A comparison of fresh and used aircraft oil for the identification of toxic substances linked to aerotoxic syndrome.

David Megson [1], Xavier Ortiz [2] Karl J. Jobst [2], Eric J. Reiner [1,2], Michel F.A. Mulder [3], Jean-Christophe Balouet [4]

1 Department of Chemistry, University of Toronto, Toronto, ON, Canada

2 Ontario Ministry of the Environment and Climate Change, 125 Resources Road, Canada

3 Aviation Medical Consult, Karbouwstraat 14, 1402 VC Bussum, The Netherlands

4 Environnement International, Orrouy, France,

Abstract

Fresh and used aircraft engine lubricants (Mobil Jet Oil II) were analysed using a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICRMS) and comprehensive two dimensional gas chromatography with high resolution time of flight mass spectrometry (GCxGC-HRTOFMS). The composition of the fresh oil was established, with special focus to its tricresyl phosphate (TCP) content as this has formed the focus for most investigations into aerotoxic syndrome. The results showed that only four TCP isomers were present at detectable levels in the fresh oil: mmm-TCP, mmp-TCP, ppm-TCP

and ppp-TCP. The results indicate that the formulation of Mobile Jet Oil II does not contain the more toxic ortho substituted TCP isomers at concentrations above 0.0005%. The temperatures of jet engines during operation are greater than 200°C which creates the potential to alter the composition of the original oil and create other toxic compounds. The results show there may be a significant risk from alkylated cresyl phosphates, which were identified in the used oils at concentrations calculated in the range of 0.13 to 0.69%. w/w. Several xylenyl and ethylphenyl phosphates have been shown to exhibit a similar toxicity to ortho substituted TCP isomers which makes there discovery in used oil significant. These compounds should be included in future aircraft air quality studies and when assessing the risks and causes of aerotoxic syndrome.

Key words

Aerotoxic, multidimensional chromatography, high resolution mass spectrometry, cresyl phosphates, organophosphates

1. Introduction

Due to their widespread use as pesticides, plasticizers and flame retardants organophosphates are routinely detected in environmental samples. However, a specific concern has arisen in recent decades as aircraft crew have developed symptoms consistent with exposure to toxic fumes and organophosphates (Abou-Donia et al. 2013; Harrison and Mackenzie Ross, 2016; Liyasova et al. 2011; Payne, 2015). There have been reports of headaches, loss of balance, numbness and neurobehavioral abnormalities such as emotional instability, depression and cognitive dysfunction, including impaired short term memory, blurred vision and speech, altered coordination (de Ree et al. 2014; Abou-Donia et al. 2013). Organophosphates are not just used as pesticides on crops in fields but are also used in

aircraft as flame retardants, in engine oil and hydraulic fluids, and on material surfaces. This has resulted in their link to aerotoxic syndrome by Winder and Balouet (2002). The term Aerotoxic Syndrome was first published in 1999 by an international scientific team to describe the symptoms and exposure conditions reported by aircraft crew from Australia, US, Europe. Whilst aerotoxic syndrome has not been fully accepted as a medical syndrome (Wolkoff et al., 2016) it is commonly used to refer to air quality in aircraft and the associated exposure of crew and passengers to toxic compounds. Aerotoxic syndrome is understood to be caused by long term and repeated exposure to chemicals released from smoke and fume events in aircraft.

During a flight the air cabin pressure and temperature are maintained with outside air that is passed through the jet engine. Cabin air is recycled for 50%, whereas the flight deck air is comprised in most aircraft types of a continuous stream of bleed air (de Boer et al. 2015). Engine seals in use are known as "wet-seals", an inherent design feature, whereby a thin film of oil prevents rotating surfaces to come into mechanical contact with each other. Pressure differentials over the seals cause a constant loss of oil and vapours into the core engine. These enter the inlet of the high pressure compressor and contaminate the bleed air which is taken downstream. Oil leaks can result in odd smells in the cabin and in more extreme cases smoke events. The tricresylphosphate chemical fingerprint from wipe samples taken from a cockpit showed a statistically significant correlation (p value 0.039) with used engine oil (Hourzager et al. 2013), indicating that this can be a significant source of exposure. Estimates of how often these events occur varies depending upon whether the information is sourced from regulatory authorities such as the UK Civil Aviation Authority (CAA), from airlines or from trade unions who represent aircrew (Harrison and Mackenzie Ross, 2016). Reported values for the frequency of smoke/fume events include 0.5% of flights (Murawski and Supplee, 2008), 0.05% (COT, 2007) and 0.02% (Shehadi et al., 2015). Lubricating oil is applied to gas turbines in aeroplanes as anti-wear agents. However, there have been several studies that have identified subchronic neurotoxicity of aviation oils

(Freudenthal et al. 1993; Daughtrey et al. 1996; Mackerer et al. 1999). Aircraft lubricating oils have remained relatively unchanged since the 1960s and are comprised of approximately 95% synthetic esters with 3% tri-cresyl phosphates and 1% phenyl-α-naphthalamines (Winder and Balouet, 2002). Tri-cresyl phosphates (TCPs) are a group of organophosphates which contain 10 structural isomers. The ortho substituted congeners are considered to be the most toxic and therefore the proportions of these compounds in oil have been reduced in recent decades (Craig and Barth. 1999). Most of the focus of investigations in aircraft air quality and aerotoxic syndrome has been focused on tri-ortho-cresyl phosphate (ooo-TCP or ToCP), however the mono-ortho and di-ortho isomers are also highly toxic with omp-TCP the most toxic (de Boer et al. 2015; De Nola et al., 2011).

There is evidence to indicate that aircraft crew and passengers have been exposed to organophosphates from traveling on aeroplanes and that it has resulted in neurotoxic effects (Abou-Donia et al. 2013; Liyasova et al. 2011). Abou-Donia et al. (2013) undertook a study of 34 flight crew members and the results suggest the possible development of neuronal injury and gliosis in flight crew members anecdotally exposed to cabin air emissions containing organophosphates. A symptom-free pilot was sampled before symptoms and then again afterward. This pilot developed clinical problems after flying for 45 hours in 10 days. Significant increases in autoantibodies were noted to most of the tested proteins in the serum of this pilot after exposure to air emissions. The levels of autoantibodies rose with worsening of his condition compared to the serum sample collected prior to exposure. After cessation of flying for a year, this pilot's clinical condition improved, and his serum autoantibodies against nervous system proteins decreased. Many crew members who have reported conditions consistent with organophosphate poisoning recover and return to their flight duties, however some staff have lost their jobs, and several have even passed away. When individuals are grounded they are no longer exposed to aircraft air their reported symptoms can gradually reduce. The underlying mechanism that caused the ill health may be an active auto immune reaction, set into motion by repeated low dose exposure to

organophosphates, causing actual damage to the Blood-Brain-Barrier and apoptosis inside the brain. Protein filaments of these decaying cells are able to re-enter the bloodstream, causing a secondary auto immune response. Memory B cells can recognise certain antigens for a long duration of time, comparable to a status after vaccination and can lead in rare instances to an anaphylactic shock (Banks et al. 2012), also known as “incapacitation”, or sudden heart failure due to lymphocytic myocarditis. UK Coroner Payne, recently issued a report to prevent further deaths following the death of a pilot, which was linked to OP poisoning (Abou-Donia et al., 2014; Payne, 2015). The post mortem investigations gave the cause of death of either pentobarbital toxicity or lymphocytic myocarditis, individually or in combination. Hair analysis had shown the use of pentobarbital during the 4 weeks prior to his death, as a form of self medication against severe headaches. Pentobarbital is not known for causing the widespread infiltration of T-lymphocytes in the brain, heart and neurological damage otherwise observed. Testing of samples taken both prior to and after death disclosed symptoms consistent with exposure to organophosphate compounds.

Due to the sporadic occurrence of smoke/fume incidents it is particularly difficult to obtain worst case air samples for analysis. No fume events were observed on any of the flights that were monitored by Crump et al., (2011) and de Ree et al., (2014). Although this is hardly surprising as they only involved analysis on 100 and 20 flights respectively. Using the estimates of the frequency of fume events from Murawski and Supplee, (2008), and Shehadi et al., (2015) a sample size of between 200 and 5000 flights would be required to identify one event. Interestingly nine smoke/odour events were recorded in 78 samples taken by De Nola (2011). de Boer et al. (2015) used data from De Nola (2011) and Craig and Barth (1999) to calculate that even using worst-case scenarios they cannot explain a relation of TCP in flight deck air to the complaints of pilots and air crew, a similar conclusion was also reached by de Ree et al. (2014) and Schindler et al. (2013). Available data was reviewed by Ramsden (2013) who used jet oil consumption as a surrogate to measure chemical contamination in aircraft cabin air. Those results show

112 that the oil concentration in a fume event, in which visible smoke appears in the cabin, was estimated at
113 50 mg/m^3. The concentration of TCP was approximately 1.5 mg/m^3.and using the ratio of ToCP to total
114 TCP from Crump et al. (2011) resulted in a ToCP concentration of 0.5 mg/m^3, which exceeded the short-
115 term workplace exposure limit (15 minute reference period). The variability in these studies highlights
116 the uncertainty in recording and reporting methodologies but also suggests that other possible
117 explanations for the reported symptoms must be considered as the effects may not be due to TCP or
118 ooo-TCP alone. The temperatures of jet engines during operation can vary, the oil may be heated to
119 several hundred °C (Ramsden, 2013), although some parts of the engine (e.g., the combustion chamber)
120 can get much hotter and exceed temperatures of 400°C. These temperatures have the potential to alter
121 the composition of the original oil and create other toxic compounds such as trimethylolpropane
122 phosphate (Winder and Balouet, 2002). Pyrolysis of the oil also has the potential to pose a health risk
123 due to the generation of toxic asphyxiants such as carbon monoxide and hydrogen cyanide (Winder and
124 Balouet, 2002). There is currently a large degree of uncertainty as to what compounds are produced and
125 how toxic they are through inhalation in the vapour phase at high altitudes (de Boer et al. 2015).

126 In this study samples of fresh and used aircraft oil were analysed by Fourier Transform Ion Cyclotron
127 Resonance Mass Spectrometry (FTICRMS) and comprehensive two dimensional gas chromatography
128 with high resolution mass spectrometry (GCxGC-HRTOFMS), to characterise the composition of the oil
129 and identify potentially toxic products that may be generated during use. Particular focus was given to
130 organophosphates as these are the group of compounds most closely linked to aerotoxic syndrome.

131

132 2. Methodology

133 Three 10 mL samples of fresh and used Mobil II Jet oil were obtained from a maintenance facility of
134 Falcon business jets in Europe. The samples were prepared for analysis by FTICRMS by diluting the oil by

a factor of 1:1000 with toluene. For analysis by GCxGC- HRTOFMS the FTICRMS samples were further diluted by a factor of 1:10 and 1:100. These samples were spiked with $^{13}C_{18}$ labeled triphenyl phosphate (TPhP) for quantification (obtained from Wellington Laboratories).

FTICRMS analysis was performed using a Varian FTICRMS (Varian Inc., Walnut Creek, CA), consisting of a 9.4 T superconducting magnet. Samples were directly infused at a rate of 5 µL/min and ionised using atmospheric pressure photo ionisation (APPI). Mass spectra were obtained using arbitrary waveform excitation (90 v) and broadband detection from m/z 150 to 1000 with a transient length of 2 seconds. The FTICRMS was operated at a resolving power of 300,000 (fwhm) at 368 m/z. Mass accuracy was less than one ppm, achieved by internal mass calibration using the Agilent ESI calibration mix, which was added to the sample at a 1:1 ratio before infusion.

GCxGC-HRTOFMS analysis was performed using a Waters Xevo G2-XS QToF fitted with a 40 m x 0.18 mm x 0.18 µm DB-5 HT GC column in the first dimension (^{1}D) and 0.8 m x 0.15 mm x 0.15 µm Rxi17 in the second dimension (^{2}D), this was then connected to a 0.8 m x 1.8 mm Custom MXT tubing (sulfinert treated). The injector temperature was set at 280 ^{o}C, the initial oven temperature was held at 70 ^{o}C for three minutes then ramped at 10^{o}C a minute to 220 ^{o}C, then 2.5 ^{o}C a minute to 300 ^{o}C and held for 5 minutes. The secondary oven was set at a 40 ^{o}C offset to the primary oven. The modulation period was set at 4 seconds with a hot pulse duration of 0.4 s and the transfer line temperature at 360 ^{o}C. The corona voltage was set at 5 µA, the cone gas at a flow rate of 175 L/hr and auxiliary gas flow set at 100 L/hr. Ionisation was undertaken using an atmospheric pressure chemical ionisation source at 150 ^{o}C with the detector run in TOF mode using a scan window of 50 amu to 1200 amu with a scan time of 0.04 (+ 0.015 interscan delay) seconds. The GCxGC-HRTOFMS was operated at a resolving power of >20,000 (fwhm), internal mass calibration was performed by using a lock mass ion (355.0699) generated from a siloxane. Limits of detection for tri-cresyl phosphates were calculated by serial dilution of the calibration solutions, the lowest concentration detected with a S:N >10 was 0.0005%)

3. Results and discussion

3.1 FTICRMS analysis

Three fresh oil and three used oil samples were analysed using the FTICRMS and compared. This was undertaken to identify the bulk compositions of the oil and identify potential differences between the fresh and used oil, based on exact mass elemental composition assignments. The data was interpreted by investigating the mass spectra and filtering the data using a Kendrick Mass defect plot, which was pioneered by Kendrick (1963) and Hughey (2001). These are created by converting the International Union of Pure and Applied Chemistry (IUPAC) mass scale (C = 12.000 Da) to one in which CH_2 = 14.000 Da by using the following equation.

Equation 1. Kendrick mass = IUPAC mass x (14/14.01565),

The exact mass is plotted against its mass defect (exact mass minus nominal mass). Using the Kendrick mass scale gives CH_2 an exact mass of 14.0000, thus aligning series of hydrocarbons.

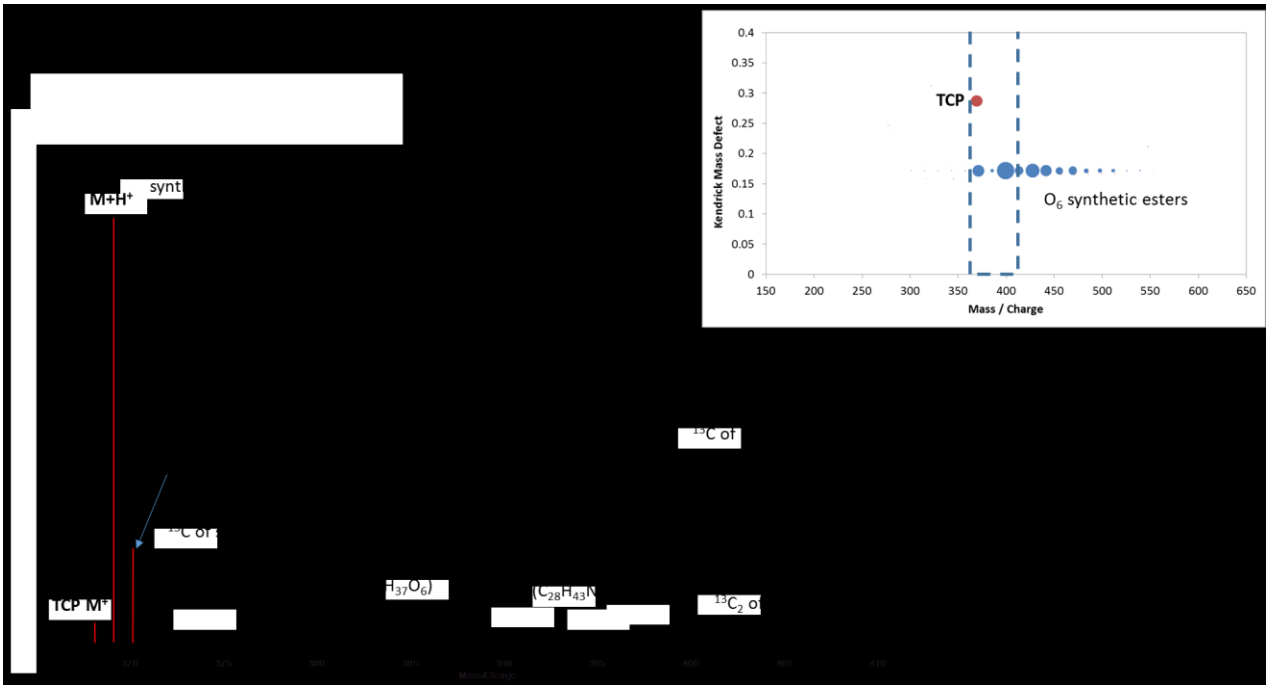
The results show that both oils were comprised of predominantly oxygen containing synthetic esters, with the O_6 series being the most abundant. The data was filtered to identify potential compounds with a PO_4 group. In both the used and the fresh oil TCP ($C_{21}H_{21}PO_4$) could be clearly identified with a mass accuracy of less than 1 ppm (Figure 1). Three ions are displayed for TCP in the plot, these represent the molecular ion of TCP, the TCP + H^+ adduct formed in the APPI source and a ^{13}C TCP + H^+ adduct.

As well as TCP, three PO_4 containing compounds were consistently identified in the three used oil samples but were not identified in any of the fresh oil samples. The molecular formula for these compounds corresponded to $C_{22}H_{23}PO_4$, $C_{23}H_{25}PO_4$, and $C_{24}H_{27}PO_4$, with a mass accuracy of 1 ppm indicating that these compounds are related to TCP but with the addition of a methyl group on one or all

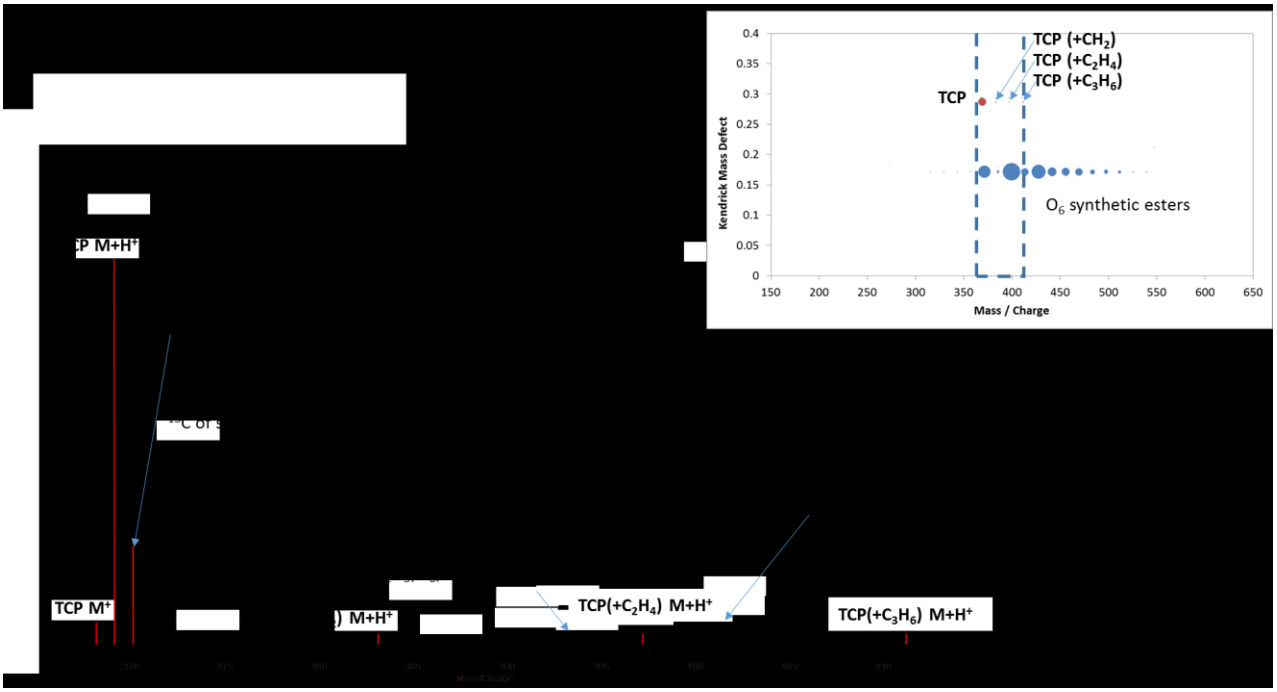
three of the cresyls. The structures are therefore hypothesised as monoxylenyl dicresyl phosphate, dixylenyl dicresyl phosphate and trixylenyl phosphate as xylenyl cresyl phosphates have been previously identified as potential contaminants in TCP solutions by Winder and Balouet (2002). Recently revised versions of the safety data sheets of some widely-used aviation engine oils also now report 0.1-1% trixylenyl phosphate (TXP) content (Exxon-Mobil, 2013). However, without the use of analytical standards we were unable to confirm that they are not another alkylated compound with the same molecular formula such as ethyl phenyl phosphates.

The discovery of alkylated cresyl phosphates in aircraft oil is a significant finding as the mono and di ortho ethyl phenyl phosphates and xylenyl phosphates have displayed a similar toxicity to ortho substituted TCP isomers (Bondey et al. 1960; Winder and Balouet 2002). Like TCP the ethyl phenyl phosphates and xylenyl phosphates have a toxicity that is position specific and so it is important to understand exactly which compounds are present. The analysis performed by FTICRMS was via direct infusion and whilst it provides an excellent mass accuracy it was not possible to identify how many different structural isomers were present. Therefore, further analysis was undertaken using GCxGC-HRTOFMS to identify the different TCP isomers and quantify the concentrations of TCP and the other alkylated cresyl phosphates detected in the used oil.

199



200



201 **Figure 1**. Kendrick mass defect plot and selected mass spectra (M/Z 365 to 413- represented by the blue

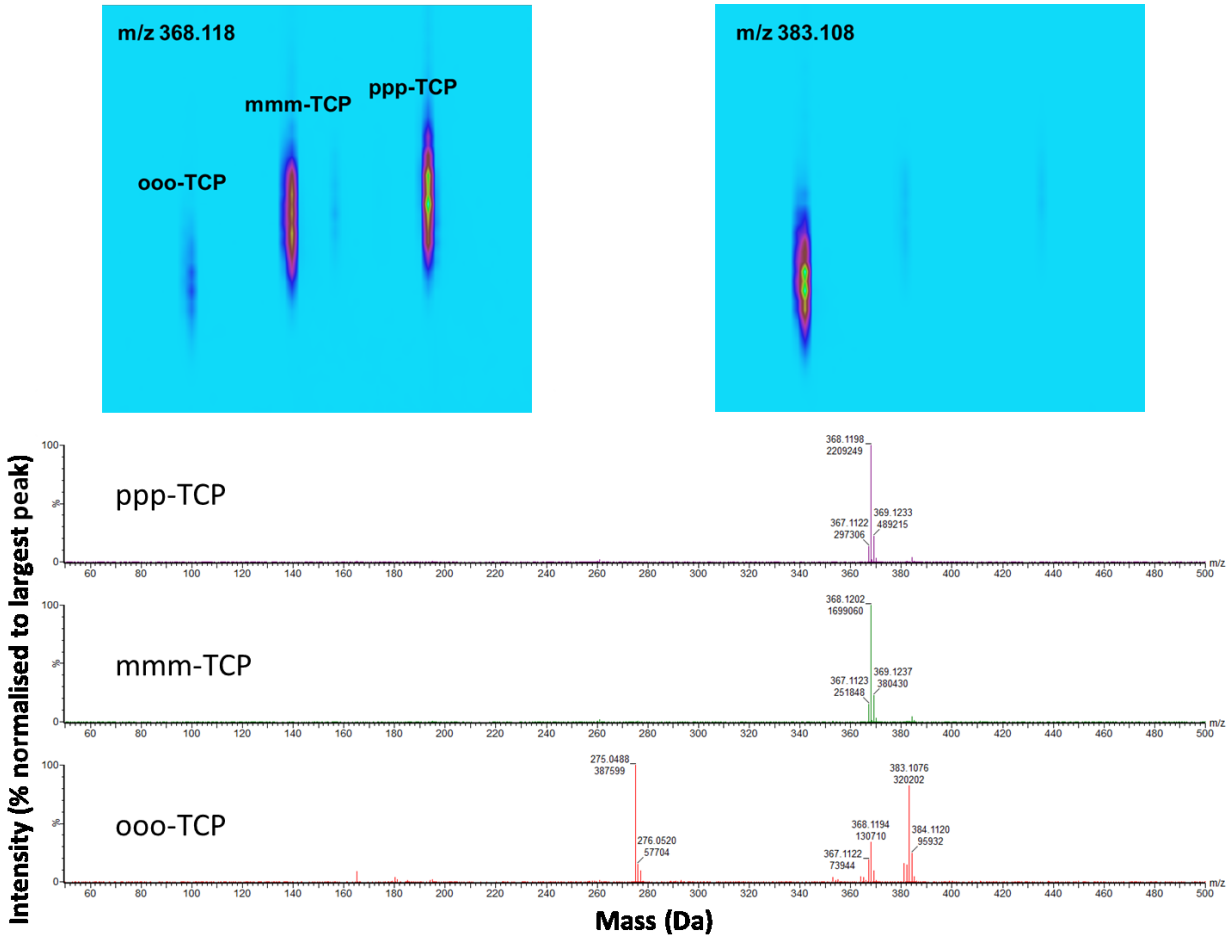
202 box in the mass defect plot) for a fresh and used oil sample. Circles on the mass defect plot are sized to

reflect the intensity of each ion recorded, and the data filtered to display potential PO_4 (red) and O_6 (blue) containing ions. These ions are annotated on the corresponding mass spectra.

3.2 GCxGC-HRTOFMS analysis

3.2.1 TCP quantification

A calibration series was produced by using $^{13}C_{18}$ labeled triphenyl phosphate (TPhP) and a native stock solution of ooo-TCP, mmm-TCP and ppp-TCP (all obtained from Wellington Laboratories). These were diluted to produce calibration solutions ranging from 0.5 to 1000 pg μL^{-1}. A mass of 368.1177 Da was selected for quantification of TCP isomers as this was the dominant molecular ion [$M^{\bullet+}$] that was expected to be generated through ionisation in the APCI source. However, when the calibration solutions were analysed the results showed generation of an $[M-H+O]^+$ ion at m/z 383.108. This ion-molecule reaction greatly favoured the ortho-substituted TCP, which along with a fragment at m/z 275.049 resulted in a significant decrease in the abundance of the molecular ion and dominance of the oxygen containing ion at m/z 383.108 for ooo-TCP (Figure 2). The formation of the $[M-H+O]^+$ ion at m/z 383.108 likely corresponds to the addition of oxygen from O_2 from residual air ion the ion source. The ion at m/z 275.049 may be generated by the loss of one of the side groups ($M-C_7H_9$) by double hydrogen transfer. These proposed ions are <3ppm of the theoretical values. The ion-molecule reaction involving O_2 appears to be structure specific. Although a mechanism is not yet known, similar ion molecule reactions between radical cations and O_2 have been observed (Jobst et al. 2009).



221

222 **Figure 2.** Mass spectra generated from ppp-, mmm- and ooo-TCP using APCI, and the corresponding SICs

223 from the M+ ion and M+O ion (+/- 0.05 da).

224

225 There are 10 different structural isomers of TCP, however Figure 3 (a&e) shows that only four TCP

226 isomers were identified at detectable levels in the fresh and used oil; mmm-TCP, mmp-TCP, ppm-TCP

227 and ppp-TCP. Quantification of mmp-TCP and ppm-TCP isomers was performed using the calibration

228 curves produced for mmm-TCP and ppp-TCP respectively. Houtzager et al. (2013) reported that ooo-TCP

229 was not identified in air and wipe samples taken from an aircraft, however ooo-TCP had been identified

230 in aircraft by Crump et al. (2011) and Rosenberger et al. (2013). The mmm-TCP, mmp-TCP, ppm-TCP and

231 ppp-TCP isomers were present in the fresh oil samples at concentrations of 0.69 % (+/ -0.01, 1σ), 1.70 %
232 (+/- 0.27, 1σ), 1.34 % (+/- 0.11, 1σ) and 0.51 % (+/-0.06, 1σ) respectively, equating to approximately 4.25
233 % total TCP (+/- 0.42, 1σ), which is consistent with the manufacturer's specifications (1-3 %). This was
234 greater than the concentrations found in the used oil which were 0.50 % (+/- 0.10, 1σ), 1.14 % (+/- 0.09,
235 1σ), 0.87 % (+/- 0.10, 1σ) and 0.24 % (+/- 0.02, 1σ) respectively, equating to 2.75 % total TCP (+/- 0.27,
236 1σ). The results are similar to those reported by Hecker et al. (2014) where total TCP concentrations in
237 Mobil II jet oil were 5.23%. Hecker et al. (2014) reported a slightly lower TCP concentration in BP 2380
238 fresh oil (4.7%) compared to used oil (5.1%). However, in this study the concentration in the used oil was
239 less than the fresh oil. This indicates that the TCP concentration in different oils can vary, and that TCP
240 may be lost during use (potentially to bleed air) or modified and converted into other compounds.

241 The non-detection of ooo-TCP (< 0.0005%) in our study significantly contrasts with earlier investigations
242 where the ooo-TCP represented between 10 and 60% of all TCP isomers in cabin air (Ramsden, 2013;
243 Rosenberger et al., 2013). Whilst this study cannot discount the presence of ooo-TCP below
244 concentrations of 0.0005% the initial results indicate that the oil is not the source of ooo-TCP in cabin
245 air. However one potential explanation for the absence of ooo-TCP in the oil but its presence in air
246 samples is the catalysis of meta and para isomers (by a palladium catalyst) which can generate ortho-
247 isomers (Imbert et al, 1997). The catalyst is used in units to decompose ozone and is often located after
248 the engine and upstream of the air conditioning pack. The authors are currently performing laboratory
249 testing to validate this hypothesis.

250

251 3.2 Identification of other toxic contaminants of concern

252 The results indicate that the formulation of Mobile II jet oil does not contain the more toxic ortho
253 substituted TCP isomers at detectable concentrations. However, there are several other toxic

components that may be present in jet oil. The absence of ortho containing TCP isomers does not necessarily mean that the oil does not pose a significant risk. Table 2 contains a list of potential compounds of concern that were screened for in the fresh and used oil. These compounds were selected from a literature search of organophosphates and other toxic compounds found in lubricating oils.

Table 2. Summary of screened analytes in the triplicate fresh and used oil samples

				Concentration in oil (%)					
	CAS #	[M+] m/z	Formula	Fresh oil 1	Fresh oil 2	Fresh oil 3	Used oil 1	Used oil 2	Used oil 3
ooo-TCP	1330-78-5	368.118	$C_{21}H_{21}PO_4$						
oom-TCP		368.118	$C_{21}H_{21}PO_4$						
oop-TCP		368.118	$C_{21}H_{21}PO_4$						
omm-TCP		368.118	$C_{21}H_{21}PO_4$						
omp-TCP		368.118	$C_{21}H_{21}PO_4$						
mmm-TCP		368.118	$C_{21}H_{21}PO_4$	0.68	0.70	0.70	0.40	0.52	0.59
opp-TCP		368.118	$C_{21}H_{21}PO_4$						
mmp-TCP		368.118	$C_{21}H_{21}PO_4$	1.51	2.01	1.58	1.05	1.16	1.22
mpp-TCP		368.118	$C_{21}H_{21}PO_4$	1.21	1.42	1.39	0.78	0.97	0.87
ppp-TCP		368.118	$C_{21}H_{21}PO_4$	0.45	0.55	0.53	0.22	0.26	0.24
xylenyl dicresyl phosphate	Not identified	382.133	$C_{22}H_{23}PO_4$	0.001 – 0.004	0.001 – 0.004	0.001 – 0.004	0.04 – 0.21x	0.06 – 0.30x	0.05 – 0.28x
dixylenyl monocresyl phosphate	Not identified	396.149	$C_{23}H_{25}PO_4$				0.02 – 0.13	0.03 – 0.17	0.03 – 0.15
trixylenyl phosphate	121-06-2	410.165	$C_{24}H_{27}PO_4$	0.009 – 0.003	0.009 – 0.003	0.009 – 0.003	0.04 – 0.23	0.06 – 0.31	0.05 – 0.29
dibutylphenyl phenyl phosphate	65652-41-7	438.196	$C_{26}H_{31}PO_4$						
tributylphenyl phosphate	78-33-1	494.259	$C_{30}H_{39}PO_4$						
tributyl phosphate	126-73-8	266.165	$C_{12}H_{27}PO_4$						
trimethyl phosphate	512-56-1	140.024	$C_3H_9PO_4$						
cresyl diphenyl phosphate	26444-49-5	340.086	$C_{19}H_{17}PO_4$						

cresyl saligenin phosphate	1222-87-3	276.055	$C_{14}H_{13}PO_4$						
triethylphosphate	78-40-0	182.071	$C_6H_{15}PO_4$						
Trimethylopropane phosphate	1005-93-2	213.053	$C_6H_{13}PO_6$						
tetraethyl pyrophosphate	107-49-3	290.068	$C_8H_{20}P_2O_7$						
triphenyl phosphorothionate	597-82-0	342.048	$C_{18}H_{15}PSO_3$						
N-phenyl-1-naphthalamine	90-30-2	219.105	$C_{16}H_{13}N$	x	x	x	x	x	x
dioctyldiphenylamine	68411-46-1	393.339	$C_{28}H_{43}N$	x	x	x	x	x	x
dinaphthylamine	532-18-3	269.120	$C_{20}H_{15}N$						
naphthylamine	134-32-7	143.074	$C_{10}H_9N$						
naphthol	90-15-3	144.058	$C_{10}H_8O$						

X = present in the sample with S:N greater than 10:1 but not quantified as no specific internal standard or calibration series were used

The GCxGC-HRTOFMS analysis confirmed the presence of the same group of three alkylated cresyl phosphates that were previously identified by FTICRMS for the used oil (Figure 1). The limits of detection by HRTOFMS were lower than the FTICR which enabled the detection of mono and tri xylenyl phosphates in the fresh oil extract in concentrations slightly greater than the limit of detection (0.0005% in the oil), however no dixylenyl phosphates were detected.

In all three used oil samples, 10 monoxylenyl dicresyl phosphate isomers, 7 dixylenyl monocresyl phosphate isomers and 10 trixylenyl phosphate isomers were detected (with S:N >10). Concentrations for the sum of monoxylenyl dicresyl phosphate isomers were calculated at between 0.05 and 0.26 %, for the sum of the dixylenyl monocresyl phosphate isomers at between 0.03 and 0.15 % and the sum of the trixylenyl phosphate isomers between 0.05 and 0.28 %. In all three fresh oil samples, 4 monoxylenyl dicresyl phosphate isomers, 0 dixylenyl monocresyl phosphate isomers and 3 trixylenyl phosphate isomers were detected (with S:N >10). Concentrations for the sum of monoxylenyl dicresyl phosphate

isomers were calculated at between 0.001 and 0.004 %, for and the sum of the trixylenyl phosphate isomers between 0.001 and 0.003 %. The range in potential concentrations is based on 'best' and 'worst' case calculations using calibration data from either ppp-TCP or ooo-TCP. Further analysis should be undertaken to confirm the structural identity of the xylenyl phosphates and other alkylated cresyl phosphates. Several standards are currently commercially available; however this task would be greatly aided by a comprehensive set of standards. Several alkylated cresyl phosphates have been shown to have a comparable toxicity to ortho substituted TCP (Winder and Balouet, 2002; Bondey et al. 1960) therefore, even though they are present in lower concentrations than the TCP isomers they may well pose a significant risk to human health. Further research should be undertaken to identify and accurately quantify the different xylenyl phosphates isomers as these results indicate that they should be included in further studies in aircraft air quality assessments. N-phenyl-1-naphthalamine and dioctyldiphenylamine were also identified in both the used and fresh oil samples, this has been previously identified in oil at approximately 1% (Winder and Balouet, 2002) and alkylated diphenylamines are noted at 1-5% on the MSDS for of Mobile jet oil II. These compounds should also be included in further studies.

Another potentially important source of organophosphate exposure that warrants further investigation is from flame retardants being released from fabrics, foams and plastics in the fittings and upholstery in the cabin. Schindler et al. (2012) found metabolite levels of flame retardants such as tributyl phosphate (TNBP), tris-(2-chloroethyl) phosphate (TCEP) and triphenyl phosphate (TPHP) (DBP 0.28 µg/l; BCEP 0.33 µg/l; DPP 1.1 µg/l) in urine at levels significantly higher than in unexposed persons from the general population. None of the samples contained o-TCP metabolites above the limit of detection (LOD 0.5 µg/l). Only one sample contained metabolites of m- and p-tricresyl phosphates with levels near the LOD. When assessing the risks in cabin air it is clear that assessments should not just consider ooo-TCP but investigate other compounds that may be present in oil and consider other pollutant pathways.

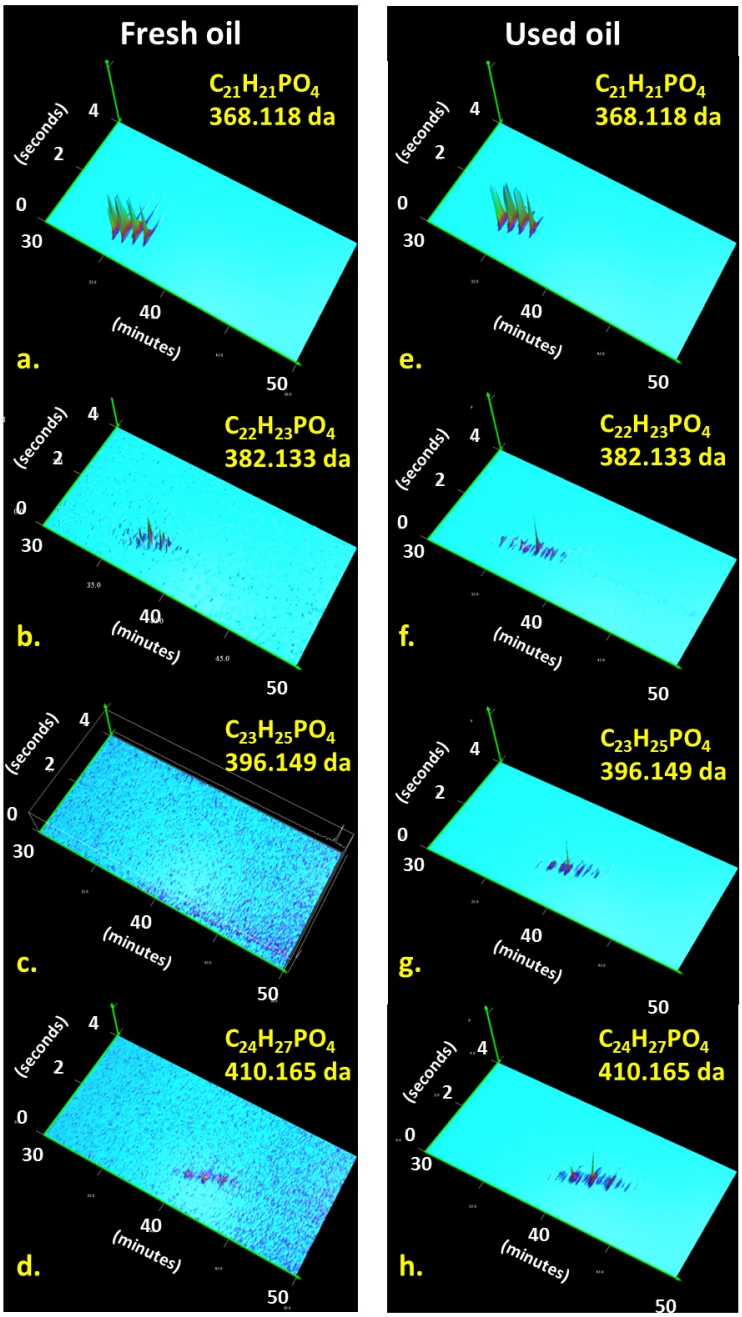


Figure 3. Selected ion chromatograms for the fresh and used oil samples displaying tricresyl phosphate isomers (a. & e.), monoxylenyl dicresyl phosphate isomers (b. & f.), dixylenyl monocresyl phosphate isomers (c. & g.) and xylenyl phosphates (d. & h.)

4. Conclusions

Flying is an important form of transportation and, for some, a rewarding past time. Although, aircraft crew are exposed to greater levels of cosmic radiation, VOCs and ozone, it is exposure to organophosphates that has been most closely linked to aerotoxic syndrome. The majority of studies on aerotoxic syndrome have focused on TCP and specifically tri-ortho-cresyl phosphate (ooo-TCP). This paper presents the findings of a wider screening method performed by FTICR MS and GCxGC-HRTOFMS to assess the presence of other organophosphates in fresh and used engine oil.

The results show that the formulation of Mobile II jet oil does not contain the more toxic ortho substituted TCP isomers at detectable concentrations. However, there may still be a significant risk from alkylated cresyl phosphates (xylenyl or ethylphenyl phosphates) which were identified in the used oils at concentrations calculated in the range of 0.13 to 0.69%. Several xylenyl and ethylphenyl phosphates have been shown to exhibit a similar toxicity to ortho substituted TCP isomers which makes there discovery in used oil significant. These compounds have not been analysed or accounted for in many of the previous exposure and air quality studies which may therefore have underestimated the actual risks from organophosphates.

More research is needed to further understand the problem of aerotoxic syndrome and establish if protective measures are necessary to ensure the health of future flight crews and passengers. These studies should include not only targeted analysis of suspected contaminants of concern such as tri-ortho-cresyl phosphate and N-phenyl-1-naphthalamine but also include non-targeted screening for other potential contaminants such as xylenyl phosphates generated during oil use. Future research should also include more detailed sampling of different matrices such as used oil, bleed air vapour, cabin air, fabric and of air crew. We can only fully understand the risks from aircraft oil when we understand,

a) what toxic compounds are in the oil, b) what is in the air, and c) what crew members have been exposed to. This paper indicates that the oil is not as safe as previously thought and so further research should be undertaken to characterise what is in the air and to measure what those adversely effected have been exposed to.

Acknowledgements

The authors are grateful for comments provided by J. Ramsden when reviewing the manuscript prior to submission.

References

Abou-Donia, M.B., Abou-Donia, M.M., ElMasry, E.M., Monro, J.A., Mulder, M.F.A., 2013. Autoantibodies to nervous system-specific proteins are elevated in sera of flight crew members: biomarkers for nervous system injury. *Journal of Toxicology and Environmental Health, Part A*. 76, 363–380.

Abou-Donia M.B., van de Goot F.R.W., Mulder M.F.A., 2014. Autoantibody markers of neural degeneration are associated with post-mortem histopathological alterations of a neurologically-injured pilot. *Journal of Biological physics and Chemistry*, 34-53 pp.

Banks, C.N., Lein, P.J., 2012. A Review of Experimental Evidence Linking Neurotoxic Organophosphorus Compounds and Inflammation. *Neurotoxicology*. 33(3). 575–584.

Craig, P.H., Barth, M.L., 1999. Evaluation of the hazards of industrial exposure to tricresyl phosphate. *Journal of Toxicology and Environmental Health, Part B.* 2, 281–300.

Committee on Toxicity of Chemicals in Food Consumer Products and the Environment (COT). (2007). Statement on the review of the cabin air environment, ill health in aircraft crews and the possible relationship to smoke/fume events in aircraft (Final Report: London).

348 Crump, D., Harrison, P., Walton, C., 2011. Aircraft Cabin Air Sampling Study; Part 1 of the Final Report.
349 YE29016V. Cranfield University, Institute of Environment and Health, pp. 1–110
350 https://dspace.lib.cranfield.ac.uk/handle/1826/5305 (accessed February 2016).

351 Daughtrey, W., Biles, R., Jortner, B., Erlich, M., 1996. Subchronic delayed neurotoxicity evaluation of jet
352 engine lubricants containing phosphorus additives. *Fundamental and Applied Toxicology*. 32, 244–249.

353 de Boer, J., Antelo, A., van der Veen, I., Brandsma, S., Lammertse, N., 2015. Tricresyl phosphate and the
354 aerotoxic syndrome of flight crew members – Current gaps in knowledge. *Chemosphere*. 119, S58-S61

355 de Ree, H., van den Berg, M., Brand, T., Mulder, G.J., Simons, R., Veldhuijzen van Zanten, B., Westerink,
356 R.H.S., 2014. Health risk assessment of exposure to TriCresyl Phosphates (TCPs) in aircraft: A
357 commentary. *NeuroToxicology*. 45, 209-215

358 De Nola, G., 2011. Determination of tricresyl phosphate air contamination in aircraft. *The Annals of*
359 *Occupational Hygiene*. 55, 710–722.

360 Exxon-Mobil, 2013. Material Safety Data Sheet for Mobil Jet II. Exxon-Mobil Corporation, Fairfax, VA.
361 Revised 12 Sept. 2013

362 Freudenthal, R.I., Rausch, L., Gerhart, J.M., Barth, M.L., Mackerer, C.R., Bisinger, E.C., 1993. Subchronic
363 neurotoxicity of oil formulations containing either tricresyl phosphate or tri-orthocresyl phosphate.
364 *Journal of the American College of Toxicology*. 12, 409–416.

365 Harrison, V. Mackenzie Ross, S.J. 2016 An emerging concern: Toxic fumes in airplane cabins. *Cortex*. 74,
366 297-302

367 Hecker, S. Kincl, L. McNeely, E. van Netten, Christiaan. Harrison, R. Murawski, J. Vallarino, J. Spangler,
368 J.D. Milton, D. Tager, I. Gale, S. Bradley, J. 2014. Cabin Air Quality Incidents Project Report. Occupational

Health Research Consortium in Aviation and Airliner Cabin Environment Research. http://www.ohrca.org/wp-content/uploads/2014/08/finalreport.pdf. Accessed May 2016.

Houtzager, M.M.G., Havermans, J.G.M.A., Bos, J.G.H. 2013. Research info presence and concentration of tricesylphosphates in cockpits of KLM Boeing 737 aircraft during normal operational conditions. TNO 2013 R11976. 1-56.

Hughey C.A., Hendrickson C.L., Rodgers R.P., Marshall A.G. 2001. Kendrick mass defect spectrum : A compact visual analysis for ultrahigh-resolution broadband mass spectra. *Analytical Chemistry*. 73(19):4676-4681

Imbert, F.E., Gnep, N. and Guisnet, M. 1997. Cresol isomerization on HZSM-5. *Journal of Catalysis*. 172, 307–313

Kendrick, E. 1963. A mass scale based on CH2 = 14.0000 for high resolution mass spectrometry of organic compounds. *Analytical Chemistry*. 35:2146–2154

Liyasova, M., Li, B., Schopfer, L.M., Nachon, F., Masson, P., Furlong, C.E., Lockridge, O., 2011. Exposure to tri-o-cresyl phosphate detected in jet airplane passengers. *Toxicology and Applied. Pharmacology*. 256, 337–347.

Mackerer, C.R., Barth, M.L., Krueger, A.J., 1999. Comparison of neurotoxic effects and potential risks from oral administration or ingestion of tricresyl phosphate and jet engine oil containing tricresyl phosphate. *Journal of Toxicology and Environmental Health, Part A*. 57, 293–328.

Murawski, J.T.L., Supplee, D.S., 2008. An attempt to characterize the frequency, health impact and operational costs of oil in the cabin and flight deck supply air on US commercial flights. ASTM Journal. 5, 15

390 Payne, Sheriff Stanthope. 2015. Regulation 28: report to prevent future deaths. Senior coroner's report
391 for The County of Dorset.

392 Ramsden, J. 2013. On the proportion of ortho isomers in the tri-cresyl phophates contained in jet oil.
393 *Journal of Biological Physics and Chemistry*, **13**, 69-72

394 Rosenberger, W., Netz-Piepenbrink, S., Wrbtzky, R. 2013, *Gefahrstoffe –Rehalting der Luft*. 73, 4, 138-
395 143

396 Shehadi, M., Jones, B., & Hosni, M. 2015. Characterization of the frequency and nature of bleed air
397 contamination events in commercial aircraft. *Indoor Air*, 1-11.

398 Schindler, B.K., Weiss, T., Schutze, A., Koslitz, S., Broding, H.C., Bunger, J., Bruning, T., 2012.
399 Occupational exposure of air crews to tricresyl phosphate isomers and organophosphate flame
400 retardants after fume events. *Analytical Toxicology*, **87**, 645-648

401 Winder, C. and Balouet, J-C., 2002. The toxicity of commercial jet oils. *Environmental Research Section A*.
402 89, 146-164.

403 Wolkoff, P. Crump, D.R. Harrison, P.T.C. 2016. Pollutant exposures and health symptoms in aircrew and
404 office workers: Is there a link? *Environment International*. **87**, 74–84