# 1 A comparison of fresh and used aircraft oil

# 2 for the identification of toxic substances

# 3 linked to aerotoxic syndrome.

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11 **Abstract**

12 Fresh and used aircraft engine lubricants (Mobil Jet Oil II) were analysed using a Fourier Transform Ion

13 Cyclotron Resonance Mass Spectrometer (FTICRMS) and comprehensive two dimensional gas

14 chromatography with high resolution time of flight mass spectrometry (GCxGC-HRTOFMS). The

15 composition of the fresh oil was established, with special focus to its tricresyl phosphate (TCP) content

16 as this has formed the focus for most investigations into aerotoxic syndrome. The results showed that

17 only four TCP isomers were present at detectable levels in the fresh oil: mmm-TCP, mmp-TCP, ppm-TCP

18 and ppp-TCP. The results indicate that the formulation of Mobile Jet Oil II does not contain the more

19 toxic ortho substituted TCP isomers at concentrations above 0.0005%. The temperatures of jet engines

20 during operation are greater than 200°C which creates the potential to alter the composition of the

21 original oil and create other toxic compounds. The results show there may be a significant risk from

22 alkylated cresyl phosphates, which were identified in the used oils at concentrations calculated in the

23 range of 0.13 to 0.69%. w/w. Several xylenyl and ethylphenyl phosphates have been shown to exhibit a

24 similar toxicity to ortho substituted TCP isomers which makes there discovery in used oil significant.

25 These compounds should be included in future aircraft air quality studies and when assessing the risks

26 and causes of aerotoxic syndrome.

27

28 **Key words**

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

30 organophosphates

# 1. Introduction

32 Due to their widespread use as pesticides, plasticizers and flame retardants organophosphates are

33 routinely detected in environmental samples. However, a specific concern has arisen in recent decades

34 as aircraft crew have developed symptoms consistent with exposure to toxic fumes and

35 organophosphates (Abou-Donia et al. 2013; Harrison and Mackenzie Ross, 2016; Liyasova et al. 2011;

36 Payne, 2015). There have been reports of headaches, loss of balance, numbness and neurobehavioral

37 abnormalities such as emotional instability, depression and cognitive dysfunction, including impaired

38 short term memory, blurred vision and speech, altered coordination (de Ree et al. 2014; Abou-Donia et

39 al. 2013). Organophosphates are not just used as pesticides on crops in fields but are also used in

40    aircraft as flame retardants, in engine oil and hydraulic fluids, and on material surfaces. This has resulted

41    in their link to aerotoxic syndrome by Winder and Balouet (2002). The term Aerotoxic Syndrome was

42    first published in 1999 by an international scientific team to describe the symptoms and exposure

43    conditions reported by aircraft crew from Australia, US, Europe. Whilst aerotoxic syndrome has not

44    been fully accepted as a medical syndrome (Wolkoff et al., 2016) it is commonly used to refer to air

45    quality in aircraft and the associated exposure of crew and passengers to toxic compounds. Aerotoxic

46    syndrome is understood to be caused by long term and repeated exposure to chemicals released from

47    smoke and fume events in aircraft.

48    During a flight the air cabin pressure and temperature are maintained with outside air that is passed

49    through the jet engine. Cabin air is recycled for 50%, whereas the flight deck air is comprised in most

50    aircraft types of a continuous stream of bleed air (de Boer et al. 2015). Engine seals in use are known as

51    "wet-seals", an inherent design feature, whereby a thin film of oil prevents rotating surfaces to come

52    into mechanical contact with each other. Pressure differentials over the seals cause a constant loss of oil

53    and vapours into the core engine. These enter the inlet of the high pressure compressor and

54    contaminate the bleed air which is taken downstream. Oil leaks can result in odd smells in the cabin and

55    in more extreme cases smoke events. The tricresylphosphate chemical fingerprint from wipe samples

56    taken from a cockpit showed a statistically significant correlation (p value 0.039) with used engine oil

57    (Hourzager et al. 2013), indicating that this can be a significant source of exposure. Estimates of how

58    often these events occur varies depending upon whether the information is sourced from regulatory

59    authorities such as the UK Civil Aviation Authority (CAA), from airlines or from trade unions who

60    represent aircrew (Harrison and Mackenzie Ross, 2016). Reported values for the frequency of

61    smoke/fume events include 0.5% of flights (Murawski and Supplee, 2008), 0.05% (COT, 2007) and 0.02%

62    (Shehadi et al., 2015). Lubricating oil is applied to gas turbines in aeroplanes as anti-wear agents.

63    However, there have been several studies that have identified subchronic neurotoxicity of aviation oils

64    (Freudenthal et al. 1993; Daughtrey et al. 1996; Mackerer et al. 1999). Aircraft lubricating oils have

65    remained relatively unchanged since the 1960s and are comprised of approximately 95% synthetic

66    esters with 3% tri-cresyl phosphates and 1% phenyl-α-naphthalamines (Winder and Balouet, 2002). Tri-

67    cresyl phosphates (TCPs) are a group of organophosphates which contain 10 structural isomers. The

68    ortho substituted congeners are considered to be the most toxic and therefore the proportions of these

69    compounds in oil have been reduced in recent decades (Craig and Barth. 1999). Most of the focus of

70    investigations in aircraft air quality and aerotoxic syndrome has been focused on tri-ortho-cresyl

71    phosphate (ooo-TCP or ToCP), however the mono-ortho and di-ortho isomers are also highly toxic with

72    omp-TCP the most toxic (de Boer et al. 2015; De Nola et al., 2011).

73    There is evidence to indicate that aircraft crew and passengers have been exposed to organophosphates

74    from traveling on aeroplanes and that it has resulted in neurotoxic effects (Abou-Donia et al. 2013;

75    Liyasova et al. 2011). Abou-Donia et al. (2013) undertook a study of 34 flight crew members and the

76    results suggest the possible development of neuronal injury and gliosis in flight crew members

77    anecdotally exposed to cabin air emissions containing organophosphates. A symptom-free pilot was

78    sampled before symptoms and then again afterward. This pilot developed clinical problems after flying

79    for 45 hours in 10 days. Significant increases in autoantibodies were noted to most of the tested

80    proteins in the serum of this pilot after exposure to air emissions. The levels of autoantibodies rose with

81    worsening of his condition compared to the serum sample collected prior to exposure. After cessation of

82    flying for a year, this pilot's clinical condition improved, and his serum autoantibodies against nervous

83    system proteins decreased. Many crew members who have reported conditions consistent with

84    organophosphate poisoning recover and return to their flight duties, however some staff have lost their

85    jobs, and several have even passed away. When individuals are grounded they are no longer exposed to

86    aircraft air their reported symptoms can gradually reduce. The underlying mechanism that caused the ill

87    health may be an active auto immune reaction, set into motion by repeated low dose exposure to

88  organophosphates, causing actual damage to the Blood-Brain-Barrier and apoptosis inside the brain.

89  Protein filaments of these decaying cells are able to re-enter the bloodstream, causing a secondary auto

90  immune response. Memory B cells can recognise certain antigens for a long duration of time,

91  comparable to a status after vaccination and can lead in rare instances to an anaphylactic shock (Banks

92  et al. 2012), also known as "incapacitation", or sudden heart failure due to lymphocytic myocarditis. UK

93  Coroner Payne, recently issued a report to prevent further deaths following the death of a pilot, which

94  was linked to OP poisoning (Abou-Donia et al., 2014; Payne, 2015). The post mortem investigations gave

95  the cause of death of either pentobarbital toxicity or lymphocytic myocarditis, individually or in

96  combination. Hair analysis had shown the use of pentobarbital during the 4 weeks prior to his death, as

97  a form of self medication against severe headaches. Pentobarbital is not known for causing the

98  widespread infiltration of T-lymphocytes in the brain, heart and neurological damage otherwise

99  observed. Testing of samples taken both prior to and after death disclosed symptoms consistent with

100 exposure to organophosphate compounds.

101 Due to the sporadic occurrence of smoke/fume incidents it is particularly difficult to obtain worst case

102 air samples for analysis. No fume events were observed on any of the flights that were monitored by

103 Crump et al., (2011) and de Ree et al., (2014). Although this is hardly surprising as they only involved

104 analysis on 100 and 20 flights respectively. Using the estimates of the frequency of fume events from

105 Murawski and Supplee, (2008), and Shehadi et al., (2015) a sample size of between 200 and 5000 flights

106 would be required to identify one event. Interestingly nine smoke/odour events were recorded in 78

107 samples taken by De Nola (2011). de Boer et al. (2015) used data from De Nola (2011) and Craig and

108 Barth (1999) to calculate that even using worst-case scenarios they cannot explain a relation of TCP in

109 flight deck air to the complaints of pilots and air crew, a similar conclusion was also reached by de Ree et

110 al. (2014) and Schindler et al. (2013). Available data was reviewed by Ramsden (2013) who used jet oil

111 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

## 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

135    a factor of 1:1000 with toluene. For analysis by GCxGC- HRTOFMS the FTICRMS samples were further

136    diluted by a factor of 1:10 and 1:100. These samples were spiked with $^{13}C_{18}$ labeled triphenyl phosphate

137    (TPhP) for quantification (obtained from Wellington Laboratories).

138    FTICRMS analysis was performed using a Varian FTICRMS (Varian Inc., Walnut Creek, CA), consisting of a

139    9.4 T superconducting magnet. Samples were directly infused at a rate of 5 μL/min and ionised using

140    atmospheric pressure photo ionisation (APPI). Mass spectra were obtained using arbitrary waveform

141    excitation (90 v) and broadband detection from m/z 150 to 1000 with a transient length of 2 seconds.

142    The FTICRMS was operated at a resolving power of 300,000 (fwhm) at 368 m/z. Mass accuracy was less

143    than one ppm, achieved by internal mass calibration using the Agilent ESI calibration mix, which was

144    added to the sample at a 1:1 ratio before infusion.

145    GCxGC-HRTOFMS analysis was performed using a Waters Xevo G2-XS QToF fitted with a 40 m x 0.18 mm

146    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

147    second dimension ($^{2}$D), this was then connected to a 0.8 m x 1.8 mm Custom MXT tubing (sulfinert

148    treated). The injector temperature was set at 280 $^{o}$C, the initial oven temperature was held at 70 $^{o}$C for

149    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

150    minutes. The secondary oven was set at a 40 $^{o}$C offset to the primary oven. The modulation period was

151    set at 4 seconds with a hot pulse duration of 0.4 s and the transfer line temperature at 360 $^{o}$C. The

152    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

153    L/hr. Ionisation was undertaken using an atmospheric pressure chemical ionisation source at 150 $^{o}$C

154    with the detector run in TOF mode using a scan window of 50 amu to 1200 amu with a scan time of 0.04

155    (+ 0.015 interscan delay) seconds. The GCxGC-HRTOFMS was operated at a resolving power of >20,000

156    (fwhm), internal mass calibration was performed by using a lock mass ion (355.0699) generated from a

157    siloxane. Limits of detection for tri-cresyl phosphates were calculated by serial dilution of the calibration

158    solutions, the lowest concentration detected with a S:N >10 was 0.0005%)

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## 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

8

181    three of the cresyls. The structures are therefore hypothesised as monoxylenyl dicresyl phosphate,

182    dixylenyl dicresyl phosphate and trixylenyl phosphate as xylenyl cresyl phosphates have been previously

183    identified as potential contaminants in TCP solutions by Winder and Balouet (2002). Recently revised

184    versions of the safety data sheets of some widely-used aviation engine oils also now report 0.1-1%

185    trixylenyl phosphate (TXP) content (Exxon-Mobil, 2013). However, without the use of analytical

186    standards we were unable to confirm that they are not another alkylated compound with the same

187    molecular formula such as ethyl phenyl phosphates.

188    The discovery of alkylated cresyl phosphates in aircraft oil is a significant finding as the mono and di

189    ortho ethyl phenyl phosphates and xylenyl phosphates have displayed a similar toxicity to ortho

190    substituted TCP isomers (Bondey et al. 1960; Winder and Balouet 2002). Like TCP the ethyl phenyl

191    phosphates and xylenyl phosphates have a toxicity that is position specific and so it is important to

192    understand exactly which compounds are present. The analysis performed by FTICRMS was via direct

193    infusion and whilst it provides an excellent mass accuracy it was not possible to identify how many

194    different structural isomers were present. Therefore, further analysis was undertaken using GCxGC-

195    HRTOFMS to identify the different TCP isomers and quantify the concentrations of TCP and the other

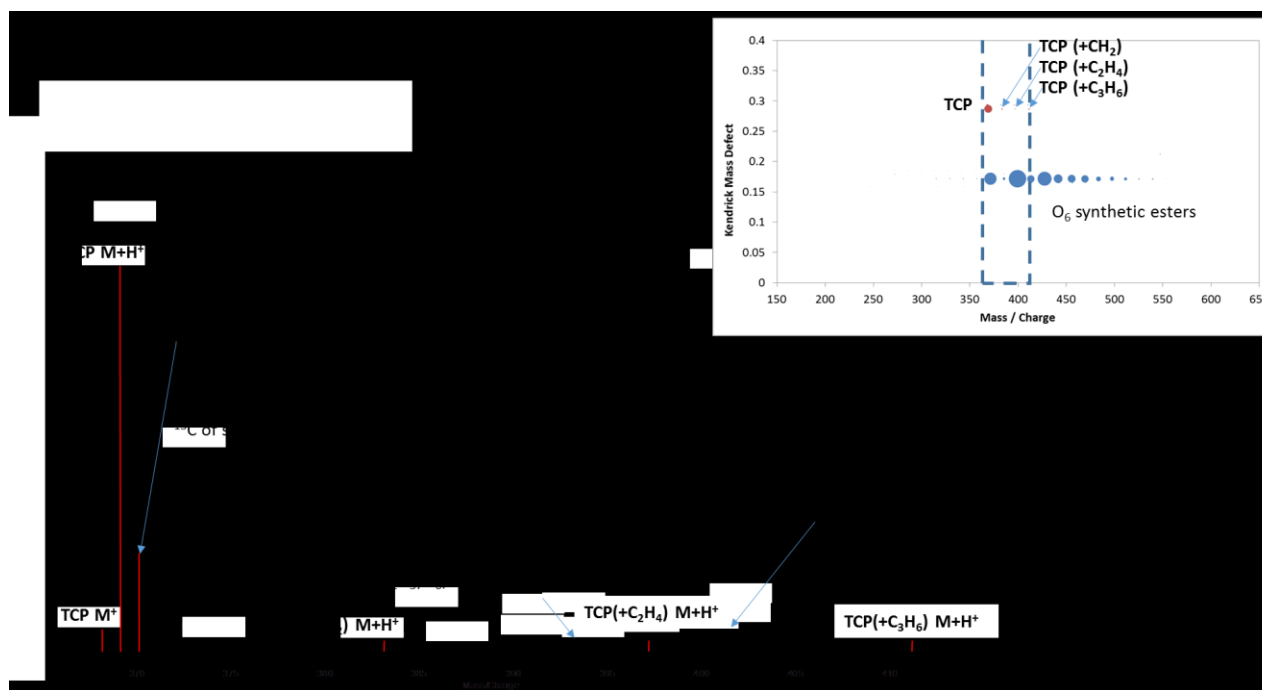196    alkylated cresyl phosphates detected in the used oil.

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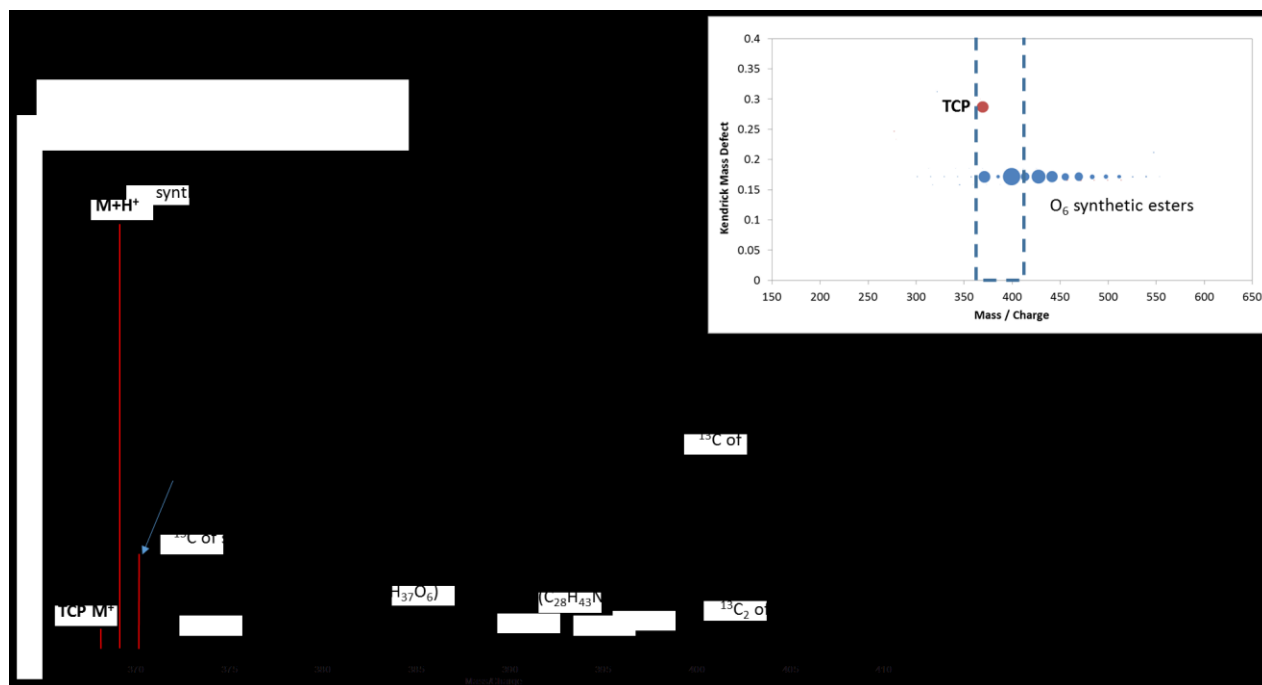


**Figure 1**. Kendrick mass defect plot and selected mass spectra (M/Z 365 to 413- represented by the blue box in the mass defect plot) for a fresh and used oil sample. Circles on the mass defect plot are sized to

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203  reflect the intensity of each ion recorded, and the data filtered to display potential $PO_4$ (red) and $O_6$

204  (blue) containing ions. These ions are annotated on the corresponding mass spectra.

205  3.2  GCxGC-HRTOFMS analysis

206  3.2.1  TCP quantification

207  A calibration series was produced by using $^{13}C_{18}$ labeled triphenyl phosphate (TPhP) and a native stock

208  solution of ooo-TCP, mmm-TCP and ppp-TCP (all obtained from Wellington Laboratories). These were

209  diluted to produce calibration solutions ranging from 0.5 to 1000 pg $\mu L^{-1}$. A mass of 368.1177 Da was

210  selected for quantification of TCP isomers as this was the dominant molecular ion $[M^{\bullet +}]$ that was

211  expected to be generated through ionisation in the APCI source. However, when the calibration

212  solutions were analysed the results showed generation of an $[M-H+O]^+$ ion at m/z 383.108. This ion-

213  molecule reaction greatly favoured the ortho-substituted TCP, which along with a fragment at m/z

214  275.049 resulted in a significant decrease in the abundance of the molecular ion and dominance of the

215  oxygen containing ion at m/z 383.108 for ooo-TCP (Figure 2). The formation of the $[M-H+O]^+$ ion at m/z

216  383.108 likely corresponds to the addition of oxygen from $O_2$ from residual air ion the ion source. The

217  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

218  transfer. These proposed ions are <3ppm of the theoretical values. The ion-molecule reaction involving

219  $O_2$ appears to be structure specific. Although a mechanism is not yet known, similar ion molecule

220  reactions between radical cations and $O_2$ have been observed (Jobst et al. 2009).
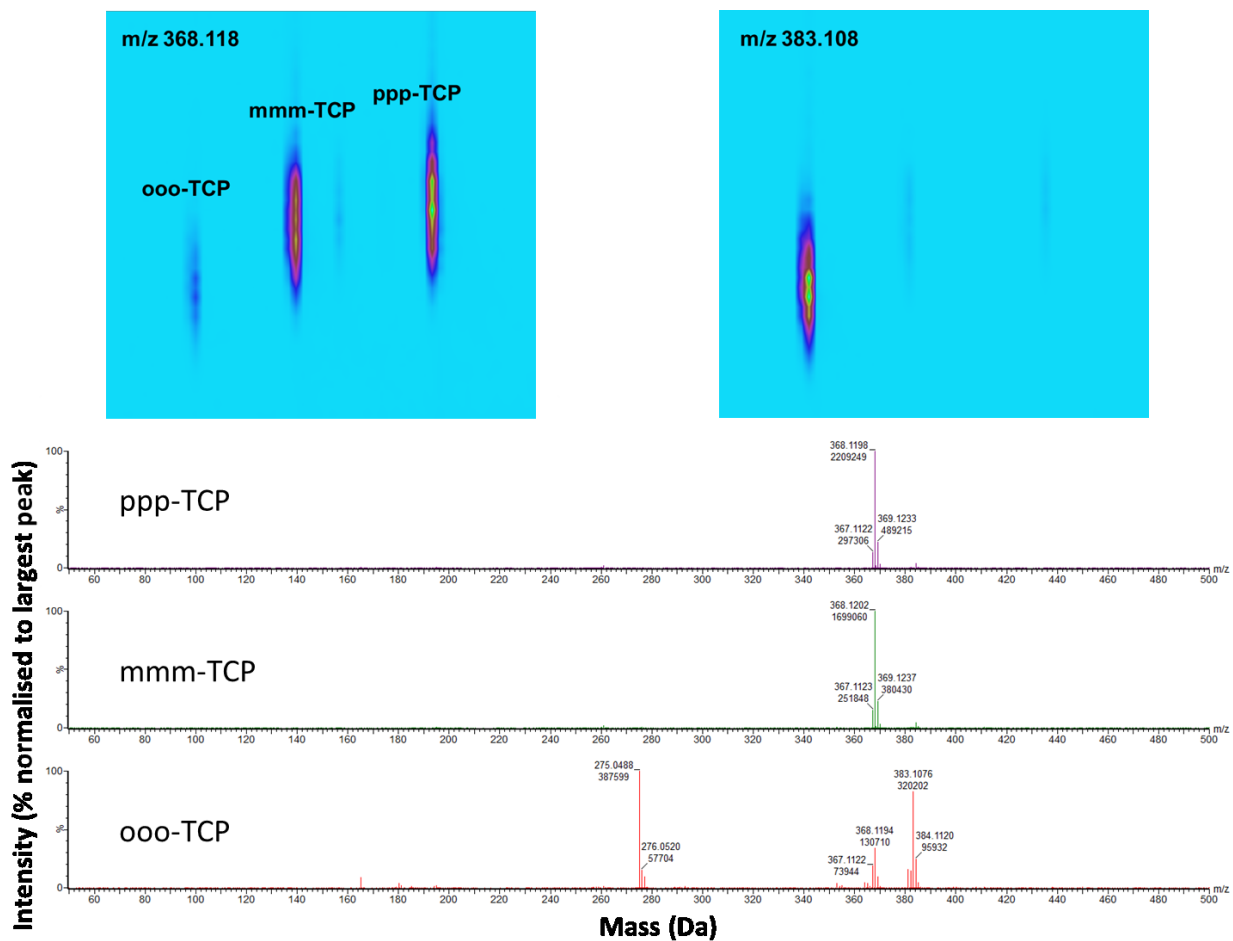
**Figure 2.** Mass spectra generated from ppp-, mmm- and ooo-TCP using APCI, and the corresponding SICs from the M+ ion and M+O ion (+/- 0.05 da).

There are 10 different structural isomers of TCP, however Figure 3 (a&e) shows that only four TCP isomers were identified at detectable levels in the fresh and used oil; mmm-TCP, mmp-TCP, ppm-TCP and ppp-TCP. Quantification of mmp-TCP and ppm-TCP isomers was performed using the calibration curves produced for mmm-TCP and ppp-TCP respectively. Houtzager et al. (2013) reported that ooo-TCP was not identified in air and wipe samples taken from an aircraft, however ooo-TCP had been identified 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

254 components that may be present in jet oil. The absence of ortho containing TCP isomers does not

255 necessarily mean that the oil does not pose a significant risk. Table 2 contains a list of potential

256 compounds of concern that were screened for in the fresh and used oil. These compounds were

257 selected from a literature search of organophosphates and other toxic compounds found in lubricating

258 oils.

259 **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 | $C_{19}H_{17}PO_4$ | | | | | | |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 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 | $C_{10}H_8O$ | | | | | | |

260  X = present in the sample with S:N greater than 10:1 but not quantified as no specific internal standard

261  or calibration series were used

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263  The GCxGC-HRTOFMS analysis confirmed the presence of the same group of three alkylated cresyl

264  phosphates that were previously identified by FTICRMS for the used oil (Figure 1). The limits of detection

265  by HRTOFMS were lower than the FTICR which enabled the detection of mono and tri xylenyl

266  phosphates in the fresh oil extract in concentrations slightly greater than the limit of detection (0.0005%

267  in the oil), however no dixylenyl phosphates were detected.

268  In all three used oil samples, 10 monoxylenyl dicresyl phosphate isomers, 7 dixylenyl monocresyl

269  phosphate isomers and 10 trixylenyl phosphate isomers were detected (with S:N >10). Concentrations

270  for the sum of monoxylenyl dicresyl phosphate isomers were calculated at between 0.05 and 0.26 %, for

271  the sum of the dixylenyl monocresyl phosphate isomers at between 0.03 and 0.15 % and the sum of the

272  trixylenyl phosphate isomers between 0.05 and 0.28 %. In all three fresh oil samples, 4 monoxylenyl

273  dicresyl phosphate isomers, 0 dixylenyl monocresyl phosphate isomers and 3 trixylenyl phosphate

274  isomers were detected (with S:N >10). Concentrations for the sum of monoxylenyl dicresyl phosphate

275  isomers were calculated at between 0.001 and 0.004 %, for and the sum of the trixylenyl phosphate

276  isomers between 0.001 and 0.003 %. The range in potential concentrations is based on 'best' and 'worst'

277  case calculations using calibration data from either ppp-TCP or ooo-TCP. Further analysis should be

278  undertaken to confirm the structural identity of the xylenyl phosphates and other alkylated cresyl

279  phosphates. Several standards are currently commercially available; however this task would be greatly

280  aided by a comprehensive set of standards. Several alkylated cresyl phosphates have been shown to

281  have a comparable toxicity to ortho substituted TCP (Winder and Balouet, 2002; Bondey et al. 1960)

282  therefore, even though they are present in lower concentrations than the TCP isomers they may well

283  pose a significant risk to human health. Further research should be undertaken to identify and

284  accurately quantify the different xylenyl phosphates isomers as these results indicate that they should

285  be included in further studies in aircraft air quality assessments. N-phenyl-1-naphthalamine and

286  dioctyldiphenylamine were also identified in both the used and fresh oil samples, this has been

287  previously identified in oil at approximately 1% (Winder and Balouet, 2002) and alkylated

288  diphenylamines are noted at 1-5% on the MSDS for of Mobile jet oil II. These compounds should also be

289  included in further studies.

290  Another potentially important source of organophosphate exposure that warrants further investigation

291  is from flame retardants being released from fabrics, foams and plastics in the fittings and upholstery in

292  the cabin. Schindler et al. (2012) found metabolite levels of flame retardants such as tributyl phosphate

293  (TNBP), tris-(2-chloroethyl) phosphate (TCEP) and triphenyl phosphate (TPHP) (DBP 0.28 µg/l; BCEP 0.33

294  µg/l; DPP 1.1 µg/l) in urine at levels significantly higher than in unexposed persons from the general

295  population. None of the samples contained o-TCP metabolites above the limit of detection (LOD 0.5

296  µg/l). Only one sample contained metabolites of m- and p-tricresyl phosphates with levels near the LOD.

297  When assessing the risks in cabin air it is clear that assessments should not just consider ooo-TCP but

298  investigate other compounds that may be present in oil and consider other pollutant pathways.

299



300
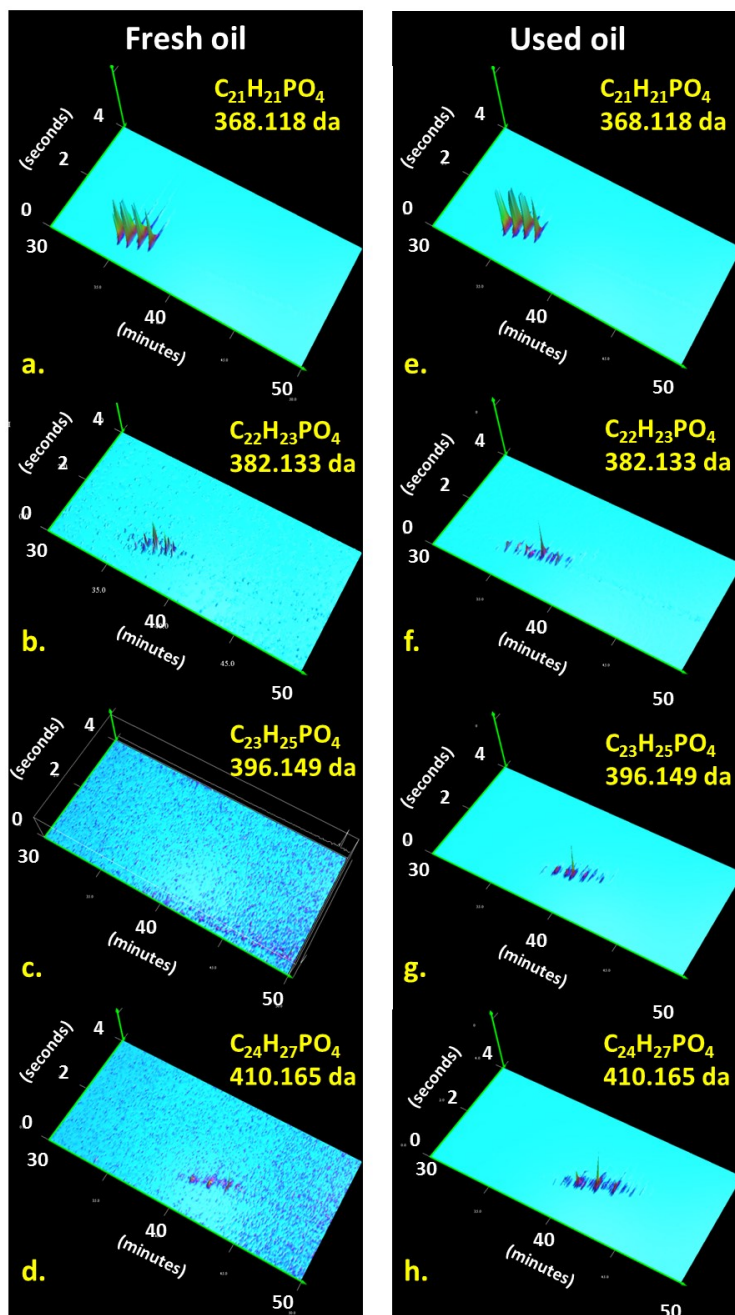
Figure 3. Selected ion chromatograms for the fresh and used oil samples displaying tricresyl phosphate isomers (a. & e.), monoxylenyl dicresyl phosphate isomers (b. & f.), dixylenyl monocresyl phosphate isomers (c. & g.) and xylenyl phosphates (d. & h.)

304

## 4. Conclusions

305

306 Flying is an important form of transportation and, for some, a rewarding past time. Although, aircraft

307 crew are exposed to greater levels of cosmic radiation, VOCs and ozone, it is exposure to

308 organophosphates that has been most closely linked to aerotoxic syndrome. The majority of studies on

309 aerotoxic syndrome have focused on TCP and specifically tri-ortho-cresyl phosphate (ooo-TCP). This

310 paper presents the findings of a wider screening method performed by FTICR MS and GCxGC-HRTOFMS

311 to assess the presence of other organophosphates in fresh and used engine oil.

312 The results show that the formulation of Mobile II jet oil does not contain the more toxic ortho

313 substituted TCP isomers at detectable concentrations. However, there may still be a significant risk from

314 alkylated cresyl phosphates (xylenyl or ethylphenyl phosphates) which were identified in the used oils at

315 concentrations calculated in the range of 0.13 to 0.69%. Several xylenyl and ethylphenyl phosphates

316 have been shown to exhibit a similar toxicity to ortho substituted TCP isomers which makes there

317 discovery in used oil significant. These compounds have not been analysed or accounted for in many of

318 the previous exposure and air quality studies which may therefore have underestimated the actual risks

319 from organophosphates.

320 More research is needed to further understand the problem of aerotoxic syndrome and establish if

321 protective measures are necessary to ensure the health of future flight crews and passengers. These

322 studies should include not only targeted analysis of suspected contaminants of concern such as tri-

323 ortho-cresyl phosphate and N-phenyl-1-naphthalamine but also include non-targeted screening for

324 other potential contaminants such as xylenyl phosphates generated during oil use. Future research

325 should also include more detailed sampling of different matrices such as used oil, bleed air vapour, cabin

326 air, fabric and of air crew. We can only fully understand the risks from aircraft oil when we understand,

327 a) what toxic compounds are in the oil, b) what is in the air, and c) what crew members have been

328 exposed to. This paper indicates that the oil is not as safe as previously thought and so further research

329 should be undertaken to characterise what is in the air and to measure what those adversely effected

330 have been exposed to.

## 331 Acknowledgements

## 334 References

335 Abou-Donia, M.B., Abou-Donia, M.M., ElMasry, E.M., Monro, J.A., Mulder, M.F.A., 2013. Autoantibodies

336 to nervous system-specific proteins are elevated in sera of flight crew members: biomarkers for nervous

337 system injury. *Journal of Toxicology and Environmental Health, Part A*. 76, 363–380.

338 Abou-Donia M.B., van de Goot F.R.W., Mulder M.F.A., 2014. Autoantibody markers of neural

339 degeneration are associated with post-mortem histopathological alterations of a neurologically-injured

340 pilot. *Journal of Biological physics and Chemistry*, 34-53 pp.

341 Banks, C.N., Lein, P.J., 2012. A Review of Experimental Evidence Linking Neurotoxic Organophosphorus

342 Compounds and Inflammation. *Neurotoxicology*. 33(3). 575–584.

343 Craig, P.H., Barth, M.L., 1999. Evaluation of the hazards of industrial exposure to tricresyl phosphate.

344 *Journal of Toxicology and Environmental Health, Part B.* 2, 281–300.

345 Committee on Toxicity of Chemicals in Food Consumer Products and the Environment (COT). (2007).

346 Statement on the review of the cabin air environment, ill health in aircraft crews and the possible

347 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

369    Health    Research    Consortium    in    Aviation    and    Airliner    Cabin    Environment    Research.

370    http://www.ohrca.org/wp-content/uploads/2014/08/finalreport.pdf. Accessed May 2016.

371    Houtzager, M.M.G., Havermans, J.G.M.A., Bos, J.G.H. 2013. Research info presence and concentration of

372    tricesylphosphates in cockpits of KLM Boeing 737 aircraft during normal operational conditions. TNO

373    2013 R11976. 1-56.

374    Hughey C.A., Hendrickson C.L., Rodgers R.P., Marshall A.G. 2001. Kendrick mass defect spectrum : A

375    compact visual analysis for ultrahigh-resolution broadband mass spectra. *Analytical Chemistry*.

376    73(19):4676-4681

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

378    307–313

379    Kendrick, E. 1963. A mass scale based on CH2 = 14.0000 for high resolution mass spectrometry of

380    organic compounds. *Analytical Chemistry*. 35:2146–2154

381    Liyasova, M., Li, B., Schopfer, L.M., Nachon, F., Masson, P., Furlong, C.E., Lockridge, O., 2011. Exposure

382    to tri-o-cresyl phosphate detected in jet airplane passengers. *Toxicology and Applied. Pharmacology*.

383    256, 337–347.

384    Mackerer, C.R., Barth, M.L., Krueger, A.J., 1999. Comparison of neurotoxic effects and potential risks

385    from oral administration or ingestion of tricresyl phosphate and jet engine oil containing tricresyl

386    phosphate. *Journal of Toxicology and Environmental Health, Part A*. 57, 293–328.

387    Murawski, J.T.L., Supplee, D.S., 2008. An attempt to characterize the frequency, health impact and

388    operational costs of oil in the cabin and flight deck supply air on US commercial flights. ASTM Journal. 5,

389    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