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Organophosphate Skin Exposure and Biological Burden of Aircraft Maintainers

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Final Report
for October 2016 to February 2019

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14. ABSTRACT
This study aimed to test the hypothesis that aircraft maintainers are exposed to organophosphate esters during maintenance processes. It also sought to test a passive sampling method in the field. Specific aims included quantifying the biological burden of organophosphate esters in aircraft maintainers before and after shifts, determining which workplaces and processes produce the highest exposures, determining the feasibility of silicone passive samplers in the maintenance setting, and comparing silicone passive sampler results to worker bioassays. Results were assessed for difference in sample means between career fields (post-shift – pre-shift) using ANOVA analysis. Logistic regression was used to assess for association between self-reported exposure to organophosphate-containing materials and inhibition of cholinesterase between pre-and post-shift samples. Other covariates were also be considered, such as location, shift type (day, swing/mid, night), and personal protective equipment worn. Overall, this study confirmed that exposure to organophosphate esters is more likely to occur through contact and absorption of chemicals through the skin then through inhalation of oil mists. Exposure to tricresyl phosphate was most common, followed by triphenyl and tributyl phosphate. Workers did experience cholinesterase inhibition, but the study was not large enough to establish a statistically significant association between exposure and disease, defined as greater than 80 percent cholinesterase inhibition. The association between passive dosimeter exposure and self-reported exposure was also very high, so workers are able to identify when they have been exposed to organophosphate-containing products in the workplace.

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1.0 SUMMARY

This study aimed to test the hypothesis that aircraft maintainers are exposed to organophosphate esters during maintenance processes. It also sought to test a passive sampling method in the field.

Specific aims:

- Quantify the biological burden of organophosphate esters in aircraft maintainers before and after shifts.
- Determine which workplaces and processes produce the highest exposures.
- Determine the feasibility of silicone passive samplers in the maintenance setting.
- Compare silicone passive sampler results to worker bioassays.

Results were assessed for difference in sample means between career fields (post-shift – pre-shift) using ANOVA analysis. Logistic regression was used to assess for association between self-reported exposure to organophosphate-containing materials and inhibition of cholinesterase between pre-and post-shift samples. Other covariates were also be considered, such as location, shift type (day, swing/mid, night), and personal protective equipment worn.

Overall, this study confirmed that exposure to organophosphate esters is more likely to occur through contact and absorption of chemicals through the skin then through inhalation of oil mists. Exposure to tricresyl phosphate was most common, followed by triphenyl and tributyl phosphate. Workers did experience cholinesterase inhibition, but the study was not large enough to establish a statistically significant association between exposure and disease, defined as greater than 80 percent cholinesterase inhibition. The association between passive dosimeter exposure and self-reported exposure was also very high, so workers are able to identify when they have been exposed to organophosphate-containing products in the workplace.

2.0 BACKGROUND

Organophosphates are known for their inhibition 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 present in aircraft turbine oils or hydraulic fluids and are cholinesterase inhibitors [1, 2]. While acute exposure can cause symptoms similar to those of nerve agents, lower-level long-term exposures are believed to cause neurological and behavioral symptoms, including personality change, mood destabilization, suicidal thoughts, and memory and attention impairment [3].

The aforementioned neurological and behavioral symptoms tie into another recent issue: U.S. Air Force (USAF) aircraft maintainers' suicide rates. Although incidence rates per 100,000 stratified by Air Force Specialty Code (AFSC) are not reported, the DoD Suicide Event Report for 2014 attributed 33.3 percent of suicides for that calendar year to "electrical/mechanical equipment repairers" [4]. Research suggests a link between cholinesterase inhibitors and depression. Several human studies have found elevated depression prevalence in populations occupationally exposed to pesticides in both farming and sheep dipping jobs as compared to those with no exposure [3, 5, 6]. In a USAF study conducted in 2014, self-reported turbine oil and hydraulic fluid exposure in aircraft maintainers was associated with prevalence and severity of depression [7]. In the same study, air sample results were inconclusive for inhalation exposure to organophosphates 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. This method captures exposures by a combination of absorption, ingestion, and inhalation exposure. Specifically, quantifying serum acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) 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]. This silicone sampler technology represents an improvement over existing methodology. It provides a better method for assessing skin exposure over long periods of time. Previous methods involve hand wiping or washing, which only capture exposure over shorter periods. It will also require less manpower to monitor during the work shift than the visual estimation method currently recommended [11].

The USAF maintains over 5,000 active aircraft including the Reserve and Air National Guard inventory [12]. Most military airframes are older than the average airline aircraft and, with recent sequestration, leadership does not anticipate replacing many airframes within the next ten years [13]. The maintenance processes required to keep these aircraft operational include high temperatures or pressures and heavy contact with fluids, elevating potential for exposure to organophosphates in engine oil and hydraulic fluid. Long-term, low-level exposure to these chemicals is also associated with depressive symptoms and mood changes, and maintainers have one of the two highest suicide rates in the USAF [14]. 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.

3.0 METHODS

3.1 Study Design

This is a cross-sectional study of aircraft maintainer chemical exposure in the field. It is partially considered a pilot study to determine which aircraft maintenance career fields are exposed to organophosphates. It is also partially an early operational assessment of a technology (silicone bands). The study had four specific aims:

- Specific Aim #1: Quantify the biological burden of organophosphate esters in aircraft maintainers before and after shifts.
- Specific Aim #2: Determine which workplaces and processes produce the highest exposures based on biomonitoring results.
- Specific Aim #3: Determine the feasibility of silicone passive samplers in the maintenance setting.

- Specific Aim #4: Compare silicone passive sampler results to worker bioassays.

Results will be used to build an exposure profile to determine which AFSCs and which processes are at highest risk for exposure.

3.2 Subject Inclusion Criteria

Biomonitoring was conducted for aircraft maintainers in various career fields, designated by the 2AXXX AFSC. The intent was to recruit six to ten workers from each AFSC to be assessed over multiple locations and shifts to capture exposure variability in accordance with AIHA statistical methods for assessing workplace exposure [11]. This was an initial study in part to determine which AFSCs to direct focus for research, so smaller sample size was acceptable and falls in line with the law of diminishing returns.

Participants were eligible if they were Active Duty USAF or Air National Guard or Reserves with an USAF Specialty Code (job code) of 2AXXX, performing work in their primary field at the time of study, and consented to participating in the study. The following career fields were included:

Table 1. Career Fields Included

AFSC	Career Field
2A0X/2A1X/2A9X3/2A3X	Avionics
2A2X/2A8X1/2A9X1	Comm/Nav
2A5X	Aerospace Maintenance (crew chief)
2A6X1	Aerospace Propulsion
2A6X2	AGE
2A6X3	Aircrew Egress Systems
2A6X4	Aircraft Fuel Systems
2A6X5	Aircraft Hydraulic Systems
2A6X6	Aircraft Electrical & Environmental Systems
2A7X1	Aircraft Metals Tech
2A7X2	NDI
2A7X3/2A7X5	Aircraft Structural Maintenance

Subjects who were currently taking physostigmine were excluded from the study because the medication interferes with blood cholinesterase levels, which would make the blood measurements inaccurate. Other substances such as caffeine and alcohol were considered as well, but would not produce enough inhibition to warrant exclusion from the study. Informed consent was obtained from each participant prior to enrolling in the study.

3.3 Data Collection

Data collection was completed over the course of six months at three Air Force bases in the Continental United States. First, approval to visit the Air Force bases was obtained from each local maintenance group commander via signed memorandum. The research team arrived at each base, briefed the commanders on study protocol, and obtained informed consent from participants prior to each sampled work shift. Research was conducted at Moody Air Force Base, Georgia; Hill Air Force Base, Utah; and Davis-Monthan Air Force Base, Arizona.

Work was divided into pre-shift and post-shift and workers were used as their own controls during blood sample data analysis. Before each shift, one vial of blood was drawn by a phlebotomist through venipuncture using a straight stick vacutainer kit with a 20 gauge needle. If a participant was found to have smaller or deeper veins, a 23 gauge butterfly was opted for use. After blood was withdrawn, a 2x2 gauze pad was used to cover the insertion site, with Coban or a Band Aid to secure the gauze in place. After each sample was obtained, it was slowly inverted four times to mix with the tube preservatives. Samples were then labeled and immediately stored in a Styrofoam container on ice, with a separation of foam so tubes did not come in direct contact with any ice products directly. Foam cushions with tube space cut-outs were used to keep the samples upright and to pad and insulate the tubes. Blood samples were preserved between 4 and 8 degrees Celsius; a thermometer was kept in the cooler and checked hourly and no variance outside the acceptable temperature range was observed. Participants were fitted with two silicone wristbands and instructed to wear them on the dominant hand during the entire work shift unless they became a safety hazard. Participants performed work as they normally would, including wearing personal protective equipment, handling chemicals, and washing hands prior to eating, drinking, or smoking. It should be noted that some workers had to remove the bands for short periods if they were doing work in tight spaces that did not allow for their wear. Certain AFSCs (propulsion and crew chiefs) were also fitted with a third wristband for replicate analysis and an SKC Low Flow air sampling pump. The pump was worn on the belt with a thermal desorption tube positioned near the breathing zone. The pump collected a single air sample for the entirety of the shift. Start times were recorded for wristbands and air sampling pumps. Workers were not visually monitored during the shift, since monitoring may affect their decisions to perform tasks as they normally would, including decisions to use personal protective equipment (PPE) while using chemicals.

At the end of each sampled shift, blood was drawn by a phlebotomist using the same methods used in the pre-shift sample. The wristbands were removed at the end of the shift and each item was labeled and placed in a separate container and transported to the laboratory at Oregon State University for analysis as described by O'Connell and colleagues [10]. Air sampling tubes were also placed in a container for transport. Workers also completed a questionnaire after each shift. This questionnaire outlined which processes the worker conducted during the shift, what personal protective equipment he or she wore, which chemicals were used, and where the process was performed (flight line, hangar, etc.). The questionnaire is included as Appendix A. Compensation in the form of a $15-30 gift card was given to each participant upon completion of the shift.

After each shift, blood samples were placed in approved clinical FedEx shipping containers with a one-time cooling system that was activated just prior to shipment. The samples were then shipped either via same-day or overnight delivery to the Air Force Research Laboratory for analysis. Both pre- and post-shift samples were paired according to participant number for each shipment so that shipping conditions would be similar for both samples. No worker's samples were separated into multiple shipments and all samples arrived at the lab within 24 hours of the blood being drawn. Wristbands were shipped to Oregon State University for analysis.

3.4 Blood Sample Analysis

All blood samples from both pre- and post-shift were analyzed within 24 hours of collection. Two aliquots were taken from each heparinized blood sample. The first aliquot was 50 µL and was used to prepare the AChE activity, and the second was 100 µL for BuChE activity, both of which were analyzed with the Ellman Assay [15]. This assay utilizes the addition of and 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. This hydrolysis leads to the formation of thiocholine, which reacts with 5-dithiobis-2-nitrobenzoate ions to produce the yellow anion 5-thio-2-nitro-benzoic acid. This anion is measurable at 412 nm in a photometer. Thus, the more organophosphate that is present in each sample, the less hydrolysis occurs, the lower the photometer reads.

For the AChE assay, the 50 µL blood samples were diluted to 10% with distilled water and then centrifuged at 19100 x g for 15 minutes at 4 degrees Celsius. This isolates the red blood cells, which is crucial as acetylcholinesterase is found on the cell membrane. The supernatant was discarded and the remaining pellet was resuspended in 1 mL phosphate-buffered saline (PBS). The samples were then centrifuged again for five minutes three times, discarding the supernatant and resuspending in 1 mL PBS between each centrifugation. The second set of aliquots that had been reserved for the BuChE assay was not diluted. Instead, they were centrifuged one time at 1300 x g for 10 minutes at 4 degrees Celsius. 25 µL of the plasma was then removed and reconstituted into a 2.5% dilution with PBS.

The samples were then analyzed in duplicate in a 384 well plate. 10 µL of the isolated RBCs and 50 µL of the diluted plasma were used for each replicate of the acetylcholinesterase activity assay and butyrylcholinesterase activity assay, respectively. PBS was then added to bring the volume up to 85 µL. The Ellman Assay reaction was started by then adding 5% 5-dithiobis-2-nitrobenzoate and 10% acetylcholine or butyrylcholine. Activity was then measured at 412 nm every 1:22 minutes for 20 minutes on a FlexStation III plate reader. The maximum rate of the enzymatic reaction (Vmax) reading for each sample was taken, and the post-shift sample for each individual was compared with the pre-shift sample to determine the percentage of cholinesterase inhibition.

3.5 Wristband Sample Analysis

Wristbands were analyzed at Oregon State University via the Food Safety and Environmental Stewardship program. Samples were cleaned individually to remove debris, water, and other potential analytic interferences prior to extraction of organic compounds. The wristbands were sequentially cleaned in two jars of solvents. First, the wristbands were submerged in a glass jar of 18 MΩ*cm water to remove debris. Next, the wristbands were quickly submerged in a second jar of isopropanol bath. The wristbands were cut into sections using clean scissors that were rinsed with ethyl acetate prior to use and between samples. The wristbands were then placed in extraction glassware; they were stored in amber jars in the freezer until extraction.

After cleaning, all samples were extracted and extracts were concentrated to 1mL in ethyl acetate and stored in amber chromatography vials at -20ºC. Extraction began by adding ~100 mL of ethyl acetate for each wristband, or enough to completely cover the sample. The samples were left to stand for at least 2 hours or overnight at room temperature on an orbital shaker set at 60 rotations per minute (rpm). The first dialysate was transferred to a TurboVap tube. For the second extraction, the same amount of ethyl acetate was added to the original amber jar still containing the silicone sample and left to soak for at least 2 hours on an orbital shaker at 60 rpm. To concentrate the extracts, solvent was reduced to approximately 1 mL using the TurboVap closed cell evaporator and transferred quantitatively to centrifuge tubes using glass pipettes. Extract was concentrated to the final volume in appropriate solvent and vortexed in a centrifuge tube, then transferred to a labeled chromatography vial. Samples and extracts were kept in the dark during processing and extracts were stored at 4ºC as soon as possible after extraction.

Due to excessive background, samples required further clean-up prior to analysis. This was done via dilution of a 100µL aliquot of the sample extract from the original extraction and dilution into 3 mL of acetonitrile. This was followed by flow through solid phase extraction. All samples were then analyzed using SIM GC/MS on an Agilent gas chromatograph (GC) 7890A equipped with an Agilent 5975C mass spectrometer (MS). Peak identification was based on retention time, qualifier ions, and mass spectral display. Confirmation of peak identification criteria included peak response at least a 3:1 signal to noise ratio; peak retention time within the retention index or unadjusted relative retention of the compound to an internal standard; at least one qualifier ratio within the qualifier to quantification ion ratio established during calibration or in the CCV; mass spectral display is a reasonable match to the reference spectrum. Analytes were identified as pg/µL by ChemStation software and reported as ng/g or wristband material. Wristbands weights were not individually reported, but the laboratory reported a typical wristband to weight 4.6 grams.

3.6 Thermal Desorption Tube Analysis

Thermal desorption tubes were analyzed at Oregon State University using an Agilent 6890/5975 GC/MSD. First, standard calibration mixes were analyzed at the beginning of the method and the calibration curve was updated prior to running samples. For analysis, tubes were loaded into the tray for the Ultra TD Autosampler. ChemStation software was used to quantify the compounds in nanograms. Quality control was performed using four calibration standards and a calibration curve of $r^2 \geq 0.97$. An instrument blank was also run with each sequence.

3.7 Human Subjects and Safety Considerations

This study was approved by the Air Force Research Laboratory Institutional Review Board (IRB). If severe cholinesterase inhibition was detected from the blood cholinesterase analysis (defined for this study as 50% inhibition using percent of baseline ChE still available), subjects were informed via encrypted e-mail within 60 days of PIs receiving results. They were told to seek medical attention from their occupational medicine physician so they would be reevaluated. A 50% decline from baseline indicates severe inhibition and may coincide with symptoms such as cramping, muscle tremors, miosis, vomiting, and headache.

3.8 Data Analysis

First, data from the post-shift questionnaire were transferred to an Excel spreadsheet for data analysis. Alpha-numeric AFSCs were translated to corresponding duty descriptions within the aerospace maintenance field (i.e. “Propulsion” or “Non-Destructive Inspection”). Two columns were added to delineate whether the participant had listed engine oil or hydraulic fluid exposures in their list of reported chemical exposures from their work shift. Any variation of a description of “engine oil” or “7808 oil” was considered a positive response, as was any variation of “hydraulic fluid” or “hydro.” Cholinesterase blood analysis, thermal desorption tube lab analysis, and passive dosimeter lab analysis results were added to each participant’s row of the spreadsheet. In order to facilitate data analysis in STATA, any non-detect results were censored as LOD/√2.

Any job that was not a 2AXXX AFSC designation was considered ineligible for inclusion in the data analysis. Of 88 subjects screened, eight were enrolled and withdrawn. Seven of those were the wrong AFSC and one went on quarters (i.e., took a sick day) during the sampled shift. One participant failed to complete the questionnaire, so this participant was excluded from data analysis because their results could not be controlled for demographics. There were four accepted participants from whom the phlebotomist was unable to draw blood due to difficulty finding a viable vein. However, they did wear the passive dosimeter and complete the questionnaire, so they are included in passive dosimeter data analysis but not in any analysis examining the cholinesterase data.

Statistical analysis was conducted using Stata/SE 15.1. The Excel spreadsheet was uploaded and categorical variables were created for AFSC, shift, rank, Air Force Base, and location of personal protective equipment (above wristband, below wristband, or not worn) based on responses to the post-shift questionnaire. Binomial variables were created for gender, self-reported hydraulic fluid exposure (yes/no), self-reported engine oil exposure (yes/no), whether the participant was around active aircraft, and alcohol use. In order to assess whether the location of PPE influenced the passive dosimeter results, a binomial variable was created for the wristband’s ability to model skin exposure. If the dosimeter was worn under gloves or a Tyvek suit, then the band would receive the same exposure as the skin. However, if the dosimeter was worn above the suit or gloves, then the wristband would receive more exposure to the chemicals than the skin. This is important when considering the analysis regarding cholinesterase inhibition versus exposure based on dosimeter results.

Demographics were analyzed overall and broken down by AFSC for age, height, weight, gender, shift, base, and rank.

Biological monitoring results were quantified before and after shifts and percent inhibition for each type of cholinesterase was computed using Eq. 1.

$$\%\ \text{ChE inhibition} = \frac{\text{average postshift ChE}}{\text{average preshift ChE}}\ \text{x}\ 100 \qquad (1)$$

Cholinesterase inhibition results were then categorized according to severity using ranges established by Strelitz and colleagues [16]. Eighty percent of baseline (20 percent inhibition) was considered a “cut point.” Subsequently, a categorical variable for severity of each type of cholinesterase was created in Stata based on the categories in Table 2.

Table 2. 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

Overall prevalence of any cholinesterase inhibition, as well as prevalence of inhibition of each type, was also calculated.

To determine whether mean cholinesterase inhibition, air sampling results, or passive dosimeter results differed by workplace, AChE and BChE 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 (ANOVA) 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.

In order to determine whether AFSC was an acceptable determinant for self-reported exposure likelihood, a self-reported exposure variable was created based on the reported chemical exposures from the post-shift questionnaire. When workers self-reported using either engine oil or hydraulic fluid, they were classified "exposed" or "unexposed" with a binomial variable for each category: engine oil, hydraulic fluid, and either engine oil or hydraulic fluid. These exposure variables were compared via logistic regression to the categorical AFSC variable. Additionally, logistic regression was performed to determine if the self-reported exposure variable is associated with cholinesterase inhibition, as defined by the post-shift sample at less than 80 percent of baseline for either AChE or BChE.

Passive sampler measurements of TBP, TPP, and TCP were compared to worker acetylcholinesterase and butyrylcholinesterase percent inhibition 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 ng/g) in the passive sampler as well as for each chemical individually. A multivariate regression was also performed to determine if other factors influenced the outcome, including base, alcohol consumption, rank, gender, and proximity to active aircraft.

To further investigate the relationship between self-reported exposure and actual exposure, odds ratios were computed for exposure as determined by total organophosphates on the wristband versus odds of self-reporting exposure.

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 cholinesterase inhibition (on the X axis).

To examine the feasibility of silicone passive dosimeters in the aircraft maintenance setting, participants were classified into categorical variables for wristband comfort (agree that it is comfortable/disagree that it is comfortable/neutral) and whether it interfered with work (did not interfere/did interfere/neutral). The data were analyzed for descriptive statistics and trends by AFSC based on these variables.

4.0 RESULTS

Seventy-nine participants were sampled for this study. Demographics are included by career field in Table 3. The study population was mostly male, young, between Airman First Class and Staff Sergeant rank, and 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.

There were four main goals in this study; the results are broken down by goal:

Quantify the biological burden of organophosphate esters in aircraft maintainers before and after shifts by calculating percent inhibition of acetylcholinesterase and butyrylcholinesterase.

Seventy-six participants gave blood for analysis. Overall, the mean cholinesterase levels increased from baseline (beginning of shift) to the end of the sampled shift for both acetylcholinesterase (108.74% of baseline) and butyrylcholinesterase (107.49% of baseline). The mean percentage of baseline for acetylcholinesterase and butyrylcholinesterase was 108.74% and 107.49%, respectively. Aerospace Ground Equipment (AGE) workers had the highest mean AChE inhibition (75.34%) while Avionics workers had the highest mean BChE inhibition (93.14%). Results are detailed by AFSC in Table 4.

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. Cholinesterase inhibition graphed as a histogram by total percent is displayed in Figures 1, 2, and 3. The pattern appears normal, centered around the 100% mark.

Table 3. Participant Demographics as Defined by Air Force Specialty Code (AFSC)

Demographic	Overall n (%) or Mean (SD)	Avionics	Crew Chief	Propulsion	AGE	Egress	Fuel Systems Repair	Electro-environmental	NDI	Structural
Age (yr)	25.1 (4.92)	27.54 (4.41)	24.43 (4.36)	24.54 (4.82)	33.33 (1.53)	27 (4.95)	21.3 (2.67)	24.13 (5.22)	23.25 (4.03)	25.67 (6.51)
Height (in)	69.7 (3.33)	70.29 (2.72)	70.29 (2.33)	70.77 (2.74)	67.67 (0.58)	70.1 (2.42)	68.45 (4.55)	69.5 (4.04)	68.5 (4.51)	67 (7)
Weight (lb)	178.48 (25.74)	187 (29.11)	186.14 (21.85)	180.92 (23.01)	179.33 (37.17)	177.3 (22.72)	170.18 (21.82)	165.38 (21.94)	166.25 (41.10)	180 (43.30)
Gender										
Male	71 (91.03)	12 (85.71)	14 (100)	13 (100)	3 (100)	9 (100)	2 (18.18)	1 (12.5)	2 (50)	1 (33.33)
Female	7 (8.97)	1 (7.14)	0 (0)	0 (0)	0 (0)	0 (0)	9 (81.82)	7 (87.5)	2 (50)	2 (66.67)
Shift										
Day	52 (65.82)	3 (21.43)	12 (85.71)	11 (84.62)	3 (100)	4 (40)	8 (72.73)	7 (87.5)	1 (25)	3 (100)
Swing	12 (15.19)	7 (50)	1 (7.14)	0 (0)	0 (0)	1 (10)	3 (27.27)	0 (0)	0 (0)	0 (0)
Mid/Night	15 (18.99)	3 (21.43)	1 (7.14)	2 (15.38)	0 (0)	5 (50)	0 (0)	1 (12.5)	3 (75)	0 (0)
Base										
Moody	33 (41.25)	5 (38.46)	7 (50)	7 (53.84)	2	0 (0)	7	1	1	2
Hill	19 (23.75)	8 (61.53)	7 (50)	0 (0)	0 (0)	4 (40)	0 (0)	0 (0)	0 (0)	0 (0)
Davis-Monthan	28 (35)	0 (0)	0 (0)	6 (46.15)	1	6 (60)	4	7	3	1
Rank										
Airman Basic	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (9.09)	0 (0)	0 (0)	0 (0)
Airman	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)
A1C	27 (33.75)	2 (14.29)	3 (21.43)	6 (42.86)	0 (0)	6 (60)	6 (54.54)	2 (25)	1 (25)	1 (33)
SrA	21 (26.25)	3 (21.43)	6 (42.86)	3 (23.08)	0 (0)	0 (0)	4 (36.36)	2 (25)	2 (50)	1 (33)
SSgt	25 (31.25)	7 (50)	4 (28.57)	4 (30.77)	3 (100)	3 (30)	0 (0)	3 (37.5)	1 (25)	0 (0)
TSgt	4 (5)	1 (71.43)	1 (71.43)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	1 (33)
Total	**79**	**13**	**14**	**13**	**3**	**10**	**11**	**8**	**4**	**3**

Table 4. Cholinesterase Inhibition Between Pre-Shift and Post-Shift as Measured by Blood Samples, by AFSC Category

AFSC Category	AChE % of baseline (95% CI)	BuChE % of baseline (95% CI)
Avionics	98.89 (80.19, 117.59)	93.14 (-151.03, 337.31)
Crew Chief[1]	111.01 (3.05, 218.97)	105.17 (73.78, 136.56)
Propulsion[1]	103.66 (38.16, 169.16)	98.57 (70.83, 126.31)
AGE	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[1]	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)**

[1]Blood was not obtained from 4 workers: 1 Crew Chief, 2 from Propulsion and 1 from Fuel Systems Repair.

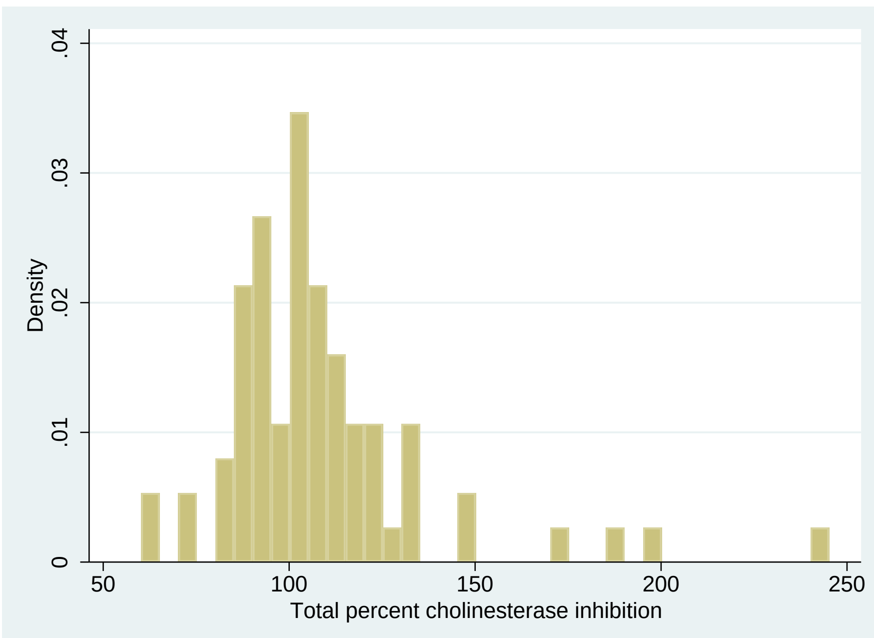

Figure 1. Total percent cholinesterase inhibition.

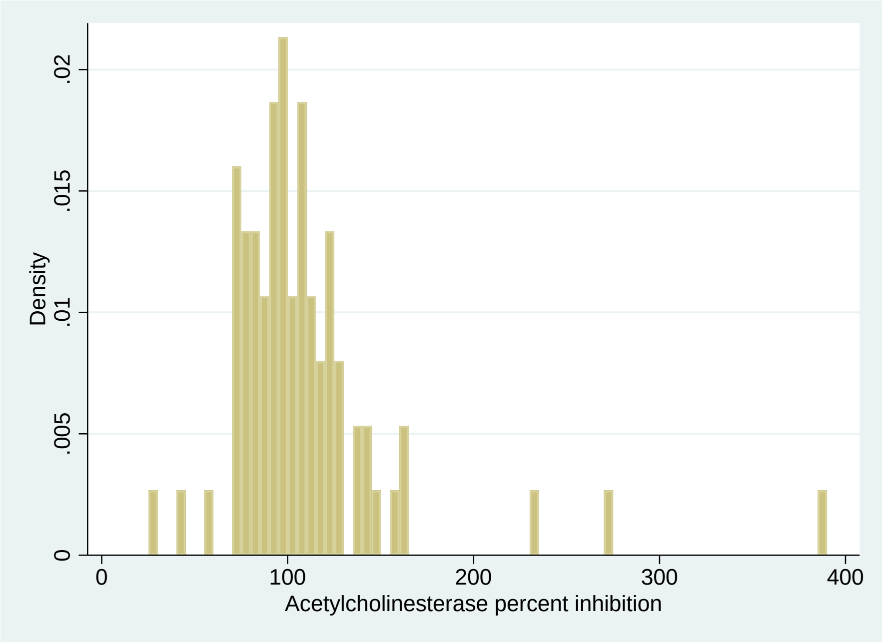

Figure 2. Acetylcholinesterase inhibition.

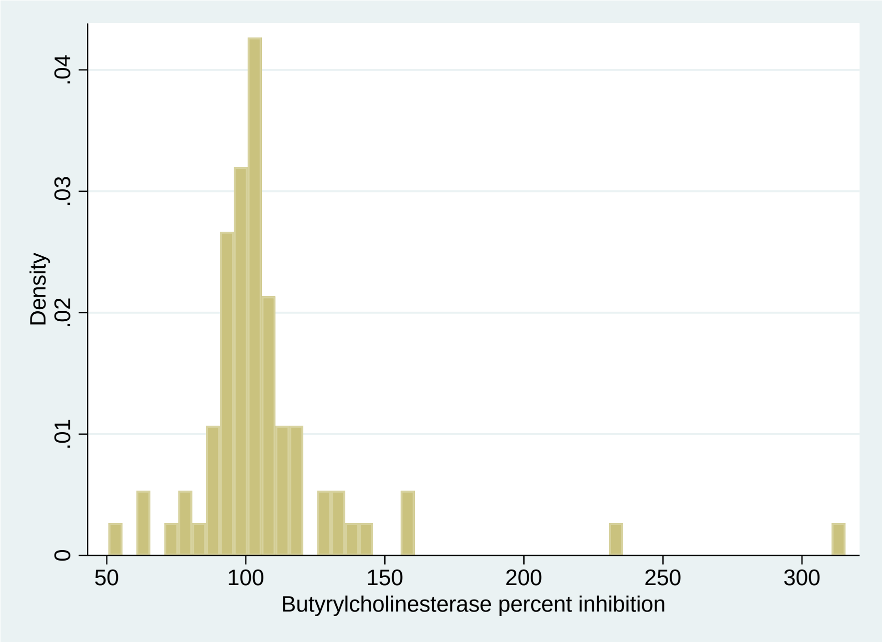

Figure 3. Butyrylcholinesterase inhibition

Severity of inhibition by category from none to severe is displayed in Table 5.

Table 5. 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

Determine whether mean cholinesterase inhibition, air sampling results, or passive dosimeter sampling results differ by workplace.

One-way ANOVA revealed no significant difference in percent inhibition of acetylcholinesterase among AFSCs, (F (8,66)=0.55, p=0.8178). However, there was a statistically significant difference in mean percent inhibition of butyrylcholinesterase (F (8,66)=2.41, p=0.0238). There was also a difference in AChE mean inhibition among bases (F (2,72)=3.37, p=0.0397) but this was not the case for BChE (F=3.06, p=0.0532).

Very little tributyl phosphate 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 nanograms of TBP per gram of wristband (ng/g) (95% CI:,-5.63, 9.05). Triphenyl phosphate was more prevalent, with only one sample below the limit of detection (mean 1386.26 ng/g, 95% CI: -7297.78, 10070.31), and TCP was found in every sample (mean 4311.65 ng/g, 95% CI: -8890.24, 17512.31). A histogram of total organophosphate results from passive dosimetry, by frequency of total, is included in Figure 4 and results are separated by AFSC in Table 6.

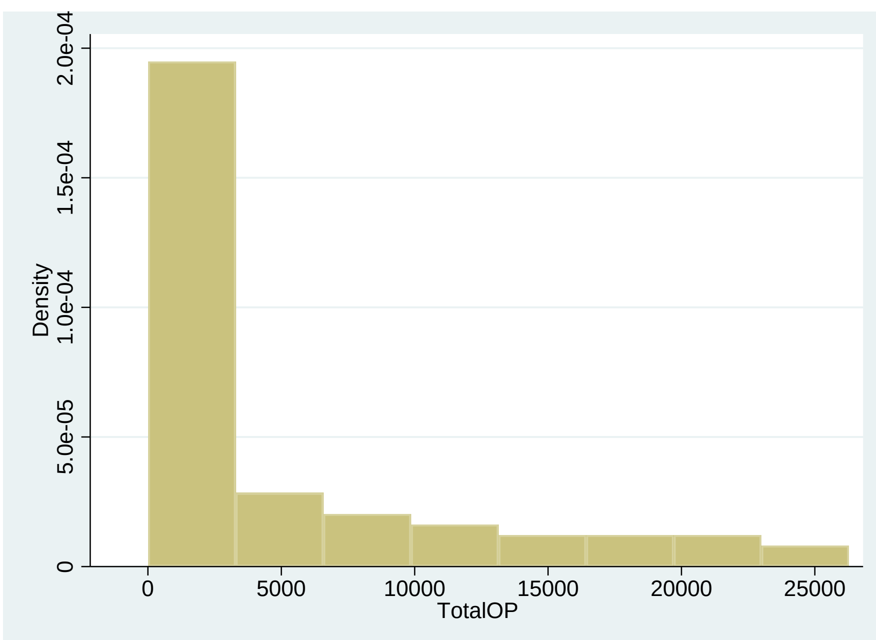

Figure 4. Histogram of total organophosphates from passive dosimetry

Table 6. Passive Dosimeter Results in Nanograms of Chemical per Gram of Wristband, by AFSC

AFSC	TBP (ng/g)	TPP (ng/g)	TCP (ng/g)
Avionics	1.06 (-1.8, 3.92)	2420.12 (-7895.44, 12735.68)	2963.93 (-7541.87, 13469.73)
Crew Chief[1]	1.67 (-7.05, 10.39)	832.36 (-2356.58, 4201.3)	2352.05 (-8070.19, 12774.29)
Propulsion[1]	0.98 (-2.46, 4.42)	3315.38 (-14584.1, 21214.86)	11997.51 (-6373.89, 30368.91)
AGE	0.49 (0.49, 0.49)	810.33 (-479.35, 2100.01)	969 (-1651.6, 3589.6)
Egress	5.19995 (-8.60, 18.99)	236.92 (-394.4, 868.24)	295.36 (-338.84, 929.56)
Fuel Systems Repair[1]	1.32 (-4.18, 6.82)	1080.22 (-2587.38, 4747.82)	4632.61 (-8567.43, 17832.65)
Electro-environmental	1.73 (-5.27, 8.73)	583.53 (-2476.65, 3643.71)	4765.25 (-4957.83, 14488.33)
NDI	0.49 (0.49, 0.49)	96.45 (-7.71, 200.61)	3476.75 (-2116.35, 9069.85)
Structural	0.49 (0.49, 0.49)	87.8 (-6.14, 241.74)	1432 (-677, 3541)
Overall mean (SD)	**1.71 (-5.61, 9.03)**	**1386.26 (-7297.78, 10070.3)**	**4311.65 (-8889.63, 17512.93)**

[1]Blood was not obtained from 4 workers: 1 Crew Chief, 2 from Propulsion and 1 from Fuel Systems Repair. However, these workers are included in the TPP, TBP, and TCP analysis.

There was a significant difference in tricresyl phosphate 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 (Table 7).

Table 7. Blood and Passive Dosimeter Results, by Base

	AChE % of baseline (95% CI)	BChE % of baseline (95% CI)	TBP (ng/g) (95% CI)	TPP (ng/g) (95% CI)	TCP (ng/g) (95% CI)
Moody AFB[1]	91.2785 (40.84, 141.71)	99.49617 (66.02, 132.97)	1.0122 (-2.64, 4.66)	2728.103 (-10354.38, 15816.38)	5773.074 (-10398.49, 22082.96)
Hill AFB	114.7477 (29.62, 199.88)	101.3689 (61.35, 141.39)	1.558651 (-6.05, 9.17)	223.8421 (-554.15, 1001.83)	639.1526 (-1866.23, 3144.53)
Davis-Monthan AFB	122.7403 (-3.24, 248.72)	119.5242 (23.36, 215.69)	2.610571 (-7.17, 12.39)	637.2921 (-1842.21, 3116.796)	5052.729 (-6992.51, 17097.96)
Overall mean (SD)	**108.9698 (12.65, 205.29)**	**107.4477 (40.16, 174.73)**	**1.71 (-5.62, 9.04)**	**1386.26 (-7297.79, 10070.31)**	**4311.04 (-8890.24, 17512.32)**

[1]Blood was not obtained from 4 workers from Moody AFB.

After logistic regression of the “exposed” and “unexposed” variable to the categorical AFSC variable, self-reported exposure to engine oil was only statistically significantly associated with the Propulsion AFSC (OR: 7.5, 95% CI: 1.31, 43.03, p=0.024), while exposure to hydraulic fluid was not associated with any particular AFSC. It is important to note, however, that the hydraulics career field was not sampled because no participants from hydraulics volunteered for the study.

Logistic regression of AChE inhibition with an 80% inhibition cut point versus binomial self-reported exposure status resulted in a fourfold association of inhibition for those with self-reported exposure to either engine oil or hydraulic fluid (OR: 4.04, 95% CI: 1.0226, 15.953, p=0.046). This was not the case for BChE (OR: 1.338, 95% CI: 0.21, 8.506, p=0.758). Regression of any inhibition resulted in a 3.23 odds ratio (95% CI: 1.024, 10.186, p=0.045).

Maintainers who had exposed wristbands indicating exposure exceeding 500 ng of organophosphates per gram of wristband were slightly more likely to have cholinesterase inhibition. When compared for maintainers exposed above 1000 ng/g of wristband, the odds ratio was slightly above 2, but neither result was statistically significant. Results are included in Table 8.

Table 8. Crude Prevalence Odds Ratios for Any Cholinesterase Inhibition for Exposed and Unexposed Groups Based on Sampling and Self-Reported Exposure Categories

	n (%)	Prevalence	Prevalence odds ratio
Exposed maintainers (dosimeter results >500)	50 (66.67)	14/50=	1.56 (0.49, 4.95) p=0.455
Unexposed maintainers (dosimeter results <500)	25 (33.33)	5/25=	1.00
Exposed maintainers (dosimeter results >1000)	40 (53.33)	13/40=0.325	2.32 (0.77, 6.99) p=0.132
Unexposed maintainers (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		

A multivariate logistic regression for any inhibition versus organophosphate passive dosimeter results with a cut-point of 1000 ng/g of wristband, using gender, shift, rank, base, alcohol intake, active aircraft (near/not near), and self-reported exposure to chemicals revealed one potential interaction. The binomial variable for active aircraft had an odds ratio of 5.2 (p=0.045, 95% CI 1.035, 26.833). In addition, the categorical base variable indicated that being at Hill AFB was protective of inhibition (OR: 0.034, p=0.020, 95% CI 0.002, 0.59). When the model was rerun with only base and active aircraft proximity as independent variables, the odds ratio as closer to one (OR: 1.16, p=0.828, 95% CI 0.31, 4.37). Results were similar when a multivariate regression for any inhibition versus a cut-point of 500 ng/g dosimetry results, with both Hill AFB and active aircraft being significant factors (Hill OR 0.025, p=0.015, 95%CI 0.001, 0.496; Active aircraft OR 6.24, p=0.03, 95% CI 1.19, 32.64). Regression with just the base and active aircraft variables resulted in an odds ratio of 0.48, but the values was not statistically significant (p=0.334, 95% CI 0.11, 2.12).

Maintainers who had exposed wristbands indicating exposure exceeding 500 ng of organophosphates per gram of wristband were nearly five times more likely to have self-reported exposure to organophosphates. When compared for maintainers exposed above 1000 ng/g of wristband, the odds ratio was nearly 10. Results are included in Table 9. This indicates a personal awareness of exposure that increases with increasing skin exposure.

Table 9. Crude Prevalence Odds Ratios for Exposure and Non-Exposure as Determined by Wristbands, as Compared to Self-Reported Exposure Categories

	n (%)	Prevalence	Prevalence odds ratio
Exposed maintainers (dosimeter results >500)	50 (66.67)	33/50=0.66	4.99 (1.75, 14.28) p=0.003
Unexposed maintainers (dosimeter results <500)	25 (33.33)	7/25=0.28	1.00
Exposed maintainers (dosimeter results >1000)	40 (53.33)	31/40=0.775	9.95 (3.44, 28.74) p=0.000
Unexposed maintainers (dosimeter results <1000)	35 (46.67)	9/35=0.257	1.00
Total	75		

Compare silicone passive sampler results to worker cholinesterase inhibition levels.

Linear regression of total organophosphate wristband result compared to total percent cholinesterase inhibition was not significant (p=0.585). Likewise, regression of each compound's wristband results compared to AChE or BChE was not significant, with one exception: TBP versus AChE percent inhibition (Coefficient=0.019, p=0.034). Tributyl phosphate was only found in 14 of 79 samples, so there may be bias involved in this association. There may be additional bias because some workers wore their wristbands underneath gloves while some wore them over gloves. Therefore, those who wore the wristbands over gloves would've been protected from actual contact with the chemical while the wristband would suggest higher exposure than they were vulnerable to. However, when analyzed by wristband status (over or under PPE), the linear regression was also not significant. It is possible that the hands were not the only exposed part of the skin, as some workers may be exposed on the arms, chest, or face and neck.

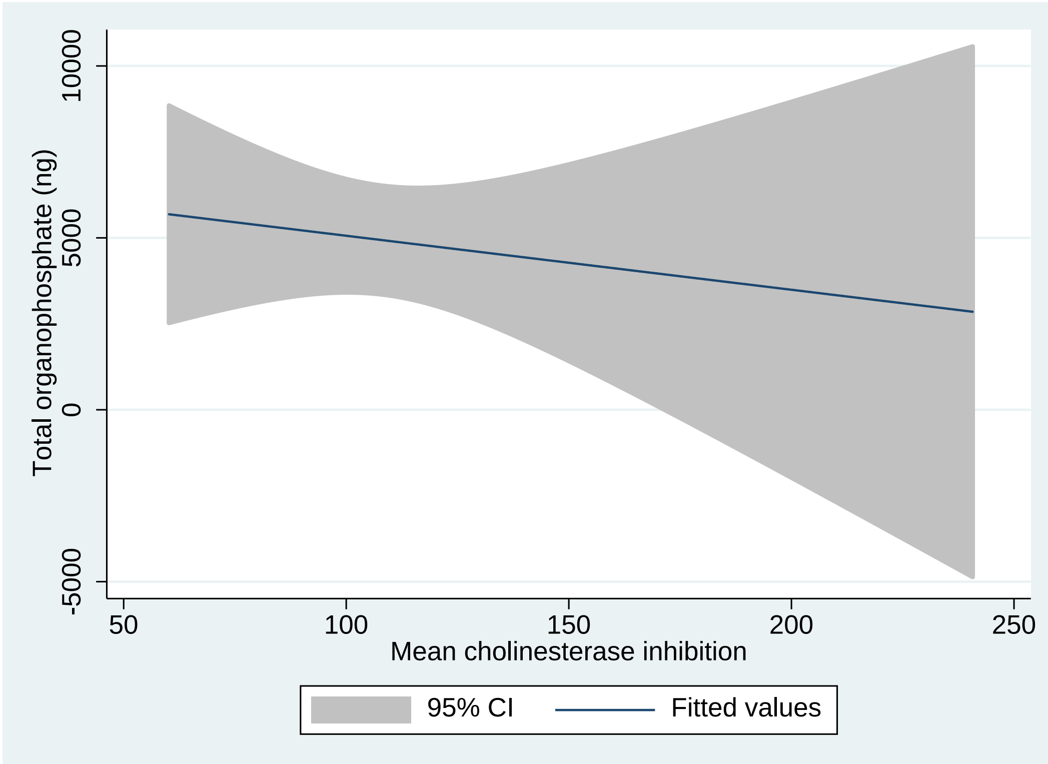

Figure 5. Linear prediction (with 95% CI) of total organophosphate on passive dosimeter versus mean cholinesterase inhibition.

Determine general statistics for the feasibility of silicone passive samplers in the maintenance setting.

Of the 79 participants who answered the questionnaire, 84.81% (n=67) agreed that the device was comfortable, while 12.66% (n=10) reported neutral feelings and 2.53% (n=2) reported that it was uncomfortable. Similarly, 82.28% (n=65) reported that the band did not interfere with their duties, while 12.66% (n=10) were neutral and 5.06% (n=4) reported that it interfered. There was no trend in reporting discomfort or interference by AFSC; discomfort was reported by one each of Fuel Systems Repair and Structural Maintenance, while interference was reported by one each of Propulsion, Egress, NDI, and Structural Maintenance.

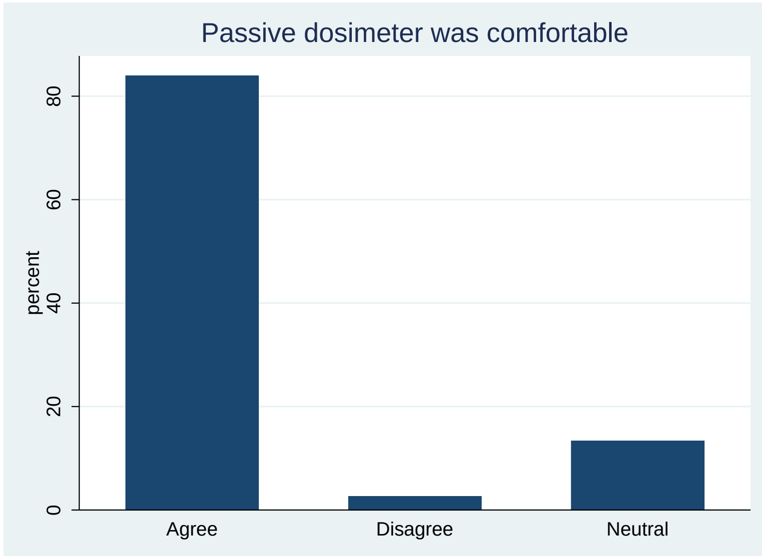

Figure 6. Participant opinions on passive dosimeter comfort.

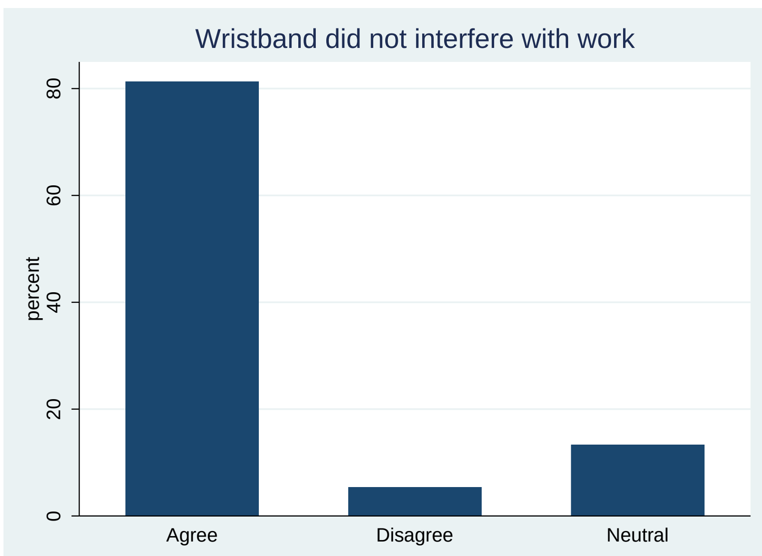

Figure 7. Participant opinions on passive dosimeter interfering with work.

5.0 CONCLUSION

This study demonstrated that 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. Air Force Specialty Code does not appear to be a good predictor of exposure to cholinesterase inhibitors. This may be explained by the close physical proximity of work done by multiple AFSCs. Some participants also conveyed that they were being used as overlap or extra manpower for other AFSCs' responsibilities. 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 Air Force Base had just deployed many of their aircraft to an exercise 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. In addition, results ruled out tributyl phosphate 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, tricresyl phosphate 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. Those workers who admitted to engine oil exposure during the work shift were more likely to be Propulsion workers.

AChE inhibition and any cholinesterase inhibition were both associated with self-reported exposure to engine oil or hydraulic fluid. When sample results were separated into several cut points of “exposed” and “unexposed” groups, exposure as determined by wristbands was associated with cholinesterase inhibition, but the numbers were not statistically significant. One significant finding is that those workers who self-reported exposure were more likely to have higher passive dosimeter results. This indicates that the dosimeter and the worker’s reported exposure status are closely associated. Combined with the finding that cholinesterase inhibition seems to occur in those individuals who are knowingly using the compounds that contain organophosphates, self-reporting of exposure may be an acceptable surrogate for screening sampling.

In general, the passive samplers were well received by participants. Most reported comfort and did not report interference with work. One major exception was the Fuel Systems Repair workers, who occasionally needed to transfer their dosimeters to their belt due to their leadership not allowing anything to be on the wrist during fuel tank entry. This was rare, but may have skewed the individuals’ dosimeter results toward less exposure. While there exists the potential to use the passive dosimeters to measure airborne exposure, in this case the dosimeters proved more useful in measuring contact exposure. This is beneficial for chemicals such as organophosphates that are able to reach their target organs via absorption through the skin. There are several limiting factors of this technology, however. First, total body dose depends somewhat on the time the chemical is in contact with the skin; if the worker washes his or her hands soon after exposure, there will not be as much opportunity for absorption. There was no procedure identified to either prevent or ensure that the dosimeters were washed along with the worker’s hands, so the dosimeter results may not be as reflective of actual exposure as possible. In the same vein, the wristbands were worn on the wrist, whereas exposure to the hands is more likely. Additionally, the upper extremities may not be the only point of exposure for workers, so additional locations for dosimeters may need to be used, such as the collar or chest, depending on the nature of the work being done. For this reason, the actual body exposure may be underestimated. Another potential limiting factor to the passive dosimeter data is the discrepancy between exposure to the band when the worker wears it above versus below his or her gloves or other personal protective equipment. If worn under the gloves, it more closely approximates actual skin exposure. However, if worn over the gloves or further up the arm than the gloves can reach, it may better approximate other regions of skin exposure.

As with all studies, this study has sources of bias. First, the population studied was very small and was intended to be a screening cross-section of the overall aircraft maintenance population. This means that 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 the study outcome. 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. Further, the relationship between exposure awareness (self-reported exposure) and passive dosimeter exposure may be examined in greater depth, to include investigating whether workers can delineate their degree of exposure rather than just a binomial representation of whether they were or were not exposed.

Overall, this study confirmed that exposure to organophosphate esters is more likely to occur through contact and absorption of chemicals through the skin than through inhalation of oil mists. Exposure to tricresyl phosphate was most common, followed by triphenyl and tributyl phosphate. Workers did experience cholinesterase inhibition, but the study was not large enough to establish a statistically significant association between exposure and disease, defined as greater than 80 percent cholinesterase inhibition. The association between passive dosimeter exposure and self-reported exposure was also very high, so workers are able to identify when they have been exposed to organophosphate-containing products in the workplace.

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APPENDIX: POST-SHIFT DATA COLLECTION FORM

Please fill out this form to the best of your ability.

Rank: ______________________

Squadron: __________________________

Gender (circle one): Male Female

Age: _______ years

Height: ____ ft ____ in

Weight: _____lbs

Shift (circle one): Day Mid Night Other:_____________

AFSC category (please check one):

	2A0X/2A1X/2A9X3/2A3X	Avionics
	2A2X/2A8X1/2A9X1	Comm/Nav
	2A5X	Aerospace maintenance (Crew chief)
	2A6X1	Aerospace propulsion
	2A6X2	AGE
	2A6X3	Aircrew Egress Systems
	2A6X4	Aircraft Fuel Systems
	2A6X5	Aircraft Hydraulic Systems
	2A6X6	Aircraft Electrical & Environmental Systems
	2A7X1	Aircraft Metals Tech
	2A7X2	NDI
	2A7X3/2A7X5	Aircraft Structural Maintenance
	Other (please specify)	

Please describe the processes or tasks you performed during this shift, starting with the first hour of the shift. Include the chemicals or aircraft fluids you used or came into contact with during each process.

	Processes	Chemicals or aircraft fluids used
Hour 1		
Hour 2		
Hour 3		
Hour 4		
Hour 5		
Hour 6		
Hour 7		
Hour 8		
Hour 9		
Hour 10		
Hour 11		
Hour 12		

Describe your work location (indoors, outdoors, hangar, flightline, backshop)?

____

If you were on the flightline, were there active aircraft nearby (circle one)? Yes No N/A

Did you come into contact with any other chemicals or fluids during this shift (e.g. fluids leaking from aircraft or parts)? If so, what chemicals or fluids?

____

Have you taken physostigmine, a glaucoma medication, in the past 24 hours? (circle one) Yes No

Have you consumed alcohol in the past 24 hours? (circle one) Yes No

PART II.

Please indicate whether you agree or disagree with the following statements (circle one).

The silicone wristband was comfortable to wear. Agree Neutral Disagree

The wristband did not interfere with my work. Agree Neutral Disagree

If you wore personal protective equipment (PPE) like gloves or a Tyvek suit during your shift, how did you wear the wristband in relation to the PPE? (circle one)

Wristband over PPE Wristband under PPE N/A, didn't wear PPE

Please use this section to provide any additional comments on the wristband itself:

____

____

____

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LIST OF ABBREVIATIONS AND ACRONYMS

AChE	acetylcholinesterase
AFSC	Air Force Specialty Code
ANOVA	analysis of variance
BChE	butyrylcholinesterase
GC	gas chromatograph
TBP	tributyl phosphate
TCP	tricresyl phosphate
TPP	triphenyl phosphate
USAF	United States Air Force