Cholinesterase Inhibition and Exposure to Organophosphate Esters in Aircraft Maintenance Workers

Jennifer E. Hardos; Mitch Rubenstein; Sarah Pfahler; Trevor Sleight

INTRODUCTION: Aircraft maintenance workers may be exposed to organophosphates in hydraulic fluid and engine oil. Previous research has indicated that inhalation may not be the primary exposure route. This study sought to measure dermal contact and inhalation in conjunction with cholinesterase inhibition and determine if Air Force Specialty Code serves as an exposure predictor.

METHODS: Aircraft maintenance workers were sampled for changes in acetylcholinesterase and butyrylcholinesterase. Dermal contact was measured using wrist-worn silicone passive dosimeters and inhalation exposure was measured using thermal desorption tube air sampling.

RESULTS: Overall prevalence of any cholinesterase inhibition in the study population was 25.33%. Prevalence of inhibition of acetylcholinesterase and butyrylcholinesterase was 18.67% and 6.67%, respectively. The mean tributyl phosphate result was 1.71 ng of tributyl phosphate per gram of wristband ($ng \cdot g^{-1}$) [95% confidence interval (CI): −5.63, 9.05]. Triphenyl phosphate was more prevalent, with only one sample below the limit of detection (mean 1386.26 $ng \cdot g^{-1}$; 95% CI: −7297.78, 10,070.31), and tricresyl phosphate was found in every sample (mean 4311.65 $ng \cdot g^{-1}$; 95% CI: −8890.24, 17,512.31). No organophosphates were detected via air sampling.

DISCUSSION: Workers experienced organophosphate exposure and cholinesterase inhibition, but the study was not large enough to establish a statistically significant association between exposure and disease. Exposure to organophosphate esters is more likely to occur through contact and absorption of chemicals through the skin than through inhalation of oil mists. Air Force Specialty Code does not appear to be a good predictor of exposure to organophosphates. Future studies should consider using a larger sample size.

KEYWORDS: cholinesterase, organophosphate, aircraft maintenance.

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Organophosphates are known for their inhibition of cholinesterase activity, a component of nervous system function. Previous research has indicated Air Force use of products containing organophosphates in aircraft maintenance processes. There are three primary compounds of concern in the aircraft industry: tricresyl phosphate (TCP), tributyl phosphate (TBP), and triphenyl phosphate (TPP). All three chemicals are cholinesterase inhibitors.[1,3] While acute exposure can cause symptoms similar to those of nerve agents, lower level long-term exposures are speculated to cause neurological and behavioral symptoms, including personality change, mood destabilization, suicidal thoughts, and memory and attention impairment.[4] This hypothesis has not been scientifically validated, but it warrants consideration.

Research suggests a link between organophosphates and depression.[4,11,12] In an Air Force study conducted in 2014, self-reported turbine oil and hydraulic fluid exposure in aircraft maintenance workers was associated with prevalence and severity of depression.[7] In the same study, air sample results were inconclusive for inhalation exposure to organophosphates

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during aircraft engine and hydraulic maintenance processes, so quantifiable exposure could not be compared to depression prevalence. However, skin absorption potential was noted. Workers surveyed via online questionnaire also self-reported routine exposure via all routes, including ingestion of chemicals.[7]

Contact and ingestion exposure are difficult to measure, but may be significant contributors to total exposure. The most comprehensive method of estimating total dose is by measuring biomarkers in blood or urine. Specifically, quantifying red blood cell acetylcholinesterase (AChE) and serum butyrylcholinesterase (BuChE) inhibition in tandem captures an efficient measure of exposure to all organophosphates.[8,9]

Dermal exposure to organophosphates in the aircraft maintenance environment has yet to be measured, although it has been visually estimated in prior studies.[7] Silicone wristbands have been demonstrated as passive samplers for TPP, TBP, and several isomers of TCP and may be a viable method in the workplace.[10] Previous methods involve hand wiping or washing, which only capture exposure over shorter periods.

The U.S. Air Force maintains over 5000 active aircraft, including the Reserve and Air National Guard inventory.[2] The maintenance processes required to keep these aircraft operational include high temperatures or pressures and heavy contact with fluids, elevating the potential for exposure to organophosphates in engine oil and hydraulic fluid. Long-term, low-level occupational exposure to these chemicals has been associated with depressive symptoms and mood changes, and aircraft maintenance workers have one of the two highest suicide rates in the Air Force.[5,11,12] It is not currently known which maintenance career fields have significant exposures. With maintenance being performed throughout the world and in austere environments, a proven skin sampling technique is also needed to assess exposure when other methods are not sufficient or manpower prohibits extensive sampling.

METHODS

Subjects

Subjects were recruited from the total population of active duty aircraft maintenance workers at Moody Air Force Base (AFB), GA, USA; Hill AFB, UT, USA; and Davis-Monthan AFB, AZ, USA. Thus, subjects were active duty Air Force aircraft maintenance workers with a 2AXXX Air Force Specialty Code (AFSC) (XXX indicating various codes for subspecialties), or job code, performing work in their primary field at the time of study, who consented to participate. The 2AXXX subspecialties include those who work in propulsion, hydraulics, aerospace maintenance (e.g., crew chiefs), aerospace ground equipment, communications and navigation systems, avionics, nondestructive inspection, structural maintenance, metals technology, electrical and environmental systems, and aircraft egress systems. Subjects gave informed consent at enrollment. Subjects taking physostigmine were excluded from the study due to the medication's interference with blood cholinesterase levels.

Design

Prior to commencement, the study was approved by the Institutional Review Board at the Air Force Research Laboratory. Pre- and postshift blood samples were drawn from a cross-sectional sample of military personnel who were working in the aircraft maintenance field. In addition, subjects wore two silicone passive dosimeters on the dominant wrist throughout the sampled work shift, and a smaller subset of subjects underwent thermal desorption tube personal air sampling for the duration of the sampled shift. The study was conducted at three Air Force bases: Moody AFB, GA, USA; Hill AFB, UT, USA; and Davis-Monthan AFB, AZ, USA. Blood samples were analyzed for BuChE and AChE activity using the Ellman assay method.[6] This assay uses the addition of acetylthiocholine and butyrylthiocholine to initiate enzymatic activity. If there were increased levels of organophosphate in the blood, then this would be evident by its interference in the hydrolysis of acetylthiocholine and butyrylthiocholine by endogenous AChE/BuChE, as quantified by measuring hydrolysis products with a photometer. The more organophosphate that is present in each sample, the less hydrolysis occurs, the lower the photometer reads. Results are a measurement of the maximum rate of enzymatic reaction (V_{max}). Passive dosimeters were analyzed via gas chromatography/mass spectrometry using a solvent extraction method. Thermal desorption tubes were analyzed using gas chromatography/mass spectrometry.

Statistical Analysis

Statistical analysis was conducted using Stata/SE 15.1 (StataCorp LLC, College Station, TX, USA). Power was not calculated because this study was designed as an initial attempt to determine where to focus future research because there were no data on which career fields, if any, have exposures. Thus, a smaller sample size was deemed acceptable. Biological monitoring results were quantified in duplicate before and after shifts, and each participant's postshift percent of baseline for each type of cholinesterase was computed by dividing the participant's average postshift cholinesterase by average preshift cholinesterase and multiplying the result by 100 to get a percentage. Cholinesterase inhibition results were then categorized according to severity using ranges established by Strelitz et al.[13] The "cut point" for inhibition was considered to be 80% of baseline (20% inhibition), with categorical variable for severity as displayed in **Table I**. Some participants' blood samples demonstrated an increase in cholinesterase rather than inhibition during the work shift and were thus placed in the category of no inhibition.

To determine whether mean cholinesterase percent of baseline, air sampling results, or passive dosimeter results differed by workplace, AChE and BuChE inhibition and passive dosimeter results were first summarized by AFSC. The continuous variables (cholinesterase inhibition, TCP concentration, TBP concentration, and TPP concentration) were then analyzed using one-way analysis of variance to determine if there was a difference between AFSC groups. The same analysis was performed for each chemical and for total organophosphates between bases. Air sample results were examined, but because the results were all "none detected" for organophosphates, air sample results could not be statistically analyzed for differences by workplace.

Table I. Cholinesterase Inhibition Categories Based on Post-Shift Percentage of Pre-Shift Baseline.

INHIBITION CATEGORY	ACETYLCHOLINESTERASE (% OF BASELINE)	BUTYRYLCHOLINESTERASE (% OF BASELINE)
None	>80	>80
Mild	70–80	60–80
Moderate	50–70	50–60
Severe	<50	<50

Passive sampler measurements of TBP, TPP, and TCP were compared to worker AChE and BuChE percent of baseline to determine if increase in skin exposure is associated with a similar increase in cholinesterase inhibition. Results of all participants as a whole were analyzed via linear regression with sampler results as a continuous dependent variable and bioassay results as a continuous independent variable. This was conducted for a combination of TBP, TPP, and TCP [total organophosphates summed together in nanograms of organophosphate per gram of wristband ($ng \cdot g^{-1}$)] in the passive sampler as well as for each chemical individually. A linear prediction graph was also constructed in Stata to demonstrate the relationship between total organophosphates on the passive dosimeter (on the Y axis) and total percent of baseline cholinesterase (on the X axis).

RESULTS

For this study, 76 participants were sampled. The study population was 91% male with a mean age of 25 yr old, with 73% between Airman First Class and Staff Sergeant rank, and 52% working day shift. No hydraulics, communications/navigation, or metals tech workers volunteered for the study, so there is a gap in representation for those groups.

Overall prevalence of any cholinesterase inhibition in the study population was 25.33%. Prevalence of inhibition of AChE and BuChE was 18.67% and 6.67%, respectively. The mean cholinesterase levels increased from baseline (beginning of shift) to the end of the sampled shift for both AChE (108.74% of baseline) and BuChE (107.49% of baseline). Aerospace ground equipment workers had the highest mean AChE inhibition (75.34% of baseline), while avionics workers had the highest mean BuChE inhibition (93.14% of baseline). Results are detailed by AFSC in **Table II** and by categorical severity in **Table III**.

One-way analysis of variance revealed no significant difference in percent of baseline of AChE between AFSCs [$F(8,66) = 0.55$, $P = 0.818$]. However, there was a difference in mean inhibition of BuChE [$F(8,66) = 2.41$, $P = 0.0238$]. There was also a difference in AChE mean inhibition between bases [$F(2,72) = 3.37$, $P = 0.0397$], but this was not the case for BuChE [$F(2,72) = 3.06$, $P = 0.0532$].

None of the target organophosphate compounds were detected using thermal desorption tube analysis. Very little TBP was found in the passive dosimeters, with only 14 of 79 samples above the laboratory limit of detection. The mean TBP result was $1.71\ ng \cdot g^{-1}$ [95% confidence interval (CI): −5.63, 9.05]. TPP was more prevalent, with only one sample below the limit of detection (mean $1386.26\ ng \cdot g^{-1}$; 95% CI: −7297.78, 10,070.31), and TCP was found in every sample (mean $4311.65\ ng \cdot g^{-1}$; 95% CI: −8890.24, 17,512.31).

There was a significant difference in TCP exposure between AFSCs [$F(8,70) = 4.02$, $P = 0.0006$], but this was not the case for TBP ($F = 1.47$, $P = 0.1838$) or TPP ($F = 0.65$, $P = 0.7342$). There was also a significant difference in total organophosphates by AFSC [$F(8,70) = 2.56$, $P = 0.0163$], likely because TCP made up the majority of the total amount of organophosphates found on the dosimeters. Passive dosimeter and cholinesterase inhibition results differed slightly by base, with Moody AFB workers exhibiting the highest mean cholinesterase inhibition, as well as the highest means for both TPP and TCP passive dosimeter sampling.

Maintenance workers who had exposed wristbands indicating exposure exceeding $500\ ng \cdot g^{-1}$ were slightly more likely to have cholinesterase inhibition. When compared to maintenance workers exposed above $1000\ ng \cdot g^{-1}$, the odds ratio was slightly above 2, but neither result was statistically significant. Results are included in **Table IV**.

Linear regression of total organophosphate wristband result compared to total percent cholinesterase inhibition suggested an increasing cholinesterase inhibition with increasing

Table II. Mean Cholinesterase Inhibition Between Pre-Shift and Post-Shift, as Measured by Blood Samples, by AFSC Category.

AFSC CATEGORY	AChE PERCENT OF BASELINE (95% CI)	BuChE PERCENT OF BASELINE (95% CI)
Avionics	98.89 (80.19, 117.59)	93.14 (−151.03, 337.31)
Crew Chief*	111.01 (3.05, 218.97)	105.17 (73.78, 136.56)
Propulsion*	103.66 (38.16, 169.16)	98.57 (70.83, 126.31)
Aerospace Ground Equipment	75.34 (71.92, 78.76)	173.19 (−70.97, 417.35)
Egress	128.31 (−62.45, 319.07)	120.75 (31.85, 209.65)
Fuel Systems Repair*	118.75 (32.13, 205.37)	112.33 (87.15, 137.51)
Electro-Environmental	110.03 (56.41, 163.65)	102.08 (64.89, 139.27)
NDI	112.41 (58.75, 166.07)	101.32 (92.3, 110.34)
Structural	92.44 (−36.14, 221.02)	107.99 (61.798, 154.18)
Overall	108.74 (12.98, 204.5)	107.49 (40.65, 174.33)

* Blood was not obtained from four workers: one crew chief, two from Propulsion, and one from Fuel Systems Repair. Those participants are not included in this table.

Table III. Cholinesterase Inhibition by Categorical Severity.

	NONE *N* (%)	MILD *N* (%)	MODERATE *N* (%)	SEVERE *N* (%)	TOTAL
AChE	61 (81.33)	11 (14.67)	1 (1.33)	2 (2.67)	75
BChE	70 (93.33)	4 (5.33)	1 (1.33)	0 (0)	75

exposure level, but was not significant ($P = 0.585$, **Fig. 1**). Likewise, regression of each compound's wristband results compared to AChE or BuChE was not significant, with one exception: TBP vs. AChE percent inhibition (coefficient = 0.019, $P = 0.034$).

DISCUSSION

This study demonstrated that aircraft maintenance workers do have the potential for cholinesterase inhibition and exposure to organophosphate esters. Mild cholinesterase inhibition was most common, but there were participants who exhibited moderate to severe inhibition. AFSC does not appear to be a good predictor of exposure to organophosphate esters. This may be explained by the small sample size or the close physical proximity of work done by multiple AFSCs. Some participants also conveyed that they were being used as long-term overlap for other AFSCs' responsibilities; therefore, their normal duties had been altered. Although there was a difference in inhibition between bases, this result may be influenced by the fact that each base tended to have a pool of participants from different career fields. Because some bases had more maintenance work going on during data collection than others, this may have created the appearance of an exposure difference among the career fields. For example, at the time of the research visit, Hill AFB had just deployed many of its aircraft, so there were fewer aircraft to maintain during the week of sampling.

Passive dosimeters proved useful in identifying chemical contact and absorption exposures in the workplace. Compared to the nondetectable concentration on the thermal desorption tubes, this provides some evidence that the route of exposure is from contact rather than inhalation for the group studied. In addition, passive dosimeter results ruled out TBP as a hazard in the sampled workplaces. This confirmed results of a previous study which did not find any TBP as a component in products used by the sampled workplaces. Conversely, TCP was found in every passive dosimeter sample and in much higher amounts on average than the other two organophosphates. Much like the cholinesterase inhibition results, the organophosphate results differed by location, likely based on the varied amount of work among locations.

Based on a review of the existing literature, this may be the first study of aircraft maintenance worker cholinesterase inhibition and exposure to organophosphate esters. Side-by-side air sampling and passive dosimeter sampling enabled confirmation of the hypothesis that exposure is not primarily by inhalation, but by other means. The aircraft maintenance population is also a relatively young and healthy worker population, reducing the amount of medical issues and medications that may affect cholinesterase inhibition, as well as confounding factors related to age.

The population studied was very small and was intended to be a screening cross-section of the overall aircraft maintenance population. Thus, the power for the study was very low. There were also AFSCs that were not represented during the study. In particular, the absence of hydraulics workers, who are known to work with fluids that contain organophosphates, may significantly influence overall outcomes. Also omitted from participants were communications/navigation workers and metals tech workers. There also seemed to be a trend for workers in the same AFSC to volunteer as groups from one base. Because of the difference in both airframe and workload between bases, those workers' exposures may not be representative of workers in their AFSC at other bases. Additional bias may be inherent because those workers who volunteered for the study may be the ones who have time to spare due to lighter workload, or they may feel more strongly about the study topic because of personal experience.

Further research may be useful in determining whether certain activities or operations lead to greater exposure to organophosphate esters and, as a result, cholinesterase inhibition. A long-term study with more participants as well as more frequent follow-ups and blood draws would capture the variation of work and exposure throughout the seasons, changing weather, or cyclical operations tempo, as well as provide more power. Ideally, additional research would also include assessment of ingestion exposure by examining worker habits.

This study confirmed that exposure to organophosphate esters is more likely to occur through contact and absorption of

Table IV. Crude Prevalence Odds Ratios for Any Cholinesterase Inhibition for Exposed and Unexposed Groups Based on Sampling and Self-Reported Exposure Categories.

GROUP	*N* (%)	PREVALENCE	PREVALENCE ODDS RATIO
Exposed workers (dosimeter results > 500)	50 (66.67)	14/50 = 0.28	1.56 (0.49, 4.95), $P = 0.455$
Unexposed workers (dosimeter results < 500)	25 (33.33)	5/25 = 0.20	1.00
Exposed workers (dosimeter results > 1000)	40 (53.33)	13/40 = 0.325	2.32 (0.77, 6.99), $P = 0.132$
Unexposed workers (dosimeter results < 1000)	35 (46.67)	6/35 = 0.171	1.00
Self-reported exposed	40 (53.33)	14/40 = 0.35	3.23 (1.025, 10.19), $P = 0.045$
Self-reported unexposed	35 (46.67)	5/35 = 0.0143	1.00
Total	75		

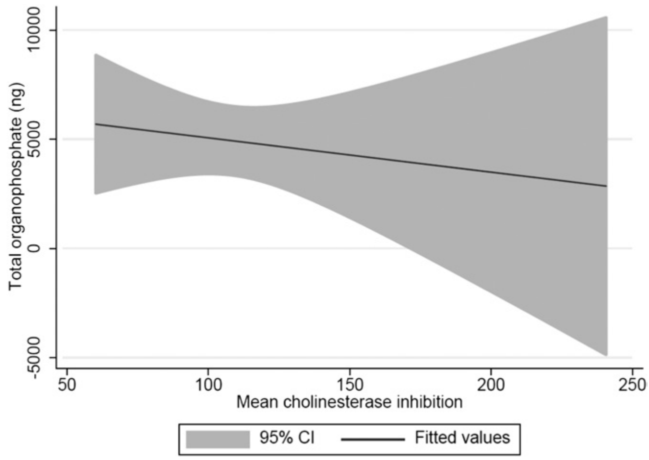

Fig. 1. Linear prediction (with 95% CI) of total organophosphate on passive dosimeter vs. cholinesterase inhibition, defined as mean postshift percent of baseline.

chemicals through the skin than through inhalation of oil mists. Dermal exposure to TCP was most common, followed by TPP and TBP, while no airborne exposure was detected. Workers did experience cholinesterase inhibition, but the study was not large enough to establish a statistically significant association between exposure and disease, defined as postshift cholinesterase at less than 80% of cholinesterase baseline.

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