthy that a number of confounding variables remain, especially when considering individual aircraft. Aircraft cleaning procedures may vary between aircraft type and aircraft operator. This is partially mitigated in that individual aircraft within the A321 series, and Boeing 787 series examined were each operated by at least two different airlines. Aircraft age also may result in variability. The youngest possible 737-600 series in this data set at the time of sampling is 14 years old, and the average age of retirement for similar narrow-body jets is 26.6 years, while the oldest possible 787 was 5 to 6 years old (Forsberg, 2015; Singh, 2020).

Following the results of the multivariate screening it was evident that the comparison between aircraft pressurization types warranted more complete analysis. A database was constructed to effectively manage the data generated and to allow for efficient non-targeted qualitative assessment. For a detailed description of database constructions and queries please see the accompanying supplementary information (SI-SQL).

4.5.3 Compounds elevated in aircraft as compared to respective trip blanks

Compounds were determined to be elevated in aircraft as compared to trip blanks if they exhibited ten times the peak area for a matching peak retention window. The matched master list comprised 286 compounds, 11 of which had a greater than 90% confidence in the library match. The aldehyde Decanal dominates this portion of the list; it was identified as the most likely source of the chromatographic peak on six flights (WS39, 54, 56, 73, 76, 89) and matched in terms of library identification in five trip blanks (WS39TB identified the peak as heptanal) (Table 14).

Table 14. Compounds present in the matched master list with a greater than 90% match factor.

Sample	Library ID	Sample Area	Trip Blank Area
WS89	Decanal	87652	5976
WS73	Decanal	34283	1448
WS76	Decanal	32481	1448
WS54	Decanal	25315	2413
WS56	Decanal	33138	2413
WS39	Decanal	18648	850
WS92	Benzene,1,1'-[1,2-ethanediylbis(oxy)]bis-	78458	5328
WS92	Dodecyl acrylate	50567	1058
WS21	1-Tetradecene	10533	728
WS21	1-Tetradecene	10533	388
WS21	1-Tetradecene	17172	622

Unlike the other compounds listed, decanal is known to exist, as is nonanal, as an emission that results from the combustion of Jet fuel (Jet A-1); these compounds have been identified as present in only aircraft emissions when compared to gasoline combustion (Bendtsen et al., 2021). When semi-quantitatively comparing relative peak areas, decanal was found, on average, to have 17.8 times (std dev= 5.53) the peak area in active samples as compared to trip blanks. Nonanal would likely be found in the samples as well; however, the use of nonane as solvent necessitated an extensive solvent delay (10 minutes). Decanal was one of the first eluting compounds (Range: 10.11-10.24 minutes) (Figure 11.).

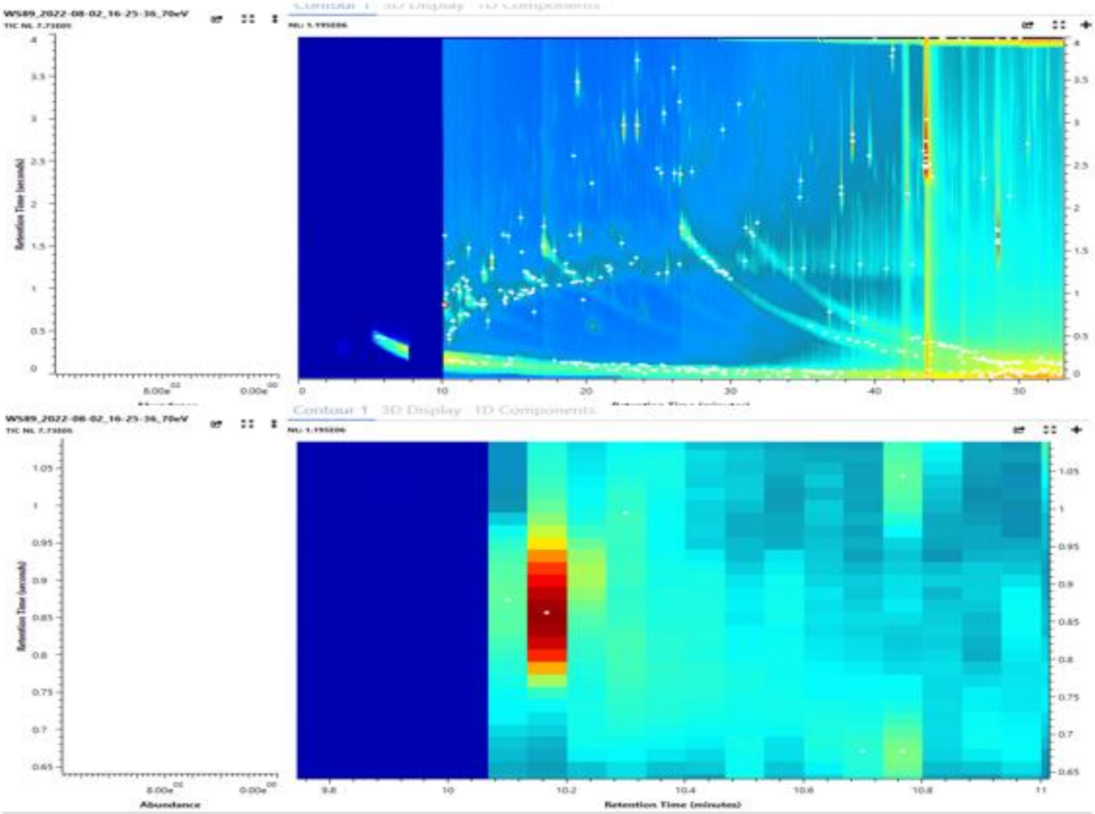
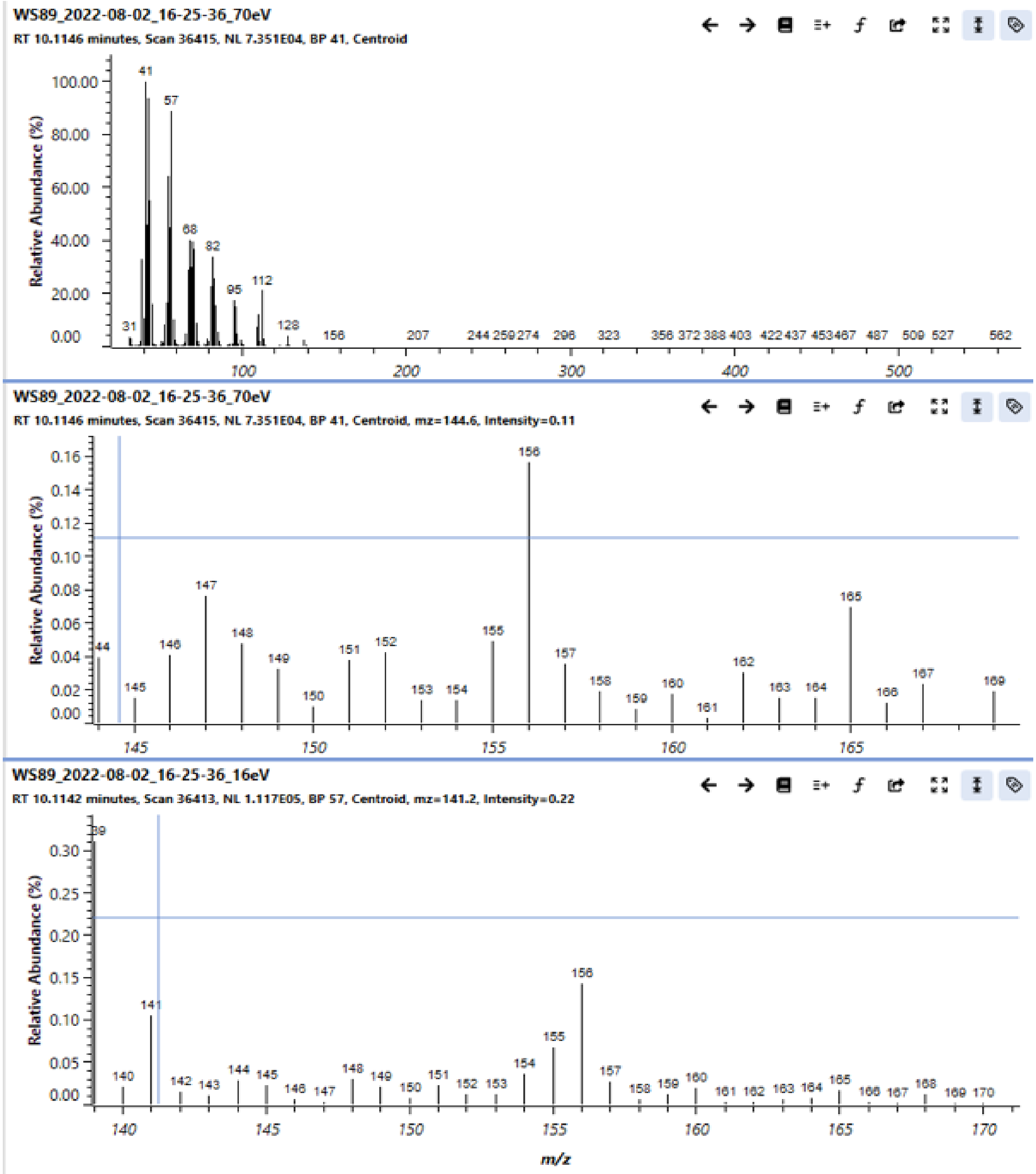


Figure 11. WS89 70eV chromatogram with decanal highlight

When examining the 70eV raw mass spectra of the suspected decanal peak in WS89, a potential small molecular ion peak was found at m/z 156. This ion peak was more prominent than surrounding peaks but not abundant (~0.16% relative abundance) (Figure 12.). Subsequent comparison of 16eV mass spectral data showed no increase in this peak (0.15% relative abundance) (Figure 12.). Other samples with suspected decanal peaks were similar. Sample WS73 demonstrated a slight increase in m/z= 156 with soft ionization but nothing notable (~0.18% to 0.23% relative abundance). Because there is little evidence to suggest that soft ionization enhanced the molecular ion in this instance, we cannot confidently state that the compound is present.



WS89_2022-08-02_16-25-36_70eV
RT 10.1146 minutes, Scan 36415, NL 7.351E04, BP 41, Centroid

WS89_2022-08-02_16-25-36_70eV
RT 10.1146 minutes, Scan 36415, NL 7.351E04, BP 41, Centroid, mz=144.6, Intensity=0.11

WS89_2022-08-02_16-25-36_16eV
RT 10.1142 minutes, Scan 36413, NL 1.117E05, BP 57, Centroid, mz=141.2, Intensity=0.22

Figure 12. Raw mass spectra WS89 decanal peak in 70 and 16eV.

While we cannot say with certainty that decanal is present in the aircraft cabin, it remains a likely possibility based on its prevalence in jet aircraft emissions (Bendtsen et al., 2021). Decanal was exclusively "identified" in the matched data in bleed air-pressurized aircraft. However, if the mechanism of entry into the cabin is the pressurization system, and the source is other aircraft exhaust, both the bleed air pressurized and non-bleed air pressurized aircraft should be equally susceptible as they both draw in outside air for the pressurization. A possible source could be the engines of the bleed air aircraft, although several variables are unaccounted for/ unexplained.

4.5.4 Compounds unique to aircraft as compared to respective trip blanks

The unmatched master list output contained 5397 compounds across all samples. To increase the analysis efficiency, the list was screened for compounds identified by software with a greater than 90% match factor. This resulted in a list of 53 compounds. Of these 53 compounds, several appear across multiple aircraft and some of these identified compounds (ex. 1-tetradecene, decanal) also appeared in the matched master list. Compounds identified on both the matched and unmatched master list can occur in circumstances where peak areas of matched compounds are too small in the trip blanks to be identified by the software as a peak during the deconvolution process. Variability in the trip blanks could potentially also be responsible. Regardless, the comparison is between individual flight samples and their respective trip blanks, and as such, compounds identified by these criteria are "unique" to that flight.

One compound from the list assessed provided an excellent opportunity to demonstrate the non-targeted assessment methods. Phosphorus containing compounds are contaminants of concern when considering occupational risk on aircraft and when screening the greater than 90% unmatched list- and only one compound of the 53 was found in multiple aircraft samples and believed to contain the element based upon the library match. N-dimethylaminomethyl-tert-butyl-isopropylphosphine (NDTPI) was identified by library match (mean = 92.26% confidence) in the greatest number from the unmatched list (n=8) and did not appear in the matched master list implying its absence from trip blanks and further emphasizing the uniqueness to the aircraft samples. Second-dimension retention times were mostly stable; however, first-dimension retention times were scattered across the chromatogram, diminishing the confidence in the library match. When analyzing repeats of the first-dimension retention time, 4 of the samples were split into two distinct groups: First-dimension RT = 18.3- 5 minutes and 22.8-9 minutes (Figure 13).

Upon visual inspection of the sample and trip blank chromatograms with NDTPI as a suspected compound, it is evident that the peaks are present in the sample and absent from trip blanks; however, significant tailing in both directions for the peak is evident in Figure 13. Better chromatography and peak resolution were determined in other injections (Figure 14.). Regardless of chromatography, peaks corresponding to both identified intervals in the first dimension (18- 19 min and 22-23 min) along the same second dimension band (~0.8 seconds) are apparent (Figure 13). Examining the raw spectra of each peak on a pixel-by-pixel basis, it is evident that the two peaks share the same base peak (m/z = 58) and second most abundant peak (m/z = 43). This is likely why both peaks are identified as NDTPI at both retention times across multiple chromatograms (Figure 15.).

The 70eV raw spectrum was examined in both WS13 and WS92, which "identified" NDTPI at 22-23 min 1-d RT for the compounds' molecular ion 189; this ion was not identified in the component spectra (Figure 15.). A 189 peak was found in both samples at very low relative abundance, 0.02 and 0.2%, respectively, and was not the most prominent ion peak in the m/z range.

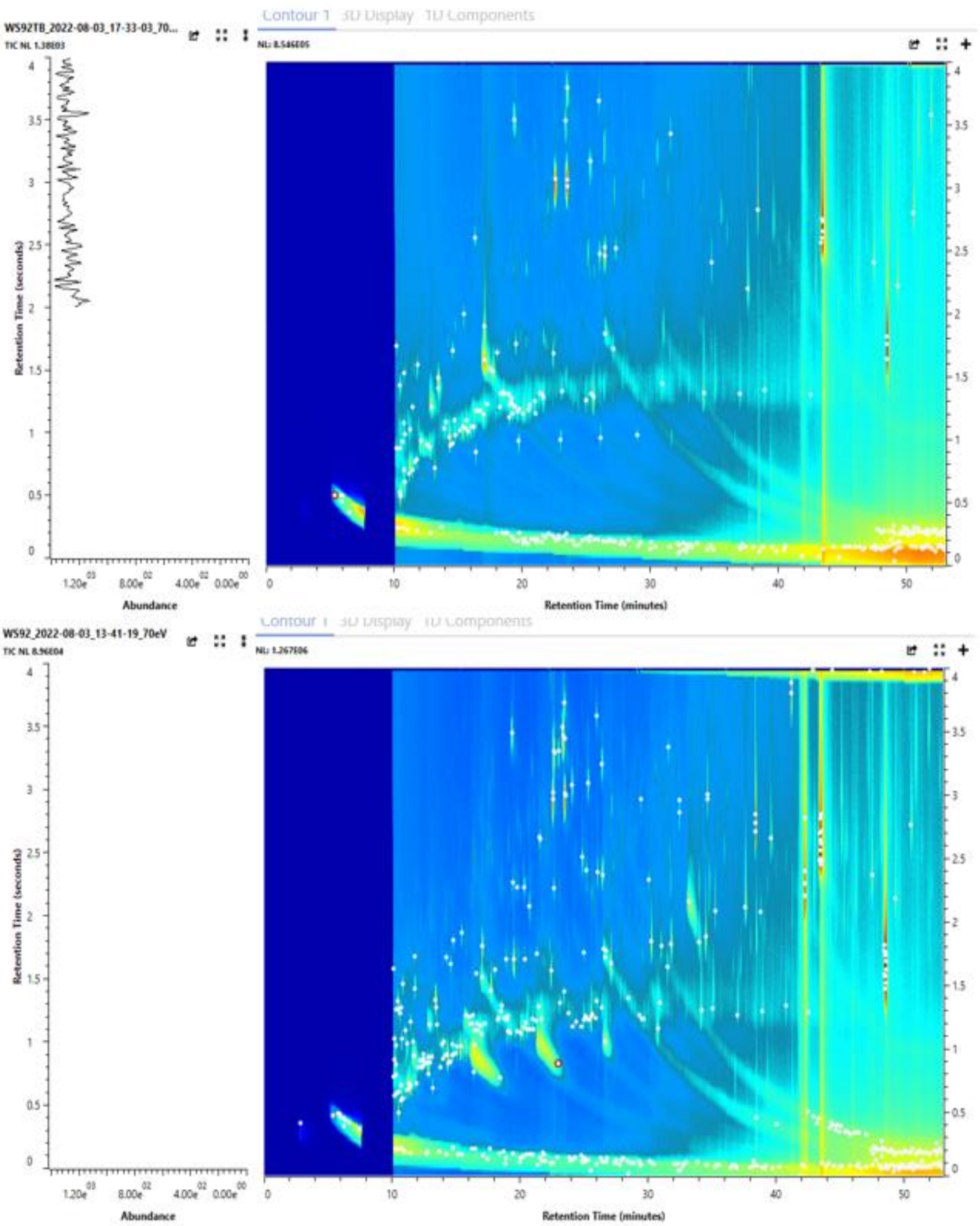


Figure 13. Chromatogram comparison between trip blank and sample WS92. Highlighted peak is library matched as NDTPI.

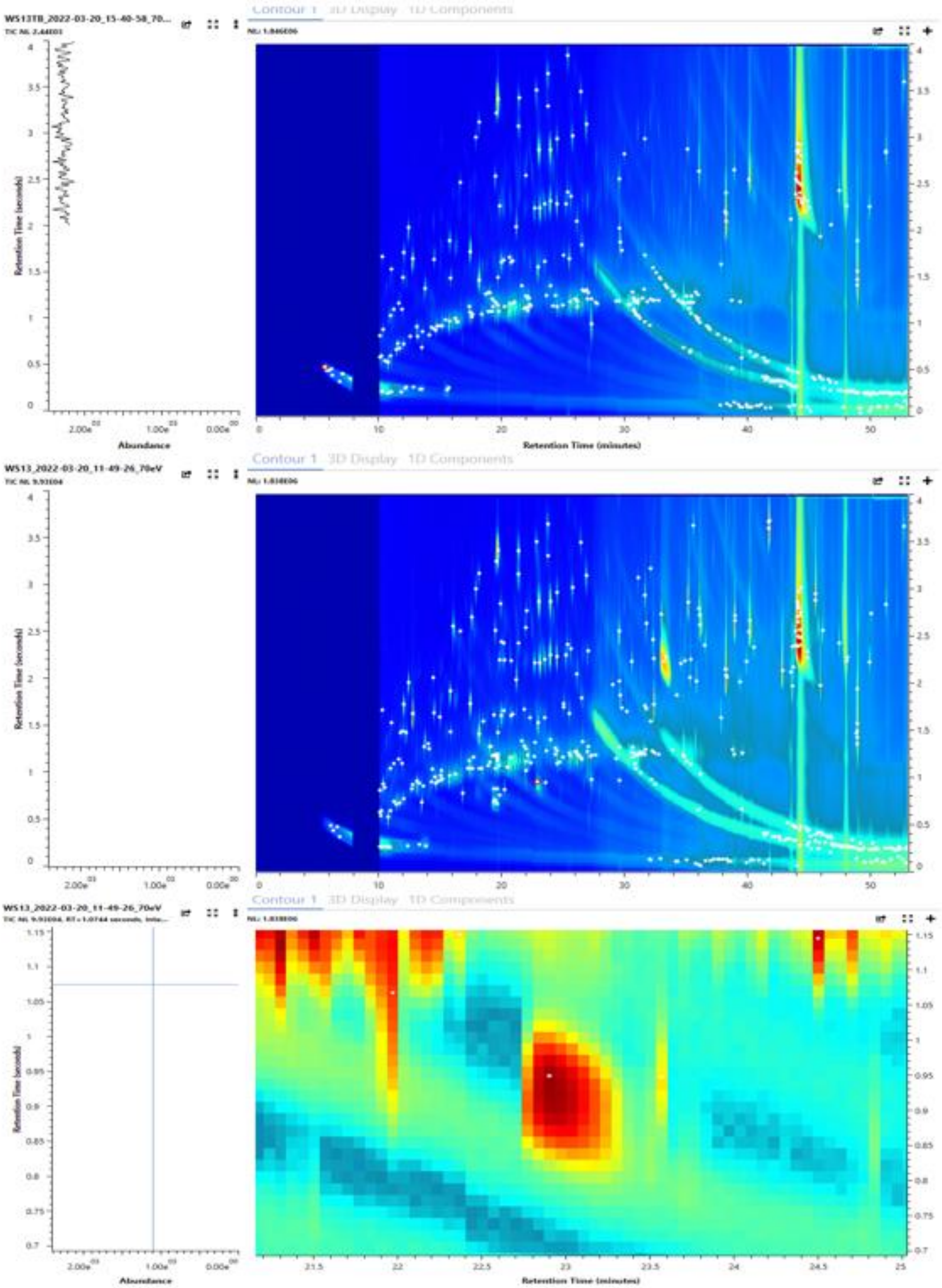
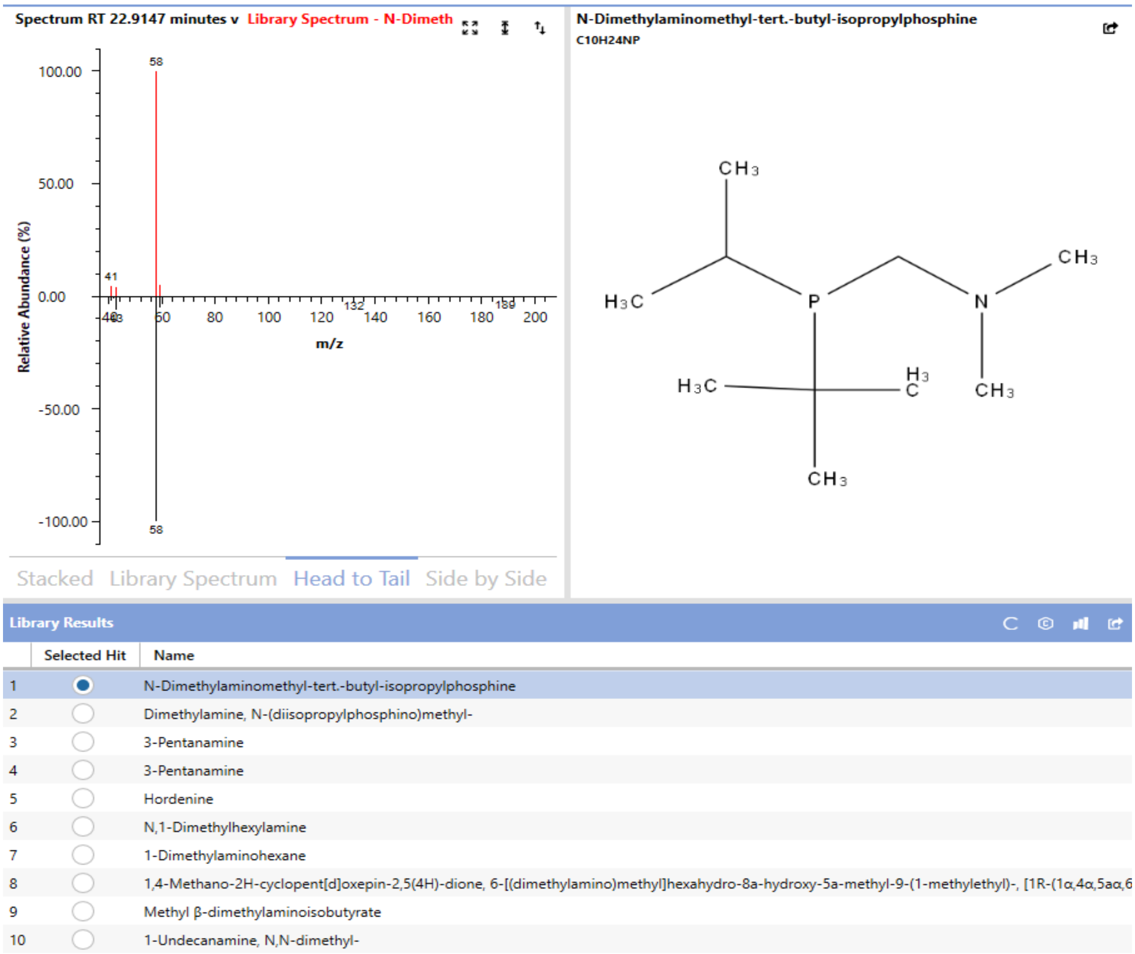


Figure 14. Trip blank and sample WS13 demonstrating absence from blank and improved chromatography for peak(s).

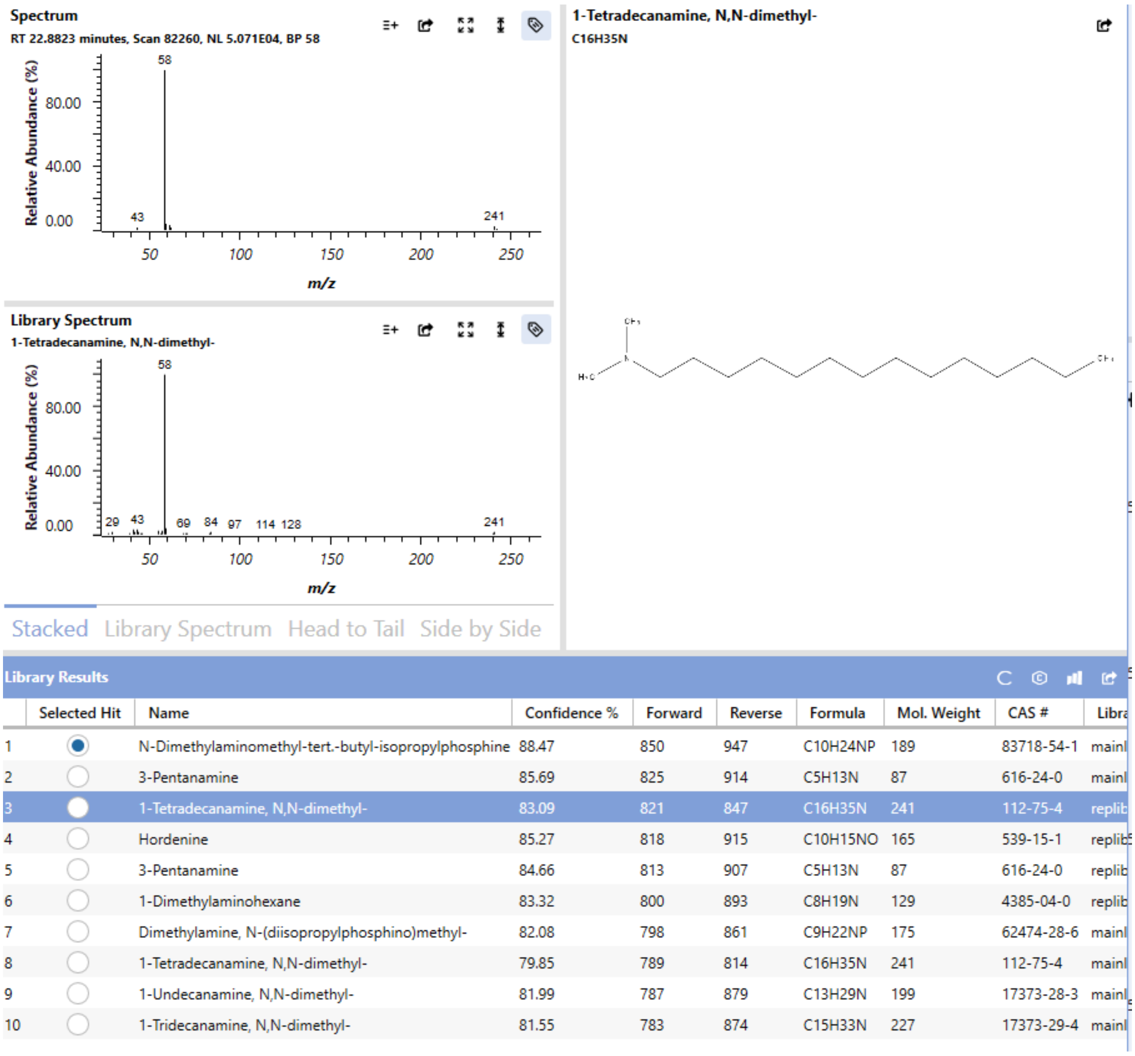


	Selected Hit	Name
1	●	N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine
2	○	Dimethylamine, N-(diisopropylphosphino)methyl-
3	○	3-Pentanamine
4	○	3-Pentanamine
5	○	Hordenine
6	○	N,1-Dimethylhexylamine
7	○	1-Dimethylaminohexane
8	○	1,4-Methano-2H-cyclopent[d]oxepin-2,5(4H)-dione, 6-[(dimethylamino)methyl]hexahydro-8a-hydroxy-5a-methyl-9-(1-methylethyl)-, [1R-(1α,4α,5aα,6
9	○	Methyl β-dimethylaminoisobutyrate
10	○	1-Undecanamine, N,N-dimethyl-

Figure 15. NIST suspected match for NDTPI for WS92 at 70eV.

The 16eV chromatograms were then examined for both samples. The suspect chromatographic compound peak was not software identified in WS92 at this ionization. However, the raw spectra of the peak on a pixel-by-pixel examination demonstrated m/z peaks at 58, 43, and a more prominent 241 peak than was present at 70eV; the 241-ion peak increased from ~1.4% in 70eV to approximately 2 to 3% relative abundance in 16eV for this sample. In WS13, the software identified the chromatographic peak (likely due to the improved chromatography), and m/z 241 was included in the component spectrum at ~3% abundance; in 70eV, this peak had a relative abundance of less than 1%. The increase of this ion peak abundance, relative to other peaks in the raw and deconvoluted spectra, implies the possibility that this is a large fragment or the molecular ion of the suspect compound. Based on the relatively early elution, confidence that this is the molecular ion is increased.

This information, coupled with the absence of a m/z= 189 peak in even the raw spectrum of the WS13 16eV sample, effectively removes NDTPI as a possibility as the compound responsible for the chromatographic peak. NIST match of the WS13 16eV chromatographic peaks erroneously suggests that NDTPI is the best fit; however, N,N-dimethyl-1-tetradecylamine appears as a matching possibility with a reasonably high match confidence (83.09) and a molecular mass of 241 (Figure 16.).



	Selected Hit	Name	Confidence %	Forward	Reverse	Formula	Mol. Weight	CAS #	Libra
1	●	N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine	88.47	850	947	C10H24NP	189	83718-54-1	mainl
2	○	3-Pentanamine	85.69	825	914	C5H13N	87	616-24-0	mainl
3	○	1-Tetradecanamine, N,N-dimethyl-	83.09	821	847	C16H35N	241	112-75-4	replib
4	○	Hordenine	85.27	818	915	C10H15NO	165	539-15-1	replib
5	○	3-Pentanamine	84.66	813	907	C5H13N	87	616-24-0	replib
6	○	1-Dimethylaminohexane	83.32	800	893	C8H19N	129	4385-04-0	replib
7	○	Dimethylamine, N-(diisopropylphosphino)methyl-	82.08	798	861	C9H22NP	175	62474-28-6	mainl
8	○	1-Tetradecanamine, N,N-dimethyl-	79.85	789	814	C16H35N	241	112-75-4	mainl
9	○	1-Undecanamine, N,N-dimethyl-	81.99	787	879	C13H29N	199	17373-28-3	mainl
10	○	1-Tridecanamine, N,N-dimethyl-	81.55	783	874	C15H33N	227	17373-29-4	mainl

Figure 16. WS13 16eV NIST match for 22-23 minute peak.

When more closely examining N,N-dimethyl-1-tetradecylamine, the suspected electron-induced fragmentation is consistent with what is found. The base peak of m/z = 58 can be accounted for with the homolytic (α) cleavage of the methyl group stabilized amine at the first R-group carbon, resulting in an $NC_3H_8^+$ cation. The second most prominent peak, m/z= 43, is likely a propyl cation, and 241 matches the molecular ion. This allowed for the examination of the chromatographic peaks with similar mass spectra at the other first dimension retention time (18-19 minutes) to be viewed with this compound class as a likely candidate.

The WS13 16eV chromatographic peak present at 18-19 minute retention time was identified by the software. The raw spectrum of the peak indicates the same base and secondary ion peaks and also a relatively prominent m/z= 213 peak, which was not apparent in the 70eV spectra (Figure 17.). NIST library matching suggests that the compound responsible for the chromatographic peak is N,N-dimethyl-1-dodecylamine (Figure 18.).

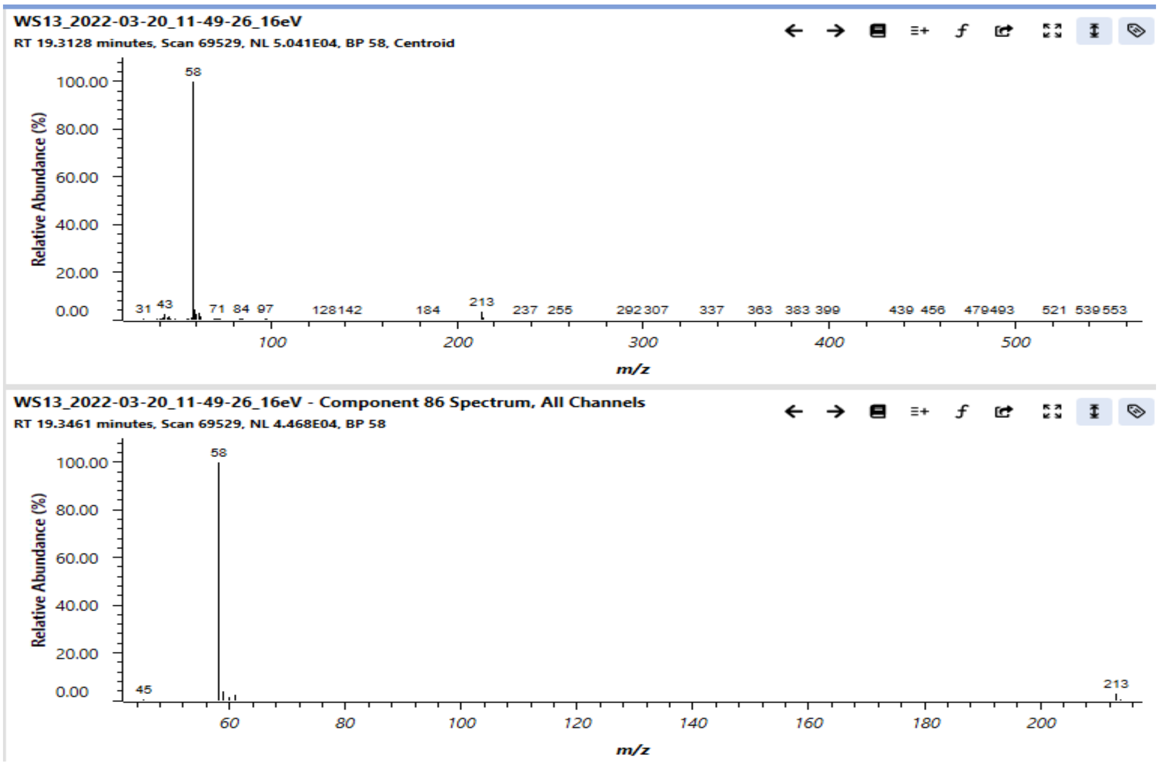
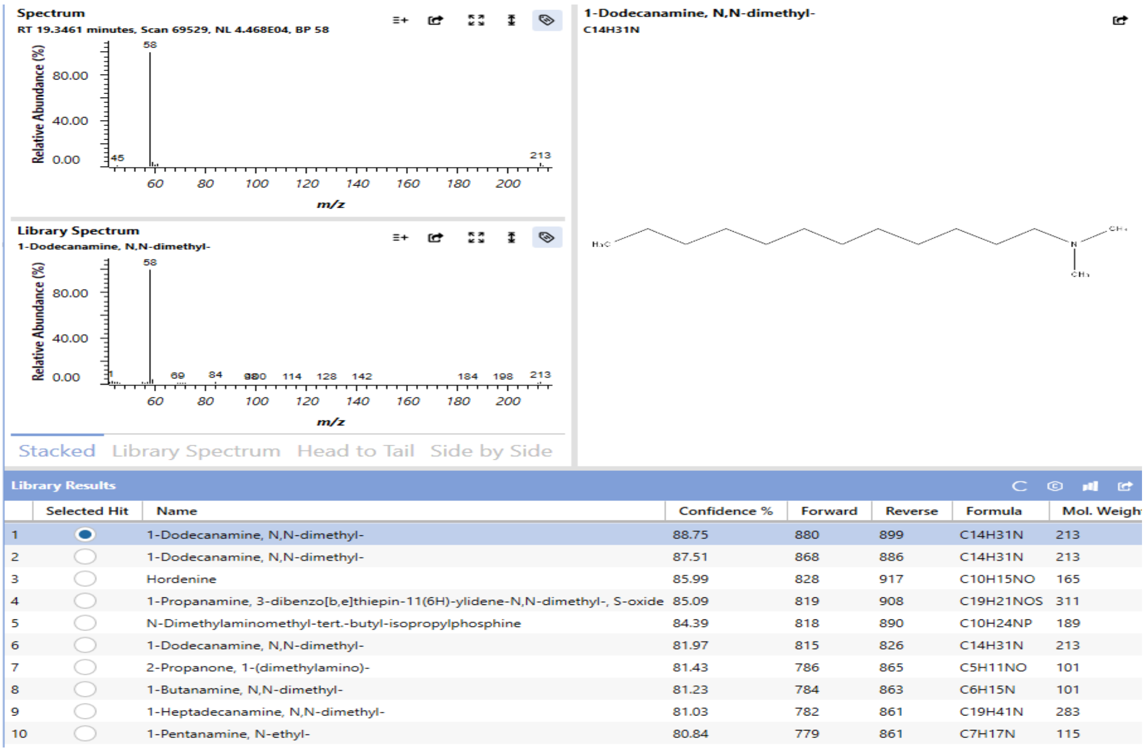


Figure 17. WS13 16eV 19 min peak. Component and raw spec m/z 213 is evident



	Selected Hit	Name	Confidence %	Forward	Reverse	Formula	Mol. Weigh
1	●	1-Dodecanamine, N,N-dimethyl-	88.75	880	899	C14H31N	213
2	○	1-Dodecanamine, N,N-dimethyl-	87.51	868	886	C14H31N	213
3	○	Hordenine	85.99	828	917	C10H15NO	165
4	○	1-Propanamine, 3-dibenzo[b,e]thiepin-11(6H)-ylidene-N,N-dimethyl-, S-oxide	85.09	819	908	C19H21NOS	311
5	○	N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine	84.39	818	890	C10H24NP	189
6	○	1-Dodecanamine, N,N-dimethyl-	81.97	815	826	C14H31N	213
7	○	2-Propanone, 1-(dimethylamino)-	81.43	786	865	C5H11NO	101
8	○	1-Butanamine, N,N-dimethyl-	81.23	784	863	C6H15N	101
9	○	1-Heptadecanamine, N,N-dimethyl-	81.03	782	861	C19H41N	283
10	○	1-Pentanamine, N-ethyl-	80.84	779	861	C7H17N	115

Figure 18. WS13 16eV NIST match for 18-19 minute peak.

In certain instances, the 16eV ionization revealed likely molecular ion peaks that did not match the 70eV library identification (Table 15). This included the "octanol" peaks with an expected molecular ion m/z of 130 but a prominent m/z= 158 peak in the 16eV spectra, suggesting that 1-decanol or nonanoic acid is a more likely compound id. The library match Nonanoic acid had a similar retention time to the octanol peaks. It had no molecular ion peak in the 70eV component spectra, but m/z= 158 was in the 16eV component spectra (Figure 19.).

Table 15. 16eV examination of bleed air pressurized unique peaks.

Sample	RT	RTm	Area	Base Peak	Base Peak	Classifier	ChemName	Mol Ion	MI in 16EV	Notes
WS49	15.5161	0.9662	173015	57	71	211	1-Iodo-2-methylundecane	296	Raw Spec	Not likely based on surrounding spectra
WS21	15.7169	1.0157	32860	57	43	169	1-Iodo-2-methylundecane	296	Raw Spec	Not likely based on surrounding spectra
WS1	21.7842	1.0511	56263	71	57	155	1-Iodo-2-methylundecane	296	Raw Spec	Not likely based on surrounding spectra
WS56	21.4536	1.2186	11811	57	71	153	1-Nonene, 4,6,8-trimethyl-	168	Raw Spec	
WS89	11.7157	0.9417	28639	55	56	112	1-Octanol	130	Raw Spec	158 more prominent likely decanol
WS49	11.5832	0.9934	6597	55	56	97	1-Octanol	130	Raw Spec	158 more prominent likely decanol
WS24	13.0481	0.8888	19760	43	41	196	1-Tetradecene	196	Comp Spec	Extra peaks in 16 that are useful
WS21	13.4492	0.9545	8791	55	43	196	1-Tetradecene	196	Comp Spec	
WS56	44.9435	2.6122	2113	149	71	293	Di-isononyl phthlate	418	Raw Spec	
WS49	44.9427	2.5622	4719	149	150	293	Di-isononyl phthlate	418	Raw Spec	
WS42	45.0094	2.5622	14233	149	43	294	Di-isononyl phthlate	418	Raw Spec	
WS41	44.4116	2.6965	3086	149	71	293	Di-isononyl phthlate	418	Raw Spec	
WS54	44.1438	2.6305	8384	149	43	294	Di-isononyl phthlate	418	Raw Spec	
WS41	43.4764	2.5821	19364	149	71	294	Di-isononyl phthlate	418	Raw Spec	
WS42	42.7434	2.601	12057	149	71	293	Di-isononyl phthlate	418	Raw Spec	
WS54	43.4103	2.6155	20493	149	57	294	Di-isononyl phthlate	418	Raw Spec	
WS49	43.41	2.5992	13085	149	71	294	Di-isononyl phthlate	418	Raw Spec	
WS56	27.1962	1.7717	103994	43	73	257	n-Hexadecanoic acid	256	Comp Spec	
WS41	36.7886	1.3148	1743	57	71	127	Nonane, 1-iodo-	254	Comp Spec	
WS1	11.1166	0.9934	6027	60	73	129	Nonanoic acid	158	Comp Spec	
WS1	14.4473	0.8354	3137	57	71	85	Sulfurous acid, 2-ethylhexyl isohexyl ester	278	Absent	Probably Trimethyl decane or similar '184' present
WS14	25.786	1.1582	20266	71	43	127	Sulfurous acid, 2-ethylhexyl isohexyl ester	278	Absent	Probably Trimethyl decane or similar '184' present
WS56	20.3195	1.1713	117638	57	71	211	Sulfurous acid, hexyl pentadecyl ester	376	Absent	
WS13	20.7201	1.2039	141779	43	57	169	Sulfurous acid, hexyl pentadecyl ester	376	Absent	Probably nonadecane '268' present
WS41	26.3207	1.2399	45896	71	57	169	Tridecanol, 2-ethyl-2-methyl-	242	Raw Spec	
WS42	25.8551	1.3081	146243	57	71	211	Tridecanol, 2-ethyl-2-methyl-	242	Raw Spec	
WS39	24.3208	1.2451	51198	57	71	155	Undecane, 3,8-dimethyl-	184	Raw Spec	
WS41	19.5862	1.1727	64928	57	71	196	Undecane, 3,8-dimethyl-	184	Raw Spec	
WS21	11.9796	0.7747	7838	43	71	141	Undecane, 4,7-dimethyl-	184	Raw Spec	Lots of unaccounted spectra in 70ev, no software match in 16
WS24	11.9121	0.7249	12069	43	57	169	Undecane, 4,7-dimethyl-	184	Raw Spec	Lots of unaccounted spectra in 70ev, no software match in 16
WS56	16.4511	1.0639	23446	57	71	127	Undecane, 4,7-dimethyl-	184	Raw Spec	

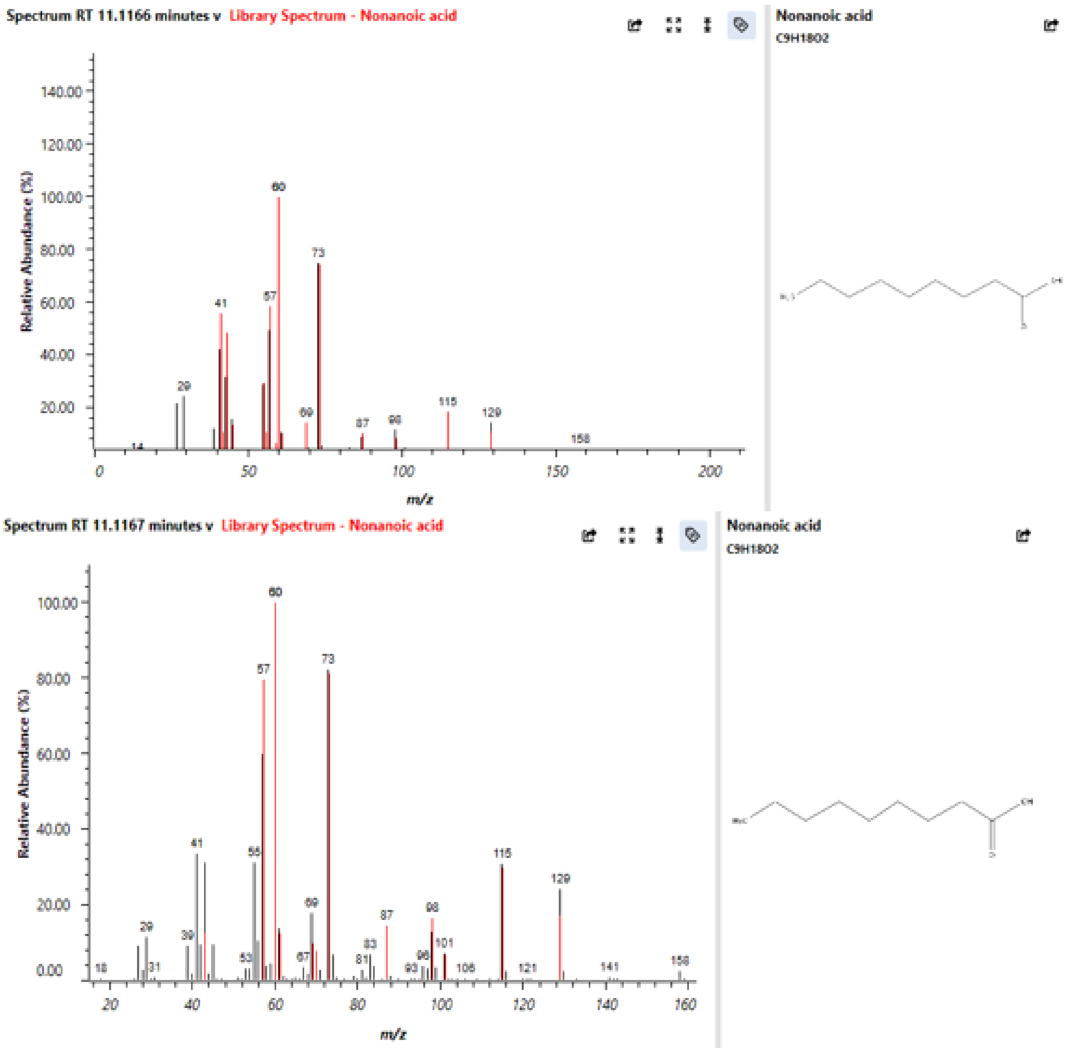


Figure 19. Component spectra of nonanoic acid (70eV top; 16eV bottom).

No compound from this could meet the previously established criteria equating to the Schymanski confidence scale. However, several compounds were present with enough evidence to support targeting them in future analysis.

4.5.5 Retention time window assessment for organophosphates

Organophosphates (OPs) are a chemical class of concern most frequently sought when assessing cabin air quality; this is largely related to their identified prevalence within the aircraft cabin and other aircraft systems (I.e., TCP as an engine oil additive, TnBP as a primary constituent of jet aircraft hydraulic oil, Chlorinated OPs in flame retardant materials) (Hayes et al., 2021; Burdon et al., 2023). Due to this, a suspect screening of the data for organophosphates was carried out via two methods. The first was the establishment, with the constructed database, of a retention time window (SI-SQL).

A visual assessment of all 70eV samples and trip blank chromatograms was completed. A "window" was selected, which demonstrated a large number of peaks in the active samples and less in the trip blanks. The window encompasses retention times on the chromatogram from 30.0000 to 43.5000 minutes in the first dimension and 1.5000 and 3.0000 seconds in the second dimension. This query generated a table with 416 rows. Then, using base peaks and qualifier ions outlined in Halloum et al. (2016), organophosphate compounds were sought. The most prominent fragment for the majority of alkyl phosphates and an expected peak in many others is m/z= 99, a fragment generated when the phosphate group is protonated via three McLafferty rearrangements (Halloum et al., 2016).

Nine chromatographic peaks from eight aircraft samples were found to have compounds within the retention time window with a base peak of m/z= 99. All identified peaks are believed to be the same compound as they share retention times (1-D= 34.7 to 35.3; 2-D= 2.2 to 2.4) and majorly share secondary and classifying ions (m/z= 43, 114) with the exception of one peak (library identified as Trioctyl phosphate in WS54). Unfortunately, little information can be gleaned from the mass spectrum of the peaks; library matches suggest the possibility of fluorinated phosphate esters, and while these would undoubtedly be capable of causing neurological injury, their presence on aircraft is doubtful (Figure 20.). The peak identified as trioctyl phosphate in WS54 could also not be further supported. A solid visual spectral match was present, but the molecular ion m/z= 435 could not be found in either ionization mode.

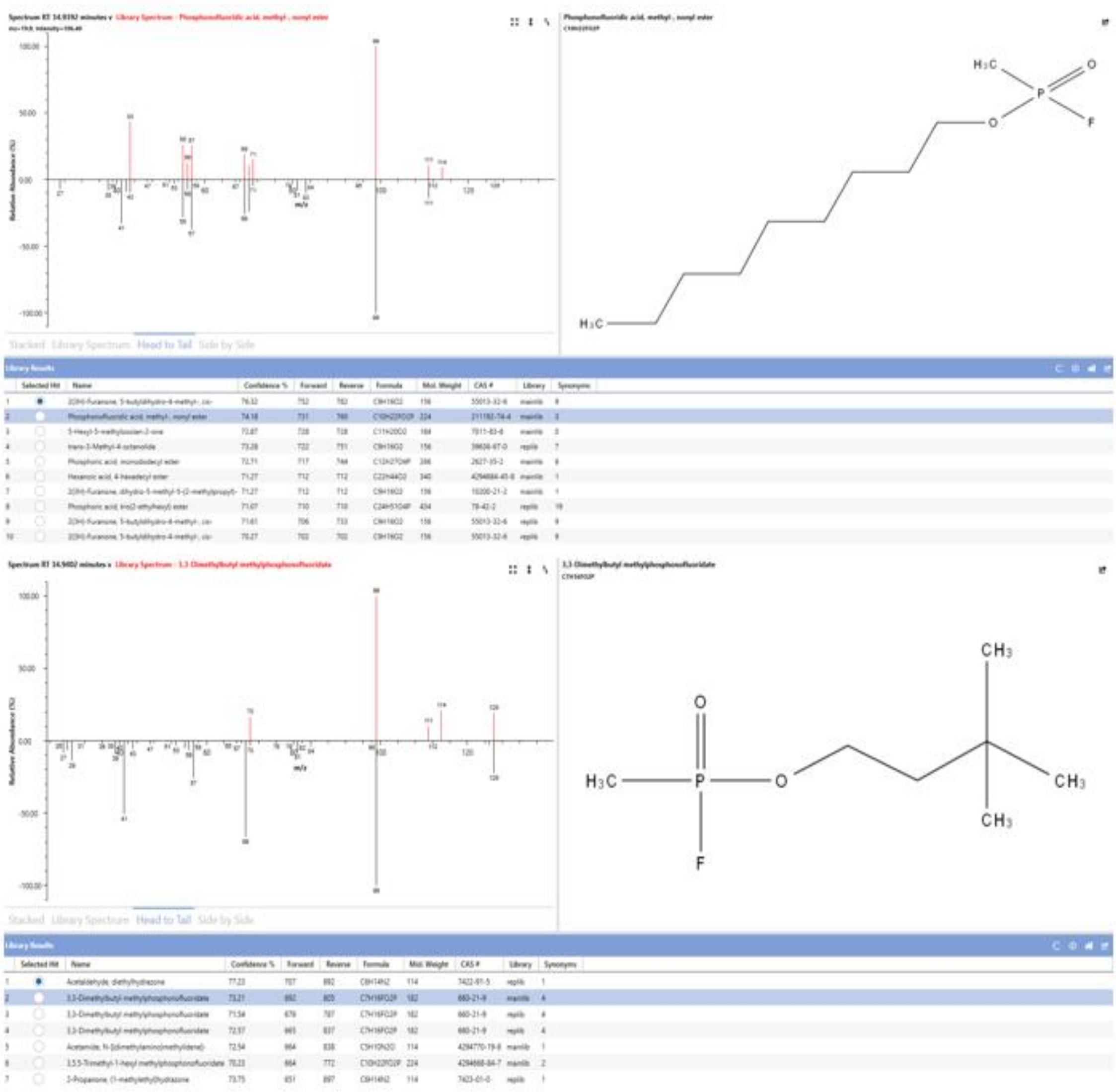


Figure 20. Suggested NIST matches for possible organophosphate peaks (70eV top, 16eV bottom).

Next, OPs were sought outside the retention window by searching the entirety of the unmatched duplicate removed list for ions identified in Halloum et al. (2016). Two probable organophosphates were identified in several samples (Table 16.)

Table 16. Possible OPs outside of retention window.

Compound	Samples	RT	RTm	Area	Basepeak			
TDCIPP	WS21	35.6611	3.668	6602	75	381	99	191
	WS13	35.5945	3.6683	4043	75	381	99	191
	WS14	35.5942	3.6514	2097	75	381	99	191
	WS54	35.6	3.66	Plausible detect based on raw spectrum and RT match.	75	381	99	191
	WS76	35.6	3.66	Plausible detect based on raw spectrum and RT match.	75	381	99	191
EHDP	WS21	37.2563	3.3808	7569	251	169	94	
	WS14	37.1888	3.3309	2088	251	169	94	
	WS76	37.2	3.33	Plausible detect based on raw spectrum and RT match.	251	169	94	

Suspected diphenyl 2-ethylhexyl phosphate (EHDP) was identified by software in two samples. A peak with matching mass spectra was present in a third. Suspected tris(1,3-

dichloro-2-propyl)phosphate peaks were found by software in three samples, with two additional unidentified peaks presenting with the same spectra at the same retention times. Molecular ion peaks could not be determined for either compound in any sample. These compounds are known to exist on aircraft (Schuchardt et al., 2019) and, while not suited for classification under the Schymanski et al. (2015) scale based on the findings of this work, should be sought with targeted methods when completing future quantitative work.

4.6 Conclusion

The results in this chapter suggest that bleed air-pressurized aircraft demonstrate an increased contaminant presence when compared to non-bleed air-pressurized aircraft. The non-targeted methods employed have proven to be effective, but will require refinement for ease of use with larger datasets. These findings, while considered preliminary or pilot scale, emphasize an exposure pathway that is unique to bleed-air pressurized aircraft, and as such, could create a unique occupational risk for the pilots, flight attendants, and passengers exposed. Larger scale sampling campaigns, including increased n-numbers of bleed-free and bleed-air pressurized aircraft types should be completed.

4.7 Acknowledgements

The author would like to acknowledge all citizen scientist sample collectors, as well as members of the Environmental Forensics and Arson Lab at Mount Royal University, James Walker, Emily Carroll, and Caleb Marx for their laboratory assistance.

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4.9 Supplementary Information

SI Table 1. Wipe sample collection information.

ID	Date of Collection	Date Storage	Aircraft Model	Seat Number	Seat Location	Flight Duration	Location Departure	Location Arrival
WS1	6/14/2019	6/24/2019	Airbus A320	2A	Window	4.5	PHX	RDV
WS2	6/15/2019	6/24/2019	Embraer E190	4A	Window	1.25	RDV	LGA
WS3	6/14/2019	6/24/2019	Boeing 757-200	1F	Window	5.5	HNL	PHX
WS4	6/12/2019	6/24/2019	Boeing 757-200	1A	Window	5.75	HNL	SLC
WS5	6/15/2019	6/24/2019	Boeing 777-200	34H	Aisle	4.5	YVR	YYZ
WS6	6/15/2019	6/24/2019	Boeing 777-200	34J	Middle	4.5	YVR	YYZ
WS7	6/13/2019	6/24/2019	Boeing 737-860/900	6A	Window	5.5	HNL	PDX
WS8	6/15/2019	6/24/2019	Bombardier Q400	14D	Window	1.75	YHZ	YYT
WS9	6/15/2019	6/24/2019	Embraer E170/175	4A	Window	1.5	LGA	YHZ
WS10	6/18/2019	6/24/2019	Boeing 777-300ER	42J	Middle	4	YVR	YYZ
WS11	6/17/2019	6/24/2019	Boeing 767-200ER	23E	Middle	5.5	HNL	YVR
WS12	6/13/2019	6/24/2019	Bombardier Q400	9D	Window	0.2	YVR	YCD
WS13	6/13/2019	6/24/2019	Airbus A321S	9B	Middle	5.5	HNL	LAX
WS14	6/13/2019	6/24/2019	Airbus A321S	18C	Aisle	5	HNL	LAX
WS15	6/13/2019	6/24/2019	Embraer 175	15D	Aisle	2	LAX	ABQ
WS16	6/18/2019	7/8/2019	Boeing 737-700	9C	Aisle	1.5	ABQ	MCI
WS17	6/13/2019	7/8/2019	Boeing 737-800/900	9C	Aisle	5.25	HNL	PDX
WS18	6/13/2019	7/8/2019	Boeing 737-800	8E	Middle	2.5	DFW	PIT
WS19	6/12/2019	7/8/2019	Boeing 777	32D	Aisle	5	HNL	LAX
WS20	6/22/2019	7/8/2019	Embraer 175	1F	Window	1.5	YHZ	PHL
WS21	6/22/2019	7/8/2019	Airbus A321	6F	Window	5	PHL	PHX
WS22	6/21/2019	7/8/2019	Bombardier Q400	11A	Window	2	YYT	YHZ
WS23	6/23/2019	7/8/2019	Boeing 747-400	30A	Window	9.5	PHX	LHR
WS24	6/30/2019	7/8/2019	Airbus A319	15A	Window	1.75	LHR	OSL
WS25	6/13/2019	7/8/2019	Embraer 175	9A	Window	2.75	LAX	OKC
WS26	7/9/2019	7/26/2019	Bombardier Q400	16D	Aisle	0.2	YVR	YCD
WS27	7/9/2019	7/26/2019	Bombardier Q400	16C	Aisle	0.2	YVR	YCD
WS28	7/9/2019	7/26/2019	Boeing 737-800	15B	Middle	2.33	SFO	YVR
WS29	7/9/2019	7/26/2019	Boeing 737-800	15A	Window	2.33	SFO	YVR
WS30	7/9/2019	7/26/2019	Bombardier Q400	16A	Window	0.2	YVR	YCD
WS31	7/9/2019	7/26/2019	Boeing 737-800/900	24A	Window	2	SFO	YVR
WS32	7/13/2019	7/26/2019	Boeing 777-300	36G	Aisle	9	HND	SFO
WS33	7/13/2019	7/26/2019	Boeing 737-800	12A	Window	0.5	SPN	GUM
WS34	7/13/2019	7/26/2019	Boeing 737-800	10A	Window	3.5	GUM	HND
WS35	7/6/2019	7/26/2019	Boeing 777-300	15H	Window	10	SFO	HND
WS36	7/8/2019	7/26/2019	Boeing 737-800	4E	Aisle	0.75	GUM	SPN
WS37	6/13/2019	6/17/2019	Boeing 737-800	7E	Middle	1	YVR	YYC
WS38	6/13/2019	6/17/2019	Boeing737-800	6E	Middle	1	YVR	YYC
WS39	6/9/2019	6/17/2019	Boeing 737-600	18F	Window	1.33	YCC	YVR
WS40	6/12/2019	6/17/2019	Boeing 737-800	12F	Window	1	YVR	YYC
WS41	6/9/2019	6/9/2019	Boeing 737-600/700	15F	Window	1	YYC	YVR
WS42	6/9/2019	6/9/2019	Boeing 737-600	14D	Aisle	1	YYC	YVR
WS43	6/9/2019	6/9/2019	Boeing 737-800	6D	Aisle	5.5	YVR	HNL
WS44	6/9/2019	6/9/2019	Boeing 737-600	14E	Middle	1	YYC	YVR
WS45	6/9/2019	6/9/2019	Boeing 737-800	6E	Middle	5.5	YVR	HNL
WS46	6/13/2019	6/17/2019	Boeing 737-800	18B	Middle	5.75	HNL	YVR
WS47	6/13/2019	6/17/2019	Boeing 737-800	18A	Window	5.75	HNL	YVR
WS48	6/16/2019	6/17/2019	Boeing 737-800	6C	Aisle	5.5	HNL	YVR
WS49	6/17/2019	6/17/2019	Boeing 737-600	5F	Window	1	YVR	YYC
WS50	6/16/2019	6/17/2019	Boeing 737-800	18B	Middle	5.5	HNL	YVR
WS51	6/16/2019	6/17/2019	Boeing 737-800	18B	Middle	5.5	HNL	YVR
WS52	6/16/2019	6/17/2019	Boeing 737-800	18A	Window	5.5	HNL	YVR
WS53	6/16/2019	6/17/2019	Boeing 737-800	18A	Window	5.5	HNL	YVR
WS54	6/17/2019	6/17/2019	Boeing 737-600	5D	Aisle	1	YVR	YYC
WS55	6/17/2019	6/17/2019	Boeing 737-600	5D	Aisle	1	YVR	YYC
WS56	6/17/2019	6/17/2019	Boeing 737-600	5E	Middle	1	YVR	YYC
WS57	6/17/2019	6/17/2019	Boeing 737-600	5E	Middle	1	YVR	YYC
WS58	6/17/2019	6/17/2019	Boeing 737-600	Bathroom	Bathroom	1	YVR	YYC
WS59	6/27/2019	7/2/2019	Bombardier Q400	11C	Aisle	1.5	YQU	YYC
WS60	6/7/2019	6/8/2019	Boeing 767-300ER	18E	Aisle	5.5	LAX	HNL
WS61	6/6/2019	6/8/2019	Boeing 737-600/700	15D	Window	1.33	YYC	YVR
WS62	6/14/2019	6/15/2019	Boeing 737-800	9B	Middle	5.5	HNL	YVR
WS63	6/14/2019	6/15/2019	Boeing 737-800	9C	Aisle	5.5	HNL	YVR
WS64	6/14/2019	6/15/2019	Boeing 737-800	9A	Window	5.5	HNL	YVR
WS65	6/15/2019	6/15/2019	Boeing 737-600/700	9E	Middle	1	YVR	YYC
WS66	6/15/2019	6/15/2019	Boeing 737-600/700	9F	Window	1	YVR	YYC
WS67	6/15/2019	6/15/2019	Boeing 737-600/700	9E	Middle	1	YVR	YYC
WS68	8/16/2019	8/26/2019	Boeing 787-9	41E	Middle	8.5	YYC	LHR
WS69	8/16/2019	8/26/2019	Boeing 787-9	41E	Middle	8.5	YYC	LHR
WS70	8/16/2019	8/26/2019	Boeing 787-9	41G	Aisle	8.5	YYC	LHR
WS71	8/16/2019	8/26/2019	Boeing 787-9	41G	Aisle	8.5	YYC	LHR
WS72	8/16/2019	8/26/2019	Boeing 787-9	41G	Aisle	8.5	YYC	LHR
WS73	8/24/2019	8/26/2019	Boeing 787-9	40D	Aisle	8.5	LHR	YYC
WS74	8/24/2018	8/26/2019	Boeing 787-9	40D	Aisle	8.5	LHR	YYC
WS75	8/24/2019	8/26/2019	Boeing 787-9	Bathroom	Bathroom	8.5	LHR	YYC
WS76	8/24/2019	8/26/2019	Boeing 787-9	40C	Aisle	8.5	LHR	YYC
WS77	8/24/2019	8/26/2019	Boeing 787-9	40C	Aisle	8.5	LHR	YYC
WS78	9/19/2019	9/27/2019	Boeing 737-700	22A	Window	4	AUS	YYC
WS79	9/19/2019	9/27/2019	Boeing 737-700	22B	Middle	4	AUS	YYC
WS80	9/12/2019	9/27/2019	Boeing 737-800	5D	Aisle	4	YYC	AUS
WS81	9/12/2019	9/27/2019	Boeing 737-800	5E	Middle	4	YYC	AUS
WS82	9/12/2019	9/27/2019	Boeing 737-800	25D	Aisle	4	YYC	AUS
WS83	9/15/2019	9/27/2019	Boeing 737-800	26C	Aisle	4	YYC	AUS
WS84	9/19/2019	9/27/2019	Boeing 737-700	4E	Middle	4	AUS	YYC
WS85	9/19/2019	9/27/2019	Boeing 737-700	4D	Aisle	4	AUS	YYC
WS86	12/14/2019	1/7/2020	Embraer 175	16D	Window	3	YYC	ORD
WS87	12/14/2019	1/7/2020	Embraer 175	16D	Window	3	YYC	ORD
WS88	12/14/2019	1/7/2020	Embraer 175	16D	Window	3	YYC	ORD
WS89	12/14/2019	1/7/2020	Airbus A330-200	25D	Aisle	7	ORD	DUB
WS90	1/4/2020	1/7/2020	Boeing 757	7F	Window	7	DUB	YYZ
WS91	1/4/2020	1/7/2020	Boeing 757	7F	Window	7	DUB	YYZ
WS92	1/4/2020	1/7/2020	Airbus A321-200	35D	Aisle	4	YYZ	YYC
WS93	2/6/2020	2/13/2020	Boeing 737-700	23B	Middle	3	YYC	PHX
WS94	2/11/2020	2/13/2020	Boeing 737-800	23C	Aisle	3	PHX	YYC
WS95	2/23/2020	3/3/2020	Boeing 787-9	Bathroom	Bathroom	9	YYC	AMS
WS96	2/25/2020	3/3/2020	Embraer 190	24B	Aisle	1	AMS	MAN
WS97	2/29/2020	3/3/2020	Boeing 737-800	32F	Aisle	1	MAN	AMS
WS98	2/29/2020	3/3/2020	Boeing 787-9	38C	Aisle	9	AMS	MAN

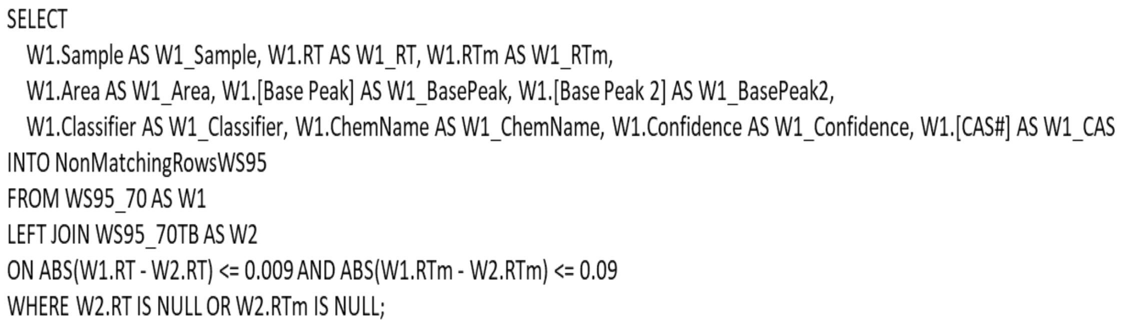
The ultimate result of the sampling campaign is 98 wipe samples with matching trip blanks from 62 unique flights. Ninety-one samples were taken on jet aircraft and seven on turboprop aircraft. The largest share of samples were taken on flights that were short in duration (0-3 hrs: 44.9%). Moderate-length flights from 3-6 hrs in duration were the next most abundant (36.7%), and long flights greater than 6 hours made up the least (18.4%) of all samples (SI Table 1.).

SI- SQL database data handling detail.

Software-generated peak tables of two-dimensional chromatographic data were extracted for each sample at both ionization energies and the trip blank for the respective sample at 70eV. The extracted files were opened in Microsoft Excel and modified to be suitable for import into Microsoft Access. Data categories maintained to Access included sample name and ionization energy, first and second dimension retention times, peak area, the base mass spectra ion peak, the second most abundant mass spectra ion peak, classifier ion peak, the predicted NIST library match name, the confidence of that match, and the predicted CAS#. Each amended peak table was placed in an individual table in Access to allow for comparisons between tables.

Several queries were then completed within the database:

In an effort to determine what peaks were present within samples but not within their respective trip blanks, the following query in SQL was developed with the assistance of ChatGPT (OpenAI).

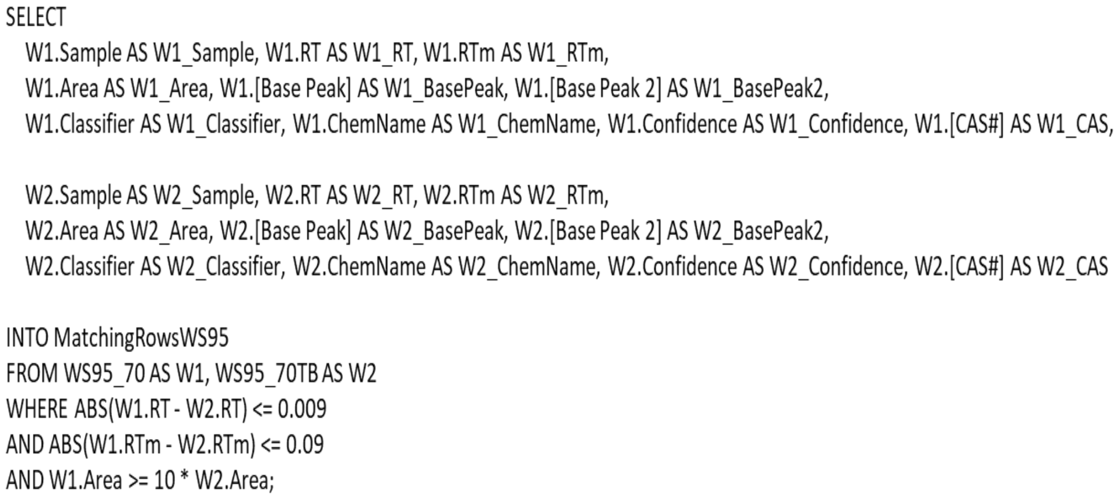


```
SELECT
   W1.Sample AS W1_Sample, W1.RT AS W1_RT, W1.RTm AS W1_RTm,
   W1.Area AS W1_Area, W1.[Base Peak] AS W1_BasePeak, W1.[Base Peak 2] AS W1_BasePeak2,
   W1.Classifier AS W1_Classifier, W1.ChemName AS W1_ChemName, W1.Confidence AS W1_Confidence, W1.[CAS#] AS W1_CAS
INTO NonMatchingRowsWS95
FROM WS95_70 AS W1
LEFT JOIN WS95_70TB AS W2
ON ABS(W1.RT - W2.RT) <= 0.009 AND ABS(W1.RTm - W2.RTm) <= 0.09
WHERE  W2.RT IS NULL OR W2.RTm IS NULL;
```

This query attempts to find matching peak retention time windows, within a small margin of error, between the sample (in this case, WS95 at 70eV ionization energy) and its matching trip blank. The error allowed in retention time difference is ±0.009 minutes in the first dimension and ±0.09 seconds in the second. This allows for a small amount of drift in both dimensions between samples, or error in instrument triggering timing, etc., but is sufficiently small enough that it is unlikely to identify a different peak within the window. Visual confirmation of chromatogram peaks is still required to confirm peak quality, identity, and spectral data. The query then identifies which rows do not meet this matching condition within the sample table and prints them in a new table (in this case, NonMatchingRowsWS95).

In addition to unmatched data, and due to the chemical complexity of our wipe matrix, it is necessary to determine what potential compounds are enriched in our samples compared to their respective trip blanks. A query was introduced to the database tables, which identified rows in the sample table and compared both dimension retention times in the same fashion and with the same RT error in both dimensions as the unmatched query. When matching rows were found, the code then compares the peak area of both peaks, and if the peak in the sample is ten times greater than that of the trip blank, it will print the row in a new "matched" table.

The query is as follows:

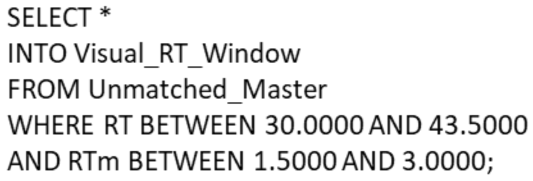


```
SELECT
  W1.Sample AS W1_Sample, W1.RT AS W1_RT, W1.RTm AS W1_RTm,
  W1.Area AS W1_Area, W1.[Base Peak] AS W1_BasePeak, W1.[Base Peak 2] AS W1_BasePeak2,
  W1.Classifier AS W1_Classifier, W1.ChemName AS W1_ChemName, W1.Confidence AS W1_Confidence, W1.[CAS#] AS W1_CAS,

  W2.Sample AS W2_Sample, W2.RT AS W2_RT, W2.RTm AS W2_RTm,
  W2.Area AS W2_Area, W2.[Base Peak] AS W2_BasePeak, W2.[Base Peak 2] AS W2_BasePeak2,
  W2.Classifier AS W2_Classifier, W2.ChemName AS W2_ChemName, W2.Confidence AS W2_Confidence, W2.[CAS#] AS W2_CAS

INTO MatchingRowsWS95
FROM WS95_70 AS W1, WS95_70TB AS W2
WHERE ABS(W1.RT - W2.RT) <= 0.009
AND ABS(W1.RTm - W2.RTm) <= 0.09
AND W1.Area >= 10 * W2.Area;
```

Following the creation of unmatched and matched tables for each of the wipe sample and trip-blanks matched pairs, the lists were combined into matched and unmatched master lists.

Retention Time Window

A visual assessment of all 70eV samples and trip blank chromatograms was completed. A "window" was selected, which demonstrated a large number of peaks in the active samples and less in the trip blanks. The window encompasses retention times on the chromatogram from 30.0000 to 43.5000 minutes in the first dimension and 1.5000 and 3.0000 seconds in the second dimension. The following query was used to create the window and collect the relevant rows from all samples:



```
SELECT *
INTO Visual_RT_Window
FROM Unmatched_Master
WHERE RT BETWEEN 30.0000 AND 43.5000
AND RTm BETWEEN 1.5000 AND 3.0000;
```

This queries all unmatched peaks from the unmatched master lists and collects those that fit within the window into a new table. By sorting from the unmatched master list, confidence is increased that the peaks found do not have a corresponding peak within the trip blank. However, a visual inspection is required for confirmation.

5.0 CONCLUSIONS AND FUTURE WORK

5.1 Overall aims of the thesis

The overarching aims of this work were to better understand what chemical exposures individuals working on aircraft are subject to, the implications of these exposures from an occupational health and safety perspective, and whether these exposures can be associated with pathways from aircraft systems to human receptors. In this thesis, these aims have been thoroughly addressed. As previously described, the aircraft environment is exceptionally complex. This thesis has helped identify multiple contaminants of concern previously unknown to exist in the aircraft cabin, identified sources to receptor pathways for both known and unknown contaminants of concern, and filled several gaps in knowledge identified in the extant published literature.

The chapters proceeding provide new knowledge which can be used to more holistically assess and mitigate occupational exposure to contaminants of concern in aviation. The following conclusion emphasizes each chapter's importance and contributions to the field.

5.2 Conclusions

The purpose of **Chapter 1** of this thesis was to systematically assess the literature regarding aircraft contamination, identify knowledge gaps, and determine if aircraft crews face increased and unaddressed occupational risk due to their professions. The successfully completed systematic review characterized the aircraft cabin exposome as detailed by published academic literature, describing chemical contaminants of concern, as well as potential confounding factors that are innately associated with flight at altitude. Cohort and biomonitoring studies demonstrated a health risk associated with aviation. However, actual measurements of individual chemical contaminants on board could not be definitively correlated to the symptomology of the affected aircrew. Nevertheless, the significant exposure risk associated with fume events, the health impacts as described by case and cohort studies, and the unknown implications of chronic low-dose exposure to contaminants of concern or exposure to a synergistic mixture of these contaminants warranted a conclusion of realized occupational risk.

This review is limited in that it was largely restricted to publicly available peer-reviewed literature. It is understood that the data in this review is not entirely complete, with a great deal of information that could be found in grey literature or information that is publicly unavailable due to prior or ongoing court cases. There is also information that has been described with conflicting results. These are fundamental challenges in the field that the review does not speak to, such as the discrepancies in the number of fume events that occur per number of flights. In these cases, we use the most recent published and publicly available data. The nature of a systematic review has also restricted the papers available to those in the databases examined and capable of being found by the search terms selected by the author.

Multiple gaps in knowledge were identified in the process of completing this systematic review, and some of these deficiencies in the literature were subsequently experimentally assessed and included as chapters two through four within this manuscript:

The pathway from the engines to the aircraft via the pneumatic system was previously established, but quantified losses of the principal contaminant of concern (tricresyl phosphate) from the source engine oil with use had not been determined. In an effort to better explain this process, the elemental analysis of new and used engine oils was completed (**Chapter 2**)**.** This experiment allowed us to look at aircraft engine oil from aircraft types that would typically be used during pilot training and recreationally, providing some additional insight into potential historical or non-occupational exposures to contaminants of concern. The major findings of the chapter describe that TCP as proxied by phosphorus concentrations in jet and turboprop engines is lost from aircraft engine oil with use in excess of the attrition of oil. While speciation in this experiment was not possible, the known practice of topping up jet aircraft with oil, as opposed to fully draining and refilling with new oil, magnifies this loss of phosphate-containing compounds. Additionally, phosphorus and lead were found in the used oil of piston engine aircraft and were absent from the new. The phosphorus has been tentatively linked to organophosphate fuel or oil additives and may constitute an exposure risk to pilots and technicians working with the aircraft. The lead (average concentration 5.77g kg^{-1}) in the oil has sourced to the tetraethyl lead present in the aviation fuel (100LL) and is concentrated several times (avg = 7.3: 1, lead in oil to AVGAS), which represents another unaddressed occupational risk.

Jet aircraft pneumatic systems were described in the literature as having multiple functions on aircraft. Cabin pressurization was chief among these and is related to human exposure risk. However, other systems pneumatically pressurized, such as the potable water system, were identified as potentially providing another exposure pathway from source to human receptor (**Chapter 3**). Chemical contamination of the water present on aircraft has not been previously assessed. This is largely because the water that is brought on board the aircraft is previously treated, and as such, at the water quality standard of the airport's municipality. This, however, neglects the possibility for contamination of the water via the interconnected components of aircraft pneumatic systems. A suspect screening detected tributyl phosphate (aircraft hydraulic fluid) in more than half of the aircraft water samples taken when compared to airport water. One sample of which demonstrated potential "leak" conditions with a substantial peak area of 2.9 times the limit of quantification. Additionally, other organophosphates (triphenyl phosphate (TPhP), tris(butoxy ethyl) phosphate (TBEP), tris(chloropropyl) phosphate (TCPP), and triethyl phosphate (TEP)), both known, and previously unknown to exist in the aircraft environment were tentatively identified in the potable water. This confirms the potable water on board aircraft as another potential exposure pathway for contaminants of concern which may have implications for both occupational and public health.

To make a further determination of the aircraft pneumatic system and engines' potential importance as a source of cabin contamination, a comparison was made between bleed air-pressurized aircraft and non-bleed air-pressurized aircraft (**Chapter 4**). Hypothetically, a bleed air-pressurized aircraft should have comparatively more *engine-specific* contamination if the pathway is present. The current, very limited literature which had made this comparison disagreed with this reasoning. To better test this hypothesis and to more fully describe the aircraft exposome, a quasi-non-targeted assessment of wipe samples of multiple aircraft types was conducted utilizing multidimensional gas chromatography. There is a definitive need for non-targeted assessments to be conducted in this field. Previous research is largely targeted and focused on a very small number of compounds which may be discounting compounds partially responsible for symptomologies of aircrew. While our assessment is somewhat targeted based upon our choices of sampling strategy, it is the first non-targeted assessment of the aircraft cabin not restricted to a particular compound class. The findings of the assessment demonstrated a greater number of statistically significant chemical components in bleed air-pressurized aircraft that were higher in concentration or unique. This trend was evident across two aircraft manufactures and four individual aircraft families. Chromatographic and mass spectral data, coupled with a literature search of known aircraft oil degradation products, allowed for the tentative identification of compounds (short-chain organic acids) that were unique to the bleed air-pressurized aircraft in the assessment. This finding strongly suggests that additional contamination of the aircraft cabin is apparent when bleed air is used to pressurize the aircraft cabin, further emphasizing the importance of this pathway.

5.3 Future Work

During the research process, a number of important “next steps” were uncovered and required to be shelved due to the scope of the thesis's work. While some of this work may be left to other researchers, two aspects will be further assessed in the near future.

First, a continuation of the quasi-non-targeted assessment of wipe samples will continue. The subset of data presented in chapter 4 demonstrates the proof of concept of the method; the work performed in the chapter was an important first step to identify a difference in bleed air and bleed free aircraft. An additional 78 samples have been collected from a wider range of different aircraft types and these will be assessed to better elucidate the factors that drive in cabin air quality in different aircraft. Ideally, upon completion of the non-targeted assessment, the compounds tentatively identified and determined to be potentially relevant in terms of occupational exposure and risk will be confirmed in the samples with analytical standards. Additionally, the further exploration of this data should allow us to determine other trends as more assessment of additional variables can be made. Examples include comparisons between short and long-haul aircraft, individual aircraft types between each other, and aircraft inside of type. This work will also allow for the refinement of the SQL database as a data management tool for non-targeted assessment, which will then be applied to future research.

Second, the findings of the potable water contamination on aircraft require additional and more exhaustive research. Our research was an important first step to validating the existence of a pollutant pathway from oils and hydraulic fluids to potable water onboard aircraft. It identified several potentially toxic marker compounds that indicate the presence of these fluids within drinking water. These identified compounds require further complimentary analysis using targeted methods to understand the potential human health risks. Opportunistic sample collection will begin shortly for a targeted screening of compounds found and suspected to have been found in the aircraft water. This assessment will ideally collect a greater number of samples from aircraft and will quantitatively assess the suspect compounds using isotopically labeled standards. This will allow for an improved description of the potential occupational risk. The previous screening did not sample films that had developed on the water surface of some of the collected samples in an effort to more representatively describe the “normal” condition of the potable water. It is understood that these films may have contained a higher concentration of contaminants of concern, and future analysis will include additional assessment of the films to account for this variability.

Further dissemination of the research completed is also planned. All submitted but not yet published papers presented in this thesis will be carried through the peer review process until their publication and the results will be presented and discussed at future conferences.

5.4 Summary statement

Ideally, the work contained within this thesis will aid regulators in making more informed decisions regarding the occupational risk of chemical exposure in aviation and ultimately allow them to design policy or require the placement of mitigative measures on aircraft to limit this risk. As outlined within our systematic review, it is apparent, in both anecdotal and cohort assessments, that pilots and flight attendants are experiencing ill health as a result of their occupations. This work describes multiple new pathways in which these workers could be exposed to contaminants of concern that may be wholly or partially responsible for this occupational injury. This includes historical exposures during pilot training and piston engine aircraft maintenance and potable water consumption on pressurized aircraft; both are previously unknown or not considered in the published literature but are demonstrably viable routes of exposure. Additionally, it seems clear, based on the findings of this thesis, that bleed air pressurization of the cabin does increase chemical loadings within the cabin. This contradicts previously published data and should emphasize that mitigation measures (filters, catalysis, monitoring, etc.) should be employed on this pathway. While further assessment of the information provided in this thesis is required, applying precautionary steps to prevent further unnecessary exposure seems prudent.