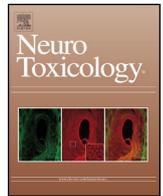


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NeuroToxicology



Commentary

## Health risk assessment of exposure to TriCresyl Phosphates (TCPs) in aircraft: A commentary

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### ABSTRACT

Possible exposure to TriCresyl Phosphates (TCPs) has led to concerns among airline crew members. One isomer, Tri-*ortho*-Cresyl Phosphate (ToCP) is known to be neurotoxic and exposure to ToCP via contaminated cabin air has been suggested to be associated with the alleged Aerotoxic syndrome. The symptoms associated with Aerotoxic syndrome are diverse, including headaches, loss of balance, numbness and neurobehavioral abnormalities such as emotional instability, depression and cognitive dysfunction. Other *ortho*-isomers are toxic as well, but the non-*ortho* isomers are regarded as less toxic.

In a collaborative effort to increase insight into the possible association between exposure to TCPs via contaminated cabin air and Aerotoxic syndrome, we performed an exposure- and toxicological risk assessment. Measurements in KLM 737 aircraft have demonstrated the presence of non-*ortho* isomers in low concentrations, though ToCP and other *ortho*-isomers could not be detected. Based on this exposure assessment, we established a toxicological risk model that also takes into account human differences in bioactivation and detoxification to derive a hazard quotient. From this model it appears unlikely that the health effects and alleged Aerotoxic syndrome are due to exposure to ToCP. Alternative explanations for the reported symptoms are discussed, but evaluation of the current findings in light of the criteria for occupational disease leads to the conclusion that the Aerotoxic Syndrome cannot be regarded as such. Additional research is thus required to unravel the underlying causes for the reported health complaints.

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## 1. Introduction

As a result of a major human exposure event in the 1930s, information on the delayed (peripheral) neurotoxicity of tricresyl phosphate (TCP) has been gathered over the decennia (Kidd and Langworthy, 1933). This major human exposure event was the result of consumption of (large) amounts of so-called Jamaica ginger as a consequence of the Prohibition laws. This Jamaica ginger was contaminated with tri-*ortho*-cresyl phosphate (ToCP), which was

later proven to be a neurotoxic compound that causes axonal damage to the nerve cells in the (human) nervous system. Other TCPs, in particular other *ortho*-cresol-containing isomers, may have similar effects as ToCP, while the *meta*- and *para*-cresol containing isomers are generally considered less toxic (Henschler, 1958).

In more recent years, TCPs have been used as e.g. plasticizer, flame retardant and additive in lubricants, hydraulic fluids and engine oil. Due to the use of TCPs in these applications, human exposure to TCPs could occur in occupational settings, e.g. in the cockpit and cabin of aircraft as a result of leakage of engine oil into the air conditioning systems during flight. The possibility of such exposures to TCP isomers in cabin air has led to concerns among airline crew members since it has been suggested that exposure to ToCP may affect the health of pilots and cabin personnel, resulting in the so-called Aerotoxic syndrome (Winder et al., 2002; Ross, 2008; Furlong, 2011; Abou-Donia et al., 2013).

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Symptoms of Aerotoxic syndrome are diverse and include headaches, confusion, loss of balance, muscle weakness, numbness and neurobehavioral problems (Abou-Donia, 2003; Michaelis, 2003; Coxon, 2002; van Netten, 1999; Montgomery et al., 1977). As a consequence of the proposed association of exposure to ToCP with Aerotoxic syndrome symptoms, the level of ToCP in commercial TCP mixtures has been reduced over time (DeNola et al., 2008). On the other hand, the suggested association of exposure to ToCP with Aerotoxic syndrome symptoms requires confirmation as (occupational) exposure to cabin air is also known to increase exposure to pathogens, carbon dioxide, barometric pressure changes, noise/vibration, radiation and numerous other factors that may affect health (Abeyratne, 2002; Hocking, 2002; Rayman, 1997). KLM Health Services therefore invited a number of experts from the Institute for Risk Assessment Sciences (IRAS-Utrecht University), the Netherlands Center for Occupational Diseases (NCvB-University of Amsterdam), the Leiden Academic Centre for Drug Research (LACDR-Leiden University) and the European Society of Aerospace Medicine (ESAM) to perform an exposure- and toxicological risk assessment of TCPs to increase insight into the possible association between exposure to TCPs via contaminated cabin air and the alleged Aerotoxic syndrome. To that aim, KLM Royal Dutch Airlines and the Netherlands Organization for Applied Scientific Research (TNO) recently collected data on TCP exposure in the cockpit during a number of flights (Houtzager et al., 2013). In this commentary, the results and implications of these analyses will be discussed in the light of known biological and toxicological effects after exposure to ToCP, the TCP isomer for which most information is available. This commentary will not address exposure during so-called fume events, but focuses on the possible risk of chronic exposure at low concentrations for air crew members.

## 2. Exposure assessment of TCP isomers in the cockpit

Boeing's 737 is the most widely used aircraft with first production in 1967 and over 8000 aircraft produced till mid-2014. The Dutch independent research organization TNO has measured concentrations of TCP isomers inside the cockpit during 20 flights of nine different Boeing 737-700, -800 and -900's aircraft (Houtzager et al., 2013). During each of the 20 flights, four air samples were taken inside the cockpit: one during climb, one during descent, one during cruise and finally one sample from the whole flight, covering all three phases thus representing a time-weighted average. In addition, wipe samples were taken from the glare shield before and after each flight.

For five TCP isomers pure analytical standards were available and used for quantification after chemical analysis using gas chromatography–mass spectrometry (GC–MS). These standards included T(*o,o,o*)CP (which is ToCP), and the four isomers with only the *p*- and *m*-cresols: T(*m,m,m*)CP, T(*m,m,p*)CP, T(*m,p,p*)CP and T(*p,p,p*)CP. Pure analytical standards of the other T(*o*)CPs are not available; however, the GC–MS method employed would have allowed their detection based on molecular mass without elucidating the isomeric structure. Detection and quantification of the TCPs by GC–MS was done as described previously (DeNola et al., 2008; Solbu et al., 2007).

Results from this exposure assessment demonstrated that ToCP levels in the cockpit air samples were below the limit of detection, which varied slightly depending on the length of the flight (0.3–0.75 ng/m<sup>3</sup>). The other TCP isomers could be detected in the ng/m<sup>3</sup> concentration range in 10 out of 20 flights (see Table 1). In the remaining flights all TCPs were below the limit of detection.

During climb, 12 of the 20 flights were negative. However, this phase also showed the single highest level of total TCPs observed in this study, 155 ng/m<sup>3</sup>. In the whole flight sample a maximum

**Table 1**

Exposure assessment of total TCPs in 20 flights with nine Boeing 737s. Samples were collected at different phases of the flight and TCP values are expressed in ng/m<sup>3</sup>. Mean and median values are calculated from the 'positive flights' only, with the number of positive flights indicated between brackets.

Total TCPs	Flight phase			
	Climb	Cruise	Descent	Whole flight
Minimum	1.8	0.53	1.3	0.27
Maximum	155	17	66	32
Mean	25 (8)	4.7 (9)	15 (10)	6.9 (9)
Median	5.9 (8)	2.9 (9)	6.0 (10)	2.9 (9)

concentration of total TCPs 32 ng/m<sup>3</sup> was observed. It should be noted that the median values for flights with positive detection of TCPs were (much) lower than their mean values. This observation reflects the comparatively very high values of the apparent incidental outliers. These incidental high maximum values may suggest that rather than gaseous TCP dissolved in air, small TCP-containing particles may be (infrequently) released in the air provided to the cockpit (CAA, 2004). This would also explain why the minimum value for the 'whole flight' measurement can be lower than the minimum values for all three separate flight phases (Table 1). Further investigations are required to substantiate this suggestion.

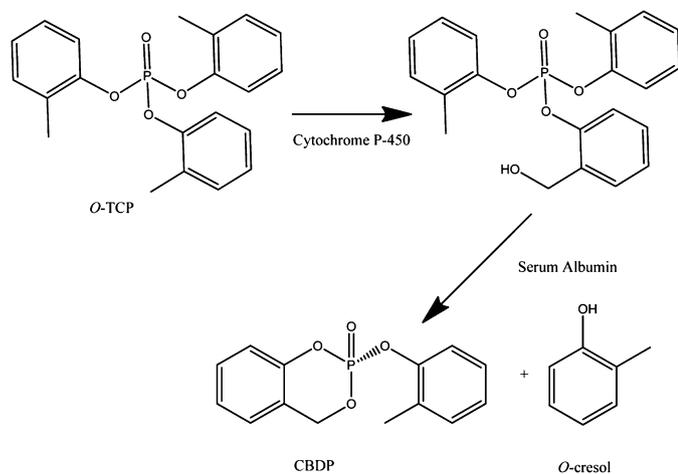
Collectively, the results from the chemical analyses show that during many of the investigated flights none of the TCPs could be measured above the detection limit of approximately 1 ng/m<sup>3</sup>. In those flights where TCPs were detected, the levels were in the ng/m<sup>3</sup>, but could vary up to two orders of magnitude (see Table 1). From a toxicological point of view it is interesting to note that ToCP was not detectable in any of the 20 flights that were studied.

Results from the wipe samples of the glare shield demonstrated the presence of small amounts of TCP isomers, at levels below 0.1 ng/cm<sup>2</sup>. Again, ToCP was not detected on the glare shield of these Boeing 737's, which is in line with its absence in the cockpit air samples. Subsequent analysis of the engine oil from BP used in these Boeing 737s also showed that ToCP was not detectable, giving further support to the absence of ToCP in the cockpit air and on the glare shield. In fact, none of the *ortho*-containing TCPs could be detected in engine oil by GC–MS. Notably, according to the manufacturer, the oil used contains less than 0.2% *ortho*-isomers (typically *ortho*-isomers range 0.03–0.06%), which would be below the current level of detection. Roughly equal shares of *meta*- and *para*-containing TCP isomers were present in the engine oil. Although the pure reference compounds were not available, the used detection method would have allowed for the detection of any peaks of *ortho*-containing TCPs. Interestingly, the chemical analysis revealed a strong correlation of the non-*ortho* TCP isomeric profile in the engine oil and the cockpit air and wipe samples. This observation supports the suggestion that the non-*ortho* TCPs found in the cockpit indeed originate from (leakage of) the engine oil.

## 3. Biomonitoring of TCP exposure

### 3.1. Adducts of ToCP with butyrylcholinesterase

Liyasova et al. (2011) developed a test to determine (long-term) exposure to ToCP, in particular to its proposed toxic metabolite, 2-(2-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide (CBDP). The basic assumption is that cytochrome P450 (CYP) mediates the conversion of ToCP to its reactive metabolite CBDP (Fig. 1), which has been proposed to be the (major) causal agent for ToCP-induced delayed neurotoxicity (Aldridge, 1954; Eto et al., 1962). At present, it still remains to be determined which human P450 enzyme(s) is (are) involved in this bioactivation.



**Fig. 1.** Schematic representation of the Cytochrome P450-mediated conversion of ToCP to its reactive metabolite CBDP.

The reactive intermediate CBDP binds covalently to a serine moiety of butyrylcholinesterase in blood and the resulting adduct can be determined by mass-spectrometry. This analytical method has a firm mechanistic base and is very sensitive, though little data on its specificity and reproducibility have been published (Liyasova et al., 2011). So far, only twelve aircraft passengers have been tested. Six of these passengers were positive for phospho-serine binding, albeit at low blood levels. Furthermore, it should be noted that none of these subjects showed symptoms related to organophosphorous intoxication or the alleged Aero-toxic syndrome.

In their discussion, Liyasova et al. (2011) mention that a further extension of their work with more individuals is required to test the validity of this newly developed method. Moreover, identification of a potential relationship between the levels of the detected adducts and adverse health effects is required if this method is going to be used for risk- and exposure assessment of ToCP(s). Whether other *ortho*-containing TCPs could eventually form similar reactive intermediates has not been investigated, but seems mechanistically plausible. However, the formation of such reactive intermediates from the *para*- or *meta*-containing TCP isomers appears unlikely from a mechanistic chemical point of view (personal communication G. Van der Marel, dept of Organic Chemistry Leiden University, The Netherlands).

### 3.2. Auto-antibodies in air crew members

In a recent exploratory study, auto-antibodies in serum against a number of proteins present in the (central) nervous system were measured (Abou-Donia et al., 2013). These auto-antibodies may be formed and released into the bloodstream upon damage to the cells of the nervous system. Serum samples of 34 flight crew members with central nervous system (CNS)-related complaints and of 12 healthy age-matched controls that had no connection with the aviation industry and no neurological symptoms were analyzed. Although the auto-antibody levels of the flight crew members and the controls showed some overlap, the levels of auto-antibodies were clearly (much) higher in many air crew members. In addition, one case subject showed an increase in auto-antibodies shortly after flying, which decreased only slowly over the subsequent months of non-flying.

There is little doubt that the presence of auto-antibodies relates to the presence of some sort of neurodegenerative process, though different auto-antibodies may not be equally predictive/sensitive. It was recently demonstrated that in particular auto-antibodies of

Glial Fibrillary Acidic Protein (GFAP) are related to (traumatic) brain injury (Papa et al., 2014). However, whether this also holds for (chronic) chemically-induced neurotoxicity needs to be confirmed. More importantly, the control group in the study from Abou-Donia et al. (2013) may not be very appropriate as it has no connection with aviation industry and also is not suffering from any CNS-related complaints. It therefore remains to be confirmed if the presence of auto-antibodies is associated with flying, with CNS-related complaints, or both. It can therefore be debated whether the selected control group in this otherwise interesting study is suited to demonstrate an association between flying and the presence of auto-antibodies. Future studies should therefore include a larger and more representative control group of crew members with the same flight history, but without health complaints. Additionally, it remains to be determined whether the auto-bodies are increased as a result of exposure to ToCP, or result from physical, physiological or chemical factors associated with flying. If the results of this study are confirmed with additional epidemiological studies, the increase or presence of these auto-antibodies in blood may prove valuable as a biomarker of exposure and/or neurodegenerative effect.

### 4. Risk assessment of ToCP exposure using a toxicological model

Based on the exposure assessment described above, a toxicological model was developed to derive a Hazard Quotient (HQ; also see EPA's National-Scale Air Toxics Assessment) for ToCP (Fig. 2). No ToCP was measured above the detection limit, so in the model the average detection limit ( $0.5 \text{ ng/m}^3$ ) was applied as maximal exposure for the cockpit crew. A daily exposure was assumed of six flight hours, equivalent with three daily flights of 2 h and an average air consumption of  $3 \text{ m}^3$ .

Based on the detection limit of ToCP ( $0.5 \text{ ng/m}^3$ ) maximum uptake via inhalation with a 100% bioavailability would amount up to  $0.02 \text{ ng/kg}$  body weight per day for a crew member of 70 kg (Step 1, Fig. 2). This level of exposure was compared with the available lowest No-Observed Adverse Effect Level (NOAEL) of ToCP that was established in chickens and amounts to  $1.25 \text{ mg/kg/d}$  after a repeated daily oral dose for 90 days (Craig and Barth, 1999). With respect to toxicological effects of ToCP it should be noted that NOAELs and Lowest Observed Adverse Effect Levels (LOAELs) were determined for two animal species; chicken and cat (reviewed in Craig and Barth, 1999; Johannsen, 1977; Ehrich and Jortner, 1999). For the model,  $1 \text{ mg/kg}$  body weight per day was used as a NOAEL. It is recognized that the NOAELs and LOAELs have been obtained from toxicity studies done several decades ago that focused exclusively on major clinical symptoms, such as neuropathology, and not more recently developed neurobehavioral tests. More subtle neurobehavioral changes are usually seen at lower dose levels than those associated with neuropathology. Therefore, an uncertainty factor (UF) of 5 was applied to the selected NOAEL. The combined toxicity studies with two non-rodent animal species – chicken and cat – indicate a rather close similarity in NOAELs and LOAELs for ToCP. From a neurotoxicity point of view these two species are also considered to represent the human sensitivity rather well. It is therefore not necessary to add an additional uncertainty factor for comparison with the human situation (UF = 1). This approach is shown in step 2 in Fig. 2.

Steps 1 and 2 in the model do not take into account any specific sensitive human subpopulation with respect to the mechanism of neurotoxicity of ToCP. Although the mechanism for individual sensitivity is not fully elucidated yet, it is known that cytochrome P450 enzymes are involved in the dearylation and bioactivation of ToCP. Given the structural resemblance of ToCP with known organophosphate-substrates of paraoxonase 1 (PON1), PON1 is

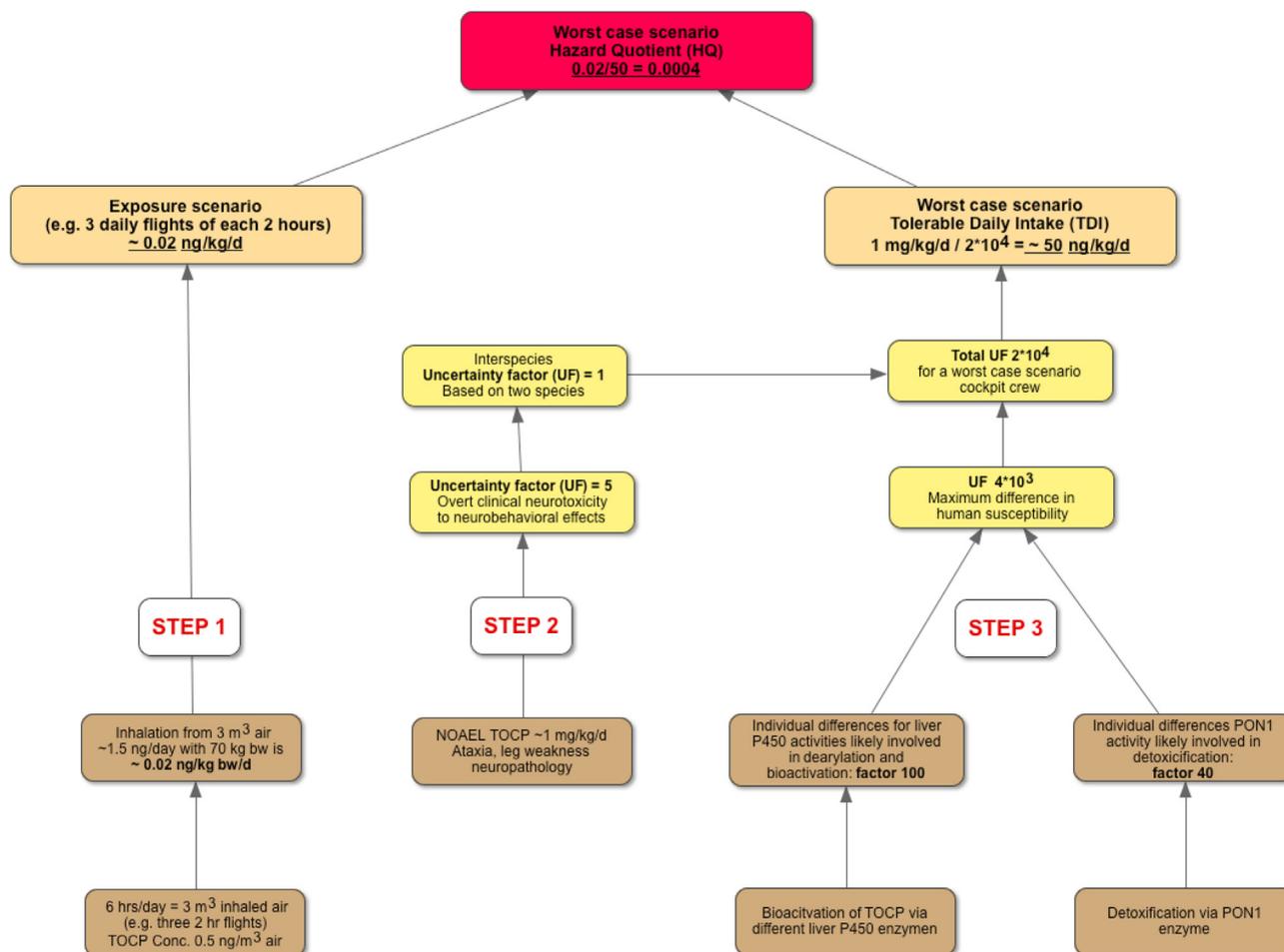


Fig. 2. Schematic presentation of the model that estimates the risk of potential ToCP exposure in KLM B737 airplanes for cockpit crews.

likely responsible for its detoxification. Therefore, the differences in human activities in both type of enzymes based on pharmacological studies and metabolism studies using organophosphate pesticides have been evaluated. The studies revealed that from cytochrome P450 2C19, 3A4, 2D6 and 1A2 one or more of these enzymes will likely play a role of importance in the metabolism of organophosphorous pesticides such as like diazinon, chlorpyrifos and parathion (Mutch and Williams, 2006). Human studies with cytochrome P450 2C19, 3A4, 2D6 and 1A2 enzymes have indicated a difference in individual constitutive hepatic activity of approximately 50–100-fold (Tamminga et al., 1999; Rendic and Di Carlo, 1997; Hägg et al., 2001). Consequently, a 100-fold uncertainty factor for P450 activity was included for bioactivation of ToCP. Similarly, a 40-fold difference in PON1 constitutive activity was found in humans (Costa et al., 2005). As a result of these inter-individual differences in P450 and PON1 enzyme activities a 4000-fold difference can be expected between individuals expressing a very low and very high sensitivity. As a result the combined uncertainty factor for metabolism and clinical/neuropathological symptoms to neurobehavioral effects in the model was set to 4000 \* 5 and applied on the ToCP NOEL of 1 mg/kg body weight per day. This approach leads to a tolerable daily intake (TDI) for the most sensitive subpopulation of cabin crew of 50 ng/kg body weight per day (see step 3 in Fig. 2). If this result is combined with the estimated exposure of 0.02 ng/kg body weight for crew members, it leads to a HQ of 0.02/50 = 0.0004, which is four orders of magnitude lower than an HQ > 1 (HQ > 1 would be a reason for health concern).

When using this model, several restrictions and limitations apply. Firstly, the calculated HQ is derived from a *worst case scenario* with respect to differences in individual enzyme activities and *focusing on a highly sensitive subpopulation*. Secondly, it only applies for the Boeing's 737 from KLM studied in the TNO exposure assessment using the BP engine oil indicated. It may very well be that the exposure situation may have been different in the past, for other aircraft models or flight schedules and other engine oils. Such a possibility is supported by the fact that an earlier study found ToCP levels in aircraft that were in the range of 1–20 µg/m<sup>3</sup> (Cranfield University, 2011), which is three to four orders of magnitude higher than our worst case scenario approach with 0.5 ng/m<sup>3</sup> (Houtzager et al., 2013) and would correspond to a HQ of 1–20 and thus a potential health risk for the few sensitive individuals that have both a high cytochrome P450 and low PON1 activity.

Additionally, the model does not take into account the occurrence of 'fume events'. Fume events can have various causes, including leakage of engine oil into the compressed air due to overfill or damaged sealants. Such fume events are characterized by smell (typically described as a wet sock or wet dog smell) or even visible smoke. The occurrence of fume events is unpredictable and the chemical composition of the fumes is likely to be highly variable. Exposure assessment during fume events is therefore challenging and exposure data in relation to fume events are virtually absent, although chemical exposure (possibly also to TCPs) is likely much higher during fume events than during normal flight scenarios as used for our model.

## 5. Assessment of effects during controlled human exposure

As yet, the information on effects of ToCP exposure on humans is limited. Except for the accidental exposure in the 1920–1930s, very limited data on epidemiology have been summarized by the ACGIH (2014). A controlled-exposure laboratory study in an inhalation chamber would make it possible to study the effects of many variables on humans (Rom et al., 2013) or laboratory animals.

So far, no method has been published for controlled ToCP exposure. ToCP is a non-volatile compound with a very low vapor pressure ( $5\text{--}10 \times 10^{-5}$  Pa), so it will easily attach to surrounding structures. Thus, obtaining a constant predictable concentration by releasing ToCP in an inhalation chamber will be very complicated. Administration through a mask is equally problematic as ToCP will attach to the inside of the tube. In order to do such experiments, a dynamic controlled gas generation system would have to be developed. However, it is rather unlikely that a medical-ethical committee will approve such an investigation with volunteers even if the concentration of the ToCP would not be higher than those in the cockpit. Moreover, the complaints of air craft personnel are difficult to measure objectively since they consist of CNS effects such as memory deficits, fatigue, headache, confusion and anxiety. Association of such effects with ToCP exposure requires a double blind experimental design to determine the causal relationship between exposure and complaints. However, there are as yet no objective physiological markers for ToCP exposure, making an objective assessment between (occupational) exposure to low concentrations of ToCP and health effects virtually impossible at this moment.

## 6. Additional modes of action and relative toxicity of TCP isomers

TCPs are structurally related to organophosphorous (OP) insecticides, which are known as acetylcholine esterase (AChE) inhibitors, although TCPs are not very potent inhibitors of AChE. However, chronic (high dose) exposure to *ortho*-containing TCPs and some OP insecticides is related to OP-induced delayed neurotoxicity (OPIDN), which appears unrelated to inhibition of AChE. This delayed neurotoxicity of TCP exposure appears primarily due to *ortho*-containing TCPs since exposure to a TCP mixture with a low (3%) *ortho*-isomer content induced less adverse effects in cats and chickens than a mixture with a high (25%) *ortho*-isomer content (Henschler, 1958). It is therefore unlikely that the delayed adverse effects are due to exposure to non-*ortho* isomers. However, TCPs may actually have more than one mode of action. Organophosphorous insecticides inhibit AChE and induce delayed neurotoxicity, likely via inhibition of neuropathy target esterase. However, organophosphorous insecticides have also been shown to inhibit ACh neurotransmitter receptors (Smulders et al., 2004) as well as voltage-gated calcium channels (Meijer et al., 2014). Yet, it is currently unknown if ToCP or any other TCP isomers also exert these neurotoxic effects. Additionally, the health effects reported in relation to the alleged Aerotoxic syndrome have to date not been associated with these additional possible modes of action. Future toxicological research should thus not be limited to investigating the effects of TCPs on known modes of action, but should rather focus on deriving a full hazard characterization of the toxicity of *ortho*-containing TCPs and non-*ortho* TCP isomers.

## 7. Alternative explanations for symptoms of the Aerotoxic syndrome

In literature, health problems that are mentioned in a possible relation with bleed air exposure or the alleged Aerotoxic syndrome

include nausea, headaches, confusion, loss of balance, lightheadedness, muscle weakness, shortness of breath, movement disorders, numbness, and paraesthesias. Neurobehavioral problems include cognitive dysfunction, emotional instability, depression, sleep and anxiety disorders (Abou-Donia, 2003; Michaelis, 2003; Coxon, 2002; van Netten, 1999; Montgomery et al., 1977). The symptoms associated with bleed air exposure or the Aerotoxic syndrome are thus diverse, the relation of these symptoms with OPIDN is unclear, and there is no common pattern of symptoms that can readily be identified as being characteristic of “cabin air quality incidents” (CAA, 2004). Among a group of 34 affected aircrew members, more than 50% reported headaches and fatigue (Abou-Donia et al., 2013). These symptoms, which are very common in general working populations (Ricci et al., 2007; Sokolovic et al., 2013; Kerber et al., 2008), can also be caused by a variety of occupational factors that may affect the health of aircrew, such as exposure to carbon-monoxide (Prockop and Chichkova, 2007), hypoxia (Simons & Krol, 1996), ozone (De Ree et al., 2000), insecticides (Murawski, 2005), de-icing fluids (SAE, 1997), exhaust fumes from ground service vehicles/other aircraft, impaired sleep, circadian disruptions, long work hours, and irregular work-rest cycles (Caldwell, 1997; Petrie et al., 2004; Jackson & Laurie, 2006; Reis et al., 2013). It can be concluded that diverse chronic symptoms are associated with contamination of aircraft cabin air and that these symptoms are not in themselves characteristic and, therefore, suggestive of any specific form of chemical toxicity (CAA, 2004).

## 8. Aerotoxic syndrome, is it an occupational disease?

To determine if an illness or syndrome is work related, in The Netherlands a five steps procedure is currently used. In the near future a sixth step will be added related to prevention.

### 1. Establishing the diagnosis

The alleged Aerotoxic Syndrome is characterized by many different symptoms, such as reduced attention and concentration as well as memory impairment and general malaise. It is suggested that these symptoms are caused by exposure to ToCP in the air inside aircraft cabins. Until now, there is no definitive scientific proof for the existence of the syndrome.

### 2. Is there a relationship between exposure to ToCP in the aircraft and the occurrence of symptoms associated with the alleged Aerotoxic Syndrome?

There is an occupational exposure limit for ToCP of 0.1 mg/m<sup>3</sup>. This is an 8-h time-weighted average. There is also a peak limit of 0.3 mg/m<sup>3</sup> for 15 min. It has been calculated that in a worst case scenario all engine oil could leak into the air conditioning system. This would then lead to an exposure of 0.025 mg/m<sup>3</sup>, which is still below the peak limit value. With this, a relationship between exposure during normal operation and the reported symptoms is unlikely.

### 3. What is the actual exposure to ToCP?

In the TNO study (Houtzager et al., 2013), no ToCP in concentrations above the detection limit was detected in the air and wipe samples or the used engine oil. Other TCP isomers in cockpit air were 6.9 ng/m<sup>3</sup> on average, with minimum 0.5 ng/m<sup>3</sup> (limit of detection) and maximum 155 ng/m<sup>3</sup>.

### 4. What is the influence of confounding factors?

Exposure is far below the limit of 100,000 ng/m<sup>3</sup>. Therefore, other possible explanations for the symptoms must be considered. One possible explanation may be increased sensitivity that may explain a relationship between exposure and symptoms.

### 5. Conclusion about the relationship between exposure and symptoms.

During this step the diagnostic process of the preceding steps is evaluated to see whether it is possible to draw a conclusion about the probability of causal relationship. It is important that the medical assessment according to protocol has been carried out.

#### 6. What can be done to prevent an occupational illness?

During this step preventive measures are indicated, carried out and implemented when applicable. To date this is not the case in this particular subject.

The research protocol to assess medical symptoms caused by neurotoxic exposure consists of five steps:

1. Intake by clinical occupational physician.
2. Neuropsychological assessment according to standard protocol looking at attention, concentration, memory, information processing speed and planning.
3. Explorative neurological screening.
4. Exposure estimation by occupational hygienist.
5. Explorative blood sampling into hematological, liver- and renal function and vitamins (B1 and B12).

De results of steps 1–5 will be discussed with various medical specialists to decide whether there is a causal relationship between exposure and symptoms. Now that the exposure has been measured (step 4 in the research protocol above) the research protocol can be used to assess the symptoms of crew members. Because the exposure to ToCP is very low a causal relationship between the symptoms and exposure to ToCP is unlikely and further study is necessary into other possible explanations for the symptoms.

## 9. Conclusions

- Exposure assessment of ToCP in 20 flights of nine Boeing 737s from KLM, a very common aircraft type with over 8000 aircraft delivered, have shown no ToCP concentrations above the detection limit of 0.5 ng/m<sup>3</sup>.
- The median concentration of non-ortho TCP isomers (total TCP) was below 6 ng/m<sup>3</sup>. Although there is little actual evidence, non-ortho isomers are regarded as less toxic than ToCP. This notion is reflected in newly proposed limits for the meta- and para-TCP isomers, which are higher than those for ToCP. The measured concentrations of non-ortho TCP isomers are far below these limits, making it unlikely that exposure to non-ortho TCPs is the explanation for the symptoms of the alleged Aerotoxic syndrome. However, additional studies are required to complement the evidence on toxicity of non-ortho isomers.
- One study has indicated that aircrew members carry more auto-antibodies than persons working on the ground. This finding must be substantiated in further studies and a possible relationship with symptoms of the Aerotoxic syndrome must be explored.
- Using a risk assessment model with detection limit values of ToCP as input and the available toxicological evidence from earlier studies leads to the conclusion that it is highly unlikely that symptoms of the Aerotoxic syndrome can be explained along the lines of ToCP intoxication.
- There are several alternative explanations for the symptoms of the Aerotoxic syndrome and these should be the subject of further study.
- With the currently available scientific evidence, the symptoms of the Aerotoxic syndrome do not constitute an occupational disease in the Netherlands.

## Conflict of interest statement

Hans de Ree and Brinio Veldhuijzen van Zanten are employed by KLM Health Services; Gerard J. Mulder acts as contracted

advisor of KLM Health Services; Teus Brand acts as independent advisor of KLM Health Services. The other authors do not have any competing financial interest or any other conflict of interest regarding the submitted article.

## Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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