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**COMPARISON OF OPERATIONAL JET FUEL AND NOISE
EXPOSURES: JAPAN STUDY REPORT**

David R. Mattie, Dirk Yamamoto, Kerrine LeGuin, Thomas Jaworek
711 HPW/RHBA

Elizabeth McKenna
711 HPW/CC-IMA

Daniel A. Williams
711 HPW/RHWE

Alex Gubler
374 MDG, Yokota AB

Patricia N. Hammer
39 MDOS/SGXW, Kadena AB

Rhonda Pitsch, Camilla Mauzy
711 HPW/RHBB

Nobuhiro Ohrui, Satoshi Maruyama
Aeromedical Laboratory, Japan Air Self-Defense Force

Asao Kobayashi
Air Development & Test Command HQ, Japan Air Self-Defense Force (ret)

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**AIR FORCE RESEARCH LABORATORY
711TH HUMAN PERFORMANCE WING,
AIRMAN SYSTEMS DIRECTORATE,
WRIGHT-PATTERSON AIR FORCE BASE, OH 45433
AIR FORCE MATERIEL COMMAND
UNITED STATES AIR FORCE**

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Date: 2024.06.25 09:37:53 -04'00'

DIRK YAMAMOTO, PhD, CIH
Chief, Force Health Protection Section
Air and Space Biosciences Division
Human Effectiveness Directorate
Air Force Research Laboratory

DURAN.CHRISTIN.
MARIE.1286379393

Digitally signed by
DURAN.CHRISTIN.MARIE.12863
79393
Date: 2024.06.25 09:11:42 -04'00'

CHRISTIN DURAN, PhD, CIH
Product Area Lead, Force Health Protection
Air and Space Biosciences Division
Human Effectiveness Directorate
Air Force Research Laboratory

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1.0 SUMMARY

The data in this report shows that flight line personnel are exposed to noise while working in and around aircraft. They are also exposed to exhaust from the jet fuel (Ohrui et al. 2023). Recent studies suggested jet fuel in combination with noise are more associated with hearing loss than with noise exposure alone (Fuente et al., 2019; Morata, Hungerford, and Konrad-Martin, 2021; Dreisbach et al., 2022). Therefore, flight line personnel are more likely to develop hearing loss compared to noise-only exposure groups (Kaufman et al., 2005; Fechter et al, 2012). The purpose of this study was to examine the effects on hearing in flight line personnel with combined exposure to jet fuel and noise and to identify and address gaps in data for flight line personnel. This study was a collaborative research effort between the Japan Air Self-Defense Force (JASDF) and the US Air Force (USAF). The JASDF analyzed jet fuel components as fifteen volatile organic compounds (VOCs) in personal breathing zone air samples, as well as blood, and urine in volunteer subjects at air bases (ABs) in Japan. The characteristics of VOC exposures for the flight line environment (air, blood, and urine) in each aircraft and fuel type are described in the JASDF final report (Ohrui, et al., 2023). The USAF analyzed noise dosimetry, microRNA (miRNA) biomarkers, metabolomic biomarkers and an audiometric test battery, consisting of otoscopy, immittance measurements, otoacoustic emissions (OAE), auditory brain response (ABR), and audiograms in the same volunteers at the same ABs in Japan. This report describes the analytical methods and results of noise dosimetry, an audiometric test battery, miRNA biomarkers, and metabolomic biomarkers.

One hundred and sixty-eight subjects voluntarily joined this study in both JASDF ABs (Hamamatsu, Matsushima, Hyakuri, Yokota and Iruma) and USAF ABs (Kadena and Misawa) in Japan. The subjects were divided into eight groups: CJ (Non-exposed control subjects in JASDF); CK (Non-exposed control subjects in Kadena); CM (Non-exposed control subjects in Misawa); T-4 (Jet A1 (jet fuel aviation 1) exposed T-4 flight line crews in Matsushima and Hamamatsu); F-2 (Jet A1 exposed F-2 flight line crews in Matsushima); F-4 (JP-4 (jet propulsion fuel 4) exposed F-4 flight line crews in Hyakuri); F-15 (JP-8 (jet propulsion fuel 8) exposed F-15 flight line crews in Kadena); F-16 (JP-8 exposed F-16 flight line crews in Misawa).

Samples were collected according to the typical sequence of events at each base. To measure personal noise levels during shift, prior to shift a noise dosimeter was attached to each subject. Blood draws were conducted post shift. Urine samples (entire void) were collected prior to shift (early morning first void) and post shift.

Total VOCs in personal air samples as reported in the JASDF final report (Ohrui, et al., 2023) were extremely low in each group compared to the permissible limits recommended by ACGIH and the Occupational Safety and Health Administration (OSHA). Demographic data was summarized and presented in tables in this report. Tinnitus and temporary hearing loss were reported in the audiological history but were also present in USAF controls. Noise levels on the flight line exceeded the U.S. Department of Defense (DoD) and OSHA exposure limits but all exposed subjects reported wearing hearing protection. Audiological tests identified significant differences between flight line personnel and control subjects, but T-4 and F-2 results were not consistently the same. Biomarker analysis of urinary metabolites and miRNA showed varying degrees of difference.

The noise level on flight lines is greater than the action level for requiring hearing protection. Hearing tests showed significant differences and trends between flight line personnel and control

subjects for both T-4, F-2, F-4, F-15 and F-16 aircraft. The differences were not consistent between aircraft even though noise levels were comparable for all exposed subjects. Since the F-4 and JP-4 jet fuel are no longer operational in the JASDF and USAF, retiring both makes the current flight line environment safer. Statistically significant alterations of miRNAs were seen between control subjects and flight line personnel as well as between bases. Metabolomics showed overlap between controls and flight line personnel with the degree of overlap varying by base and aircraft.

2.0 INTRODUCTION

2.1 Purpose of Study

Hearing loss is prevalent in various occupational environments. People who work in and around aircraft are at risk of developing hearing loss. Studies have shown that pilots, flight crews, aircraft technicians and mechanics have high rates of hearing loss compared to unexposed populations from analyses of large epidemiological databases (Fitzpatrick, 1988; Jaruchinda et al., 2005). Aircraft technicians and mechanics in the commercial aviation industry were found to have better hearing thresholds at younger ages (30 to 40 years) compared to age-matched reference groups (Smedje et al., 2011). In addition, military fighter and helicopter pilots are known to be at high risk of developing hearing loss compared to commercial pilots (Raynal et al., 2006). Hearing loss typically takes on a high frequency sloping configuration, even with the use of hearing protection such as earplugs, headsets or helmets (Jaruchinda et al., 2005).

A retrospective cohort study was conducted by Williams et al. (2015) using data from the DoD occupational and environmental health database to compare JP-8 exposed individuals with hearing test results. Hearing tests were compared for personnel exposed to noise and those exposed to both noise and JP-8 from 1996 to 2013. Personnel aged 17-44 years who were exposed to JP-8 were more likely to have an occupational hearing shift than their non-exposed peers of the same age. When the mean threshold change was examined between the first and last audiogram of the personnel, a statistically significant difference was found at 3,000 hertz (Hz) and 4,000 Hz in the JP-8-exposed group. The results of this epidemiological study suggest a synergistic effect on hearing loss when workers are exposed to both noise and JP-8.

The United States Armed Services and North Atlantic Treaty Organization (NATO) have adopted JP-8 as their standard fuel (Fechter et al., 2012; Merchant-Borna et al., 2012). The JASDF switched to Jet A1, which is JP-8 without additives. The USAF now designates Jet A with the current AF performance additives as F-24. Jet A (jet fuel aviation) and Jet A1 are very similar in chemical composition but Jet A1 has a lower freezing point. Jet A is mainly used in the U.S. commercial aviation industry.

Preliminary epidemiological analyses of auditory records from aircraft maintenance personnel exposed to both noise and jet fuel (primarily the more volatile JP-4) suggest that concurrent exposure may potentiate hearing loss at jet fuel concentrations below the currently established safe occupational exposure limit (Kaufman et al., 2005). Potentiation of hearing loss was confirmed in experiments where rats were exposed to JP-8 and non-damaging noise levels (Fechter, Gearhart and Fulton, 2010). Animal studies have revealed that exposure to JP-8 combined with noise may result in the loss of pre-neural cochlear sensitivity as shown by suppression of distortion product otoacoustic emissions (DPOAE), increased auditory thresholds and depletion of cochlear sensory cells as evidenced by cytochrome c oxidase (COX) staining that plot the

percentage of missing outer hair cells (Fechter et al., 2007, 2010, 2012). In the experimental animal models, the effect of combining jet fuel plus noise exposures was greater than that of the individual exposures to jet fuel or noise. An additional study by Guthrie et al. (2014) examined the same functional and structural assays of the presynaptic sensory cells, but added neurophysiologic studies of the auditory pathway, by conducting the ABR test. Results revealed that peripheral auditory function was not affected by individual exposures and there was no effect when the exposures were combined using this exposure paradigm and animal model. However, brainstem encoding of stimulus intensity measured by auditory brainstem response was impaired. All these findings are particularly important because military and government regulations regarding toxic exposures are often based on exposure to a single agent and less is known about combined exposure to jet fuel and noise.

A review of recent literature by (Warner, Fuente and Hickson, 2015) examined the effects of combined exposure to JP-8 jet fuel (or its aromatic solvent components) and noise on the auditory nervous system in humans. Their search found 178 articles but only 37 were relevant enough to be examined further. After articles with animal models, or about a solvent not in jet fuel or an article in a conference proceeding without sufficient data were removed, a total of six articles met the inclusion criteria and were summarized in their review. One article was by Kaufman et al. (2005) cited above where exposure was to JP-4 jet fuel only. The other five (three by the second author A. Fuente) examined the effects of individual solvents or mixed solvents. The conclusions of the literature review suggest that there is an association between aromatic solvents and jet fuel, in combination with noise and central auditory dysfunction/hearing loss. The articles considered in this review indicate that the assessment of the auditory nervous system, including pure-tone audiometry; DPOAE; auditory brainstem response, and behavioral functional assessments, should be conducted as part of a comprehensive test battery for military members exposed to both noise and solvents in the workplace. This study plus the literature in general supports the need for a closer investigational paradigm created to survey and document the auditory and environmental exposures for relating hearing decrement assessment for flight line personnel.

A previous collaborative research effort between the JASDF and the USAF correlated exposure and biomarkers for JP-4 and compared them with those from JP-8 (Kobayashi et al., 2014; Mattie et al., 2014). The JASDF measured jet fuel components in the breathing zone of workers, as well as blood and urine, while the USAF analyzed blood and urine for biomarkers. Personal exposure of 48 VOCs was measured in F-15 and C-130 flight line crews, and non-exposed control subjects (mainly hospital workers). The results of this study suggested that the level of exposure to jet fuel is dependent on the type of aircraft and fuel. Moreover, the disposition of VOCs is that workers in operational environments are exposed through the lungs with entry into the blood, are metabolized resulting in metabolites in the urine. A direct human exposure response comparison study during military operations, able to characterize jet fuel exposure and dose in air, blood, and urine, was necessary to draw substantive conclusions regarding the combined jet fuel and noise exposures and specific auditory health effects of flight line personnel.

The purpose of this study was to examine the effects on hearing in flight line personnel with combined exposure to jet fuel and noise and to identify and address gaps in data for flight line personnel. This report showed the characteristics of VOCs exposures in flight line environment in each aircraft and fuel type.

2.2 Collaborative research

This study was a collaborative research effort between the JASDF and the USAF. The JASDF analyzed jet fuel components as fifteen VOCs in the breathing zone, blood and urine collected from volunteers at ABs in Japan as reported in Ohrui, et al. (2023). The USAF analyzed noise dosimetry, an audiometric test battery consisting of otoscopy, immittance measurements, OAE, ABR, and audiograms, miRNA biomarkers in blood, and metabolomic biomarkers in urine. The analytical methods and results of noise dosimetry, the audiometric test battery, miRNA biomarkers, metabolomic biomarkers are described in this final report.

3.0 METHODS

3.1 Subjects

3.1.1 Recruitment of study subjects and demographic data

The study was designed to recruit the following total number of flight line personnel subjects: 30 to 50 individuals from F-15 or F-16 USAF ABs exposed to JP-8; 30 to 50 individuals from one or more T-4, F-2 or F-4 ABs exposed to JP-4 or Jet A1. Non-exposed control subjects needed to be mainly medical personnel sampled in an identical manner as flight line personnel: matched to the number of flight line personnel number (30 to 50) for JP-8 at U.S. ABs and matched to number of flight line personnel number (30 to 50) for JP-4/Jet A1 from JASDF ABs. Non-exposed control subjects in JASDF were recruited from Yokota and Iruma ABs because there are only a few medical personnel who are non-exposed control subjects in Hamamatsu, Matsushima and Hyakuri ABs. Subjects were volunteers who were active duty (JASDF and USAF) flight line personnel. Subjects could be male or female and the age had to be under 35 years old.

Using approved questionnaires, subjects were interviewed at end of their shift about the following demographic information: Name which was replaced with a base specific number to de-identify subjects; Career field; Rank; Years of service; Age; Gender; Hobbies (other fuel or solvent exposures; worked on car or truck engines, either on or off duty); and Last time they fueled a government or personal vehicle and type of fuel. Questions were asked about exposures during the shift as well as a brief summary of work activities associated with jet fuel and jet exhaust including time on flight line (location of job, physical activities). Additional questions were about exposure to any spills of any kind; any direct dermal exposure to jet fuel or any solvent or other exposures such as solvents and cleaning fluids; were they a smoker (if yes, number of cigarettes, cigars, heated tobacco products and/or vape smoked) and how much caffeine did they consume. They were also asked about personal protection equipment. A second questionnaire post shift was for audiological history. Questions included incidence of temporary hearing loss, tinnitus, ear infections, dizziness, ear pain, family history, neurologic or chronic medical conditions. Also asked was information about off duty and recreational noise exposures as well as usage of any herbal supplements.

3.1.2 Number of subjects and sampling date

There were 168 subjects who voluntarily joined this study at 5 JASDF ABs and 2 USAF ABs in Japan (Table 1).

Table 1. Number of subjects and sampling date at each AB

Date	AB	Aircrafts	Jet fuel	Assignment	Number of Subjects
30 May - 8 June 2018	Hyakuri JASDF	F-4	JP-4	The 301st SQ The 302nd SQ	20
		Control (Medical workers)		7th Air Wing AB Group	2
11 June - 12 June 2018	Yokota JASDF	Control (Medical workers)		Operations Support Wing	5
13 June - 15 June 2018	Iruma JASDF	Control (Medical workers)		Aeromedical Laboratory	11
19 March - 10 April 2019	Kadena USAF	F-15	JP-8	The44th SQ The 67th SQ	23
		Control (Medical workers)		The 18th Med Group	22
8 November - 12 November 2021	Matsushima JASDF	T-4	JetA1	The 21st SQ	7
		F-2		The 11th SQ	7
6 December - 10 December 2021	Hamamatsu JASDF	T-4	JetA1	The 31st SQ The 32nd SQ	22
11 July - 22 July 2022	Misawa USAF	F-16	JP-8	The14th SQ	18
		Control (Medical workers)		The 35th Med Group	15
21 October – 27 October 2022	Iruma JASDF	Control (Medical workers)		Aeromedical Laboratory	16
Total number					168

The subjects and samples were identified by their base: Hyakuri AB – HY; Hamamatsu AB – H; Matsushima AB – MA; Misawa AB – MU and MC (controls); Kadena AB – KD and KC (controls); Yokota AB (JASDF) – YJ (controls), and Iruma AB – I (controls). Samples then received an additional code based on the type of sample: Blood was B and miRNA was BR; Urine was U and metabolomics was UM. Urine was also labeled based on whether it was first void and prior to shift (B for baseline) or if it was post shift (P).

Subjects were also divided into eight groups by the Aeromedical Laboratory (AML) of JASDF in their report and in Table 4: CJ (Non-exposed control subjects in JASDF); CK (Non-exposed control subjects in Kadena); CM (Non-exposed control subjects in Misawa); T-4 (Jet A1 exposed T-4 flight line crews in Matsushima and Hamamatsu); F-2 (Jet A1 exposed F-2 flight line crews in Matsushima); F-4 (JP-4 exposed F-4 flight line crews in Hyakuri); F-15 (JP-8

exposed F-15 flight line crews in Kadena); F-16 (JP-8 exposed F-16 flight line crews in Misawa).

3.2 Sampling of noise, audiology, blood, and urine

Sampling for noise dosimetry, an audiometric test battery consisting of immittance measurements, audiogram, OAE and ABR, miRNA biomarkers in blood, and metabolomic biomarkers in urine were collected according to the typical sequence of events at each base. The sampling schedule for the study is shown in Table 2.

Table 2. Sampling schedule for the study

Prior to Shift	During Shift	Post Shift: after flight line operations
- collect urine	- perform work	- remove personal air sampler and
- place personal air	duties as usual	noise dosimeter
sampler		- draw blood
- place noise		- collect 2nd urine
dosimeter		

3.2.1 Personal noise and VOC sampling during shift

To measure noise levels during the shift, prior to shift each subject had a Quest™ Edge Noise Dosimeter (Model EG5, TSI, Oconomowoc WI) personal sound level monitor placed in the right pocket of a belt with shoulder straps designed by the Aeromedical Laboratory (AML; Figure 1). The dosimeter was removed and turned off at the end of a shift. In addition to the sound levels, the meter also measured the length of a shift. A Personal Air Monitor (PAS-500) with charcoal tube (ORBO32 small, SUPELCO) was placed in the left pocket of the belt (Figure 1) to collect VOCs for analysis by AML (Ohrui et al., 2023).

Values assigned for the noise dosimeter during sampling are shown in Table 3. The Criterion Level is the action level for noise exposure. The Exchange Rate is the decibel level increase for which the exposure time needs to be cut in half. The Upper Limit is used to calculate percent dose. The Integrating Threshold is the A-weighted sound pressure level at which a noise dosimeter begins to integrate the noise into the measured exposure. For DoD purposes this is 80 dBA. The Internal Threshold Enable equals True turns the integration on. Peak weighting can be A, C, or Z. A-weighting cuts off the lower and higher frequencies that the average person cannot hear while C-weighting is for high noise or impulse noise exposures. Z-weighting was chosen for peak weighting because it is a linear response range that would represent both A and C-weightings for peak noise levels. Projection time is the assumed time of the shift. Since the shift time was projected to be 8 hours, a slow response time was appropriate. These values are used to evaluate the noise exposure and calculate an actual time weighted average for the time of sampling. The dosimeter had two meters, so one was set to A-weighting frequency filter while the other was set to C-weighting. The two meters cover normal hearing range (A) and can better measure louder noises greater than 100 dB (C).

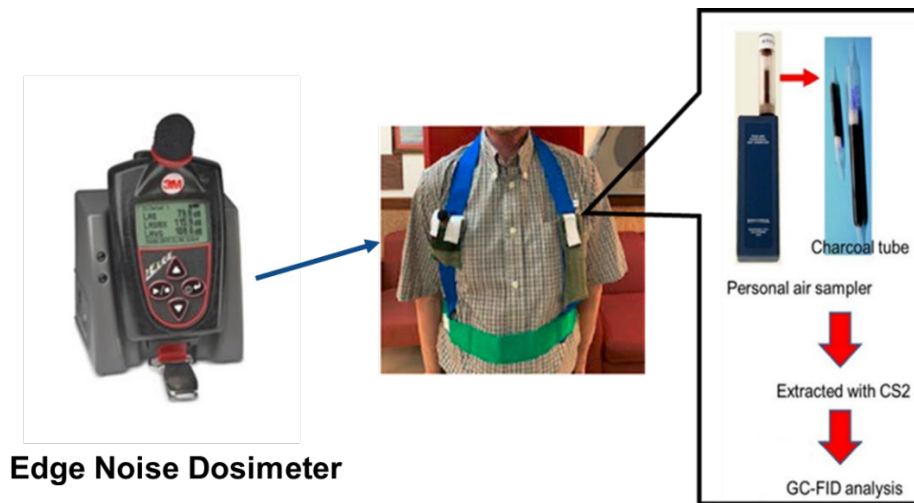


Figure 1. Personal air sampling device, Personal Air Monitor (PAS-500) and charcoal tube (ORBO32 small, SUPELCO), in the left pocket of person

On the right is also analytical scheme for VOCs (images property of JASDF/AML co-authors). On the left and in the right pocket of person is the Quest™ EG5, TSI Edge Noise Dosimeter (now discontinued; image of person is David Mattie, the first author, in photo taken by a USAF co-author)

Table 3. Assigned Noise Dosimeter Values for the Edge Noise Dosimeter (dB = decibels)

Criterion Level	85 dB
Exchange Rate	3 dB
Upper Limit (used to calculate % dose)	140 dB
Internal Threshold Enable	True
Integrating Threshold	80 dB
Peak Weighting	Z
Projection Time	480 min
Response	Slow
Weighted Frequency Filter	Meter 1 = A Meter 2 = C

3.2.2 Audiology Tests

Audiologic threshold measurements were conducted post-shift in an approved, existing sound booth or using a portable Defense Occupational and Environmental Health Readiness System-Hearing Conservation (DOEHRS-HC) audiometer in a room with a closed door and no external noise interference. The following battery of tests were conducted for each subject.

3.2.2.1 Otoscopy

The ear canals were examined prior to audiologic measurements to confirm they were free from occlusion (cerumen) and presented a normal tympanic membrane. Ear cleaning was performed

as required. A Welch Allyn diagnostic otoscope which every doctor has was used to examine ears.

3.2.2.2 Immittance

The status of the middle ear was evaluated by routine tympanometry and acoustic reflex (AR) testing (immittance audiometry) using the Interacoustics Titan Clinical Module (Middelfart, Denmark).

3.2.2.3 Otoacoustic Emissions (OAE)

OAE measurements were collected in both ears separately from 1000 to 8000 Hz using the appropriate diagnostic signal to noise ratios for response calculations with the Interacoustics Titan Clinical Module.

3.2.2.4 Auditory Brainstem Response (ABR)

ABR measurements were made with a two-channel electrophysiologic system using far-field electrode recordings (averaging), a SOAP-KALMAN Weighted Algorithm, and insert-earphones for stimulus delivery. The following electrode array was used for each test subject: surface electrodes were placed with the non-inverting electrode at Channel 1 active-Cz (high forehead or vertex), the inverting electrodes were placed at A1 and A2 ear lobes for the left and right ears respectively, while the ground electrode was placed on the low forehead. Electrodes external and internal (subject) noise interference was minimized by placement of the equipment away from light and electrical inputs and by the supine positioning of the subject. Two conditions (fast rate and slow rate) were measured to obtain findings in each ear for every test subject, with each condition using a rarefaction polarity. The first stimulus was an 80 decibels (dB) normal Hearing Level air-conducted click with a 27.5/second stimulus rate, with the second using an 80 dB normal Hearing Level air conducted click with a 85.9/second stimulus rate. Recordings were collected at least two times in each condition with a minimum of 1000 sweeps for replicability using the Vivosonic Integrity V500 8.4.1 Auditory Brainstem Response system (Toronto, ON, Canada). The waves I, III, and V latencies and amplitudes for wave I and V were manually marked by audiologist observation for the slow rate stimulus. Wave V latencies and amplitudes were manually marked by audiologist observation for the fast rate stimulus.

3.2.2.5 Audiogram

Audiometric thresholds were obtained with total dynamic head (TDH) headphones from 500 to 6000 Hz 5 dB stepwise in each ear using a modified Hughson-Westlake behavior testing method. Testing was completed using DOEHRS-HC software and hardware.

3.2.3 miRNA analysis

Blood and urine samples were collected and analyzed to determine levels of jet fuel components and potential biomarkers were present post shift. Blood draws were conducted post shift. Each subject had their blood drawn at the clinic, base hospital, or an approved designated area for that base. Each subject had 15 milliliters (mL) of blood drawn into the following tubes: One 5 mL blood collection tube for chemical analysis with heparin (Venoject II®, Terumo, Tokyo, JP) and

Two 2.5 mL PAXgene Blood RNA (ribonucleic acid) tubes for biomarkers associated with RNA in leukocytes (one 5 mL tube as alternative). The PAXgene Blood RNA tubes were handled according to the procedure in the blood RNA kit (Qiagen PAXgene RNA Kit, Qiagen, Valencia CA). The PAXgene Blood RNA tubes were frozen at -80° degrees Centigrade (°C), shipped to the USAF/711 Human Performance Wing at Wright-Patterson Air Force Base and stored frozen at -80°C until analysis.

Urine samples (entire void) were collected prior to shift (first void in early morning) and post shift. The pre-shift urine sample was required as a baseline for metabolomics. Urine weight was recorded and then 1 mL of urine was placed into one vial, 3 mL of urine each was placed into three vials, 5 mL of urine into one vial and 10 mL of urine each into three vials. The 10 mL urine samples for chemical analysis were stored chilled (4°C) in the dark until analysis by AML for jet fuel components. The 1 mL, 3 mL and 5 mL of urine were frozen, shipped to 711 Human Performance Wing, and stored at -80°C until analysis for metabolites.

3.2.3.1 2018 and 2019 Sampling Years

3.2.3.1.1 RNA Extraction

Total RNA, including miRNA, was extracted from PAXgene Blood RNA Tubes using PAXgene Blood miRNA Kits (Qiagen, Cat. No. 763134) following manufacturer's protocol. The PAXgene Blood RNA Tube was equilibrated at room temperature for 2 hours, and the thawed tube was inverted 10 times. The tube was centrifuged for 10 minutes at 3,000 times gravity (x g). The supernatant was discarded, and 4 mL of RNase-free water were added to the pellet. The tube was centrifuged a second time for 10 minutes at 3,000 x g, and the supernatant was discarded. 350 microliters (uL) of Buffer BM1 were added, and the tube was vortexed until the pellet dissolved. 300 uL of Buffer BM2 and 40 uL of proteinase K were added. The sample was incubated for 10 minutes at 55°C and 600 revolutions per minute. The sample was pipetted into a PAXgene Shredder spin column and centrifuged for 3 minutes at 20,000 x g. The flow-through was transferred to a new 1.5 mL tube, and 700 uL of 100 percent (%) isopropanol were added. The sample was vortexed, and 700 uL were pipetted into a PAXgene RNA spin column. The column was centrifuged for 1 minute at 10,000 x g. The remaining sample was added to the spin column and centrifuged for 1 minute at 10,000 x g. The flow-through was discarded. 350 uL Buffer BM3 were added to the spin column and centrifuged for 15 seconds at 10,000 x g. The flow-through was discarded, and 80 uL of DNase I incubation mix were added directly to the PAXgene RNA spin column membrane. The sample was incubated at room temperature for 15 minutes. 350 uL of Buffer BM3 were added to the spin column, and the column was centrifuged for 15 seconds at 10,000 x g. The flow-through was discarded. 500 uL Buffer BM4 were added to the column, and the column was centrifuged for 15 seconds at 10,000 x g. Another 500 uL Buffer BM4 were added to the column, and the column was centrifuged for 2 minutes at 10,000 x g followed by another minute at the same speed. The column was placed into a new tube, 40 uL Buffer BR5 were added, and the column was centrifuged for 1 minute at 10,000 x g. Another 40 uL Buffer BR5 were added, and the column was incubated for 10 minutes at room temperature before being centrifuged for 1 minute at 10,000 x g. The RNA eluate was stored at -20°C.

3.2.3.1.2 miRNA Sequencing

For sample set 1 (November 2018), miRNA quantity was assessed using an Agilent Small RNA kit (Agilent, Cat. No. 5067-1548) and Bioanalyzer 2100 (Agilent). For sample set 2 (April 2019)

miRNA quantity was assessed using a Qubit miRNA Assay Kit (Qiagen, Cat. No. Q32880) and Qubit 4 Fluorometer (Qiagen). Complimentary DNA (cDNA) libraries were constructed using an Ion Total RNA-Seq Kit v2 (Thermo Fisher, Cat. No. 4479789) and Ion Xpress RNA-Seq Barcode 1-16 Kit (Thermo Fisher, Cat. No. 4475482) following manufacturer guidelines (Ion Total RNA-Seq Kit v2 User Guide, MAN0010654, C.0). Samples were not enriched. 10 nanograms/3 uL of total RNA were hybridized and ligated. The RNA with ligated adaptors was reverse transcribed, and the Magnetic Bead Cleanup Module was used to size-select the desired cDNA products. cDNA was amplified using Ion Xpress RNA 3' Barcode primers, and the Magnetic Bead Cleanup Module was used to purify and size-select the amplified deoxyribonucleic acid (DNA). The yield and size distribution of the amplified DNA was assessed using an Agilent High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626) on a Bioanalyzer 2100 instrument (Agilent). Each of the barcoded cDNA libraries was diluted to 100 picoMole and pooled for template preparation. An Ion 540™ Kit-Chef (Thermo Fisher, Cat. No. A27759) and Ion 540™ Chip Kit (Thermo Fisher, Cat. No. A27765) was used according to manufacturer protocol on an Ion Chef™ system (Thermo Fisher) for automated template preparation and chip loading. Libraries were sequenced by multiplexing using the Ion 540™ chip on an Ion S5™ System (Thermo Fisher).

3.2.3.1.3 Analysis of RNA-Seq data

Base calling and demultiplexing were performed using Torrent Suite software, version 5.0 with default parameters. The FASTQ files were uploaded to Partek® Flow® software, version 7.0 (Partek, Inc.) on a Linux based High Performance Computing system. MicroRNA-Seq data was analyzed using the Partek® Flow® miRNA pipeline. The pre-alignment quality assurance (QA) /quality control (QC) tool was used to assess the quality of the reads imported into the pipeline. Bases were trimmed from both ends using a Phred quality score cut-off of 20 and reads shorter than 15 nucleotides in length were discarded. Reads were aligned to the *Rattus norvegicus* Rno_5.0 genome assembly (<http://www.ensembl.org>) using Bowtie 1.0.0. The post-alignment QA/QC tool was used with default parameters to assess how well the reads aligned to the rat genome assembly. Aligned reads were annotated using miRBase mature miRNAs version 21 (<http://www.mirbase.org>). Raw read counts were normalized on a per sample basis using the Total Count normalization method. Differential miRNA expression was detected using Partek's Gene Specific Analysis (GSA) statistical model. miRNA were considered differentially expressed using fold change <-2 or >2 and p-value < 0.05 cut-offs.

3.2.3.2 2021 to 2023 Sampling Years

3.2.3.2.1 Study Sample Aggregation

A total of 85 blood samples were analyzed for miRNA changes. The samples were grouped as to location collected and exposure level (Table 4). Samples were collected as reported above.

Table 4. Sample group, sample numbers, and exposure levels for miRNA samples

Group Designation	Group Location	Presumed Exposure	Personnel Tested	Sample Numbers
MA	Matsushima AB in Higashimatsushima, Yamoto, Japan	JetA1	JASDF flight line personnel	MA-1 through MA-14
HO	Hamamatsu AB in Shizuoka, Hamamatsu, Japan	JetA1	JASDF flight line personnel	HO-1 through HO-22
MU	Misawa AB in Aomori, Misawa, Japan	JP-8	US Air Force flight line personnel	MU-1 through MU-18*
MC	Misawa AB in Aomori, Misawa, Japan	None	US Air Force 35 th Medical Workers	MC-1 through MC-15
IC	Iruma AB in Kawagoe, Sayama, Japan	None	JASDF Aeromedical Laboratory Workers	I-12 through I-27

*MU-12 sample removed from study due to column failure during RNA extraction

3.2.3.2.2 RNA Preparation

Samples were sent to University of Wisconsin Biotechnology Center Gene Expression Center for miRNA analyses. There, total RNA was extracted from the whole blood, previously stabilized in PAXgene Blood RNA Tubes, using the Qiagen miRNeasy Serum/Plasma Kit as per manufacturer protocols (Qiagen, Valencia CA).

3.2.3.2.3 Quality Control

The quantity and purity of the RNA samples were analyzed by spectrophotometric wavelength scan on the NanoDrop One Spectrophotometer (Figure 2) to evaluate the purity of the RNA from each sample using a 260/280 ratio. The scan can identify contaminant peaks at other wavelengths, especially at 320 nanometers. The integrity and enrichment of small RNAs were determined on an Agilent 4200Tapestation using the High Sensitivity RNA Screentape. Small RNA species were noted in each sample.

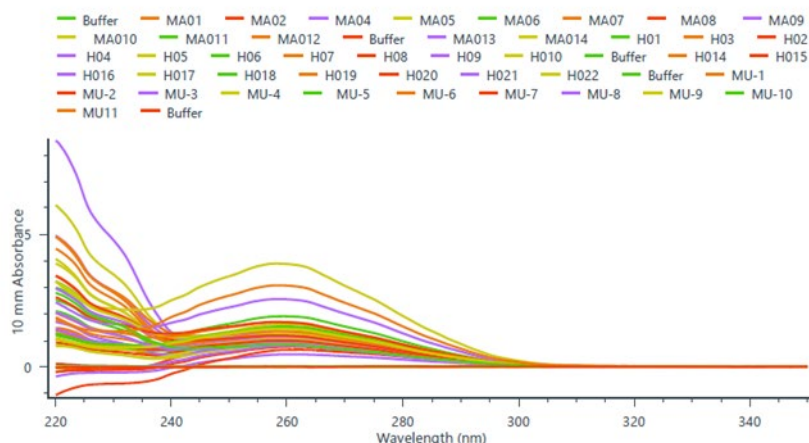


Figure 2. Quality Control of RNA preparations in the samples shown on top

3.2.3.2.4 Library Preparation and Sequencing (Seq)

Bioinformatic analysis of transcriptomic data adhered to recommended ENCODE guidelines and best practices for RNA-Seq (Encode Consortium, 2016). A total of 5 μ L of total RNA eluate was used to prepare a miRNA library following manufacturer's instructions (QIAseq miRNA UDI Library Kit Handbook, 11/2021) for sequencing on the Illumina NovaSeq X Plus sequencer and initially filtered from reads failing to meet default quality thresholds. Reads were exported to FASTQ format (bcl2fastq v2.2.0) with corresponding base quality scores. QC of the remaining reads was evaluated with fastp

(<https://academic.oup.com/bioinformatics/article/34/17/i884/5093234>). To evaluate the presence of known miRNAs, the reads were first filtered from the 3' Qiagen adapter sequence (5'-AACTGTAGGCACCATCAAT-3') using CutAdapt

(<https://journal.embnnet.org/index.php/embnnetjournal/article/view/200>). Next, the reads were aligned with bowtie (<https://genomebiology.biomedcentral.com/articles/10.1186/gb-2009-10-3-r25>) to the Rnor_6.0 reference genome (assembly accession GCA_000001895.4). The software featureCounts (<https://pubmed.ncbi.nlm.nih.gov/24227677/>) was used to assign read counts to miRNA features defined by miRbase v22.

(<https://academic.oup.com/nar/article/47/D1/D155/5179337>).

3.2.3.2.5 miRNA Data Analyses

The human samples were split into five sets with the following contrasts that were used for further analyses (Table 5):

Set01: "MC-IC", "MU-MC", "MA-MU", "HO-MU", "MA-HO"

Set02: "MU-MCIC", "MA-MCIC", "HO-MCIC"

Set03: "MAHO-MCIC"

Set04: "MAHO-MU"

Set05: "MAHO-IC", "HO-IC", "MA-IC"

To test for differential gene expression among individual groups, read counts were used as input into edgeR (v3.16.5; Robinson, et al., 2010). Inter-sample normalization was achieved with the trimmed mean of M-values (TMM; Robinson and Oshlack, 2010) method. Statistical significance of the negative-binomial regression test was adjusted with a Benjamini-Hochberg

false discovery rate (FDR) correction at the 5% level (Reiner, Yekutieli, and Benjamini 2003). Prior to statistical analysis with edgeR, independent filtering was applied and required genes to have a count-per-million (CPM) above k in n samples, where k is determined by minimum read count (10 reads) and by the sample library sizes where n is determined by the number of biological replicates in each group.

Heatmaps of the Pearson correlation difference and expression levels were generated using a regularized logarithm variance-stabilizing transform; <https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8>) on the library-normalized data and plotted with R (<https://www.r-project.org/>).

Table 5. Data comparisons of miRNA alterations completed

Group 1		Group 2	Exposure	Data Comparison
MC	vs.	IC	None	Examination of variability between control groups
MU	vs.	MC	JP-8	Examination of miRNA alterations due to Exposure
MU	vs.	MC+IC	JP-8	Examination of miRNA alterations due to Exposure compared to combined Control data set
MA	vs.	MC+IC	JetA1	Examination of miRNA alteration due to Exposure compared to combined Control data set
H0	vs.	MC+IC	JetA1	Examination of miRNA alterations due to Exposure compared to combined Control data set
MA + H0	vs.	MC + IC	JetA1	Examination of miRNA alterations due to combined Exposure data compared to combined Control data set
MA	vs.	MU	JetA1/JP-8	Examination of miRNA alterations due to additives
H0	vs.	MU	JetA1/JP-8	Examination of miRNA alterations due to additives
MA+H0	vs.	MU	JetA1/JP-8	Examination of miRNA alterations due to additives
MA	vs.	HO	JetA1 (both)	Examination of miRNA differences due to locale in similar exposure groups
MA	vs.	IC	JetA1	Examination of miRNA alterations due to JetA1 exposure in JASDF personnel only
HO	vs.	IC	JetA1	Examination of miRNA alterations due to JetA1 exposure in JASDF personnel only
MA+HO	vs.	IC	JetA1	Examination of miRNA alterations due to JetA1 exposure (two locales) in JASDF personnel only

3.2.4 Metabolomic Analysis

3.2.4.1 Overview

Samples for metabolomic analysis was urine from subjects collected pre (UB added to label) and post shift (UP added to label). Samples were randomized prior to thawing and assigned a tracking number. The proteins were removed, and the metabolites extracted from the urine. A standard was run periodically to ensure that the column was not degrading, and the instrument was not getting dirty. The metabolites were separated using hydrophilic interaction

chromatography (HILIC) followed by mass spectrometry on a Thermo QE orbitrap mass spectrometer (MS) using positive ion mode. Additionally, a pooled sample was run on a Thermo fusion lumos MS utilizing AcquireX, which provides structural identification and characterization of small molecules.

3.2.4.2 Liquid Chromatography Mass Spectrometry Analysis

To create a pooled sample, 100 μL of each urine sample ($n=98$) was aliquoted and randomized. 5 μL of each urine was sample mixed to generate a QC pooled sample. The pool was thoroughly mixed and 100 μL of this pool was used.

To each sample 100 μL of chilled acetonitrile was added, including the pooled sample and thoroughly mixed. The samples were incubated for 30 minutes at -20°C , then centrifuged at 10,000 relative centrifugal force for 30 minutes at 4°C . For the randomized samples, 1 μL of supernatant was analyzed by liquid chromatography mass spectrometer (LC-MS) with QC pooled samples run after every 10 sample injections. Blanks (mobile phase A, 5 % acetonitrile: 0.1% formic acid, 10 millimolar (mM) ammonium formate (aqueous)) were run prior to each sample injection to assure that the results did not shift from carry over of samples from one sample run to another. Separations were performed on a Thermo Scientific Vanquish H Binary Pump at 400 $\mu\text{L}/\text{minute}$ constant flow using a HILIC column (Waters Acquity BEH Amide column, 130 Åstroms, 1.7 micrometers, 2.1 millimeters (mm) $\times$ 150 mm, Milford, MA) operated at 30°C . Mobile phase A contained 5% acetonitrile: 0.1% formic acid, 10 mM ammonium formate (aqueous) and mobile phase B contained 95% acetonitrile: 0.1% formic acid, 10 mM ammonium formate (aqueous, Optima, ThermoFisher). The separation was performed at 90% B for 3.3 minutes, decreasing to 65% B at 12.3 minutes, with a hold for 3.3 minutes at 65% B, returning to 90% B at 16.3 minutes, with a hold at 90% B to 20 minutes.

Metabolites were detected from 75-375 mass-to-charge ratio (m/z) in positive mode on a Thermo Scientific Q Exactive HF MS. Profile scans were acquired at 120,000 resolution with a maximum injection time of 200 milliseconds and an automatic gain control of $3\text{E}6$. The heated electrospray ionization source was operated at 3.5 kilovolts, sheath gas of 30 (arbitrary), auxiliary gas of 5 (arbitrary), S-Lens radio frequency (RF) of 50, and the auxiliary heater at 30°C with a transfer tube temperature of 250°C the entire 30-minute separation.

3.2.4.3 Liquid Chromatography Mass Spectrometry / Mass Spectrometer Analysis

Data dependent acquisition was acquired on the pooled QC sample using Thermo Fisher Scientific's AcquireX Deep Scan data acquisition workflow on an Orbitrap Fusion Lumos MS. Following a blank injection to generate a background exclusion list preventing collection of noise fragmentation spectra, a full scan injection (MS1) of the pooled QC sample was performed. An inclusion list, consisting of m/z and retention times, was automatically generated to be isolated and fragmented in subsequent iterative pooled QC sample injections (MSn) (3 total).

With each MS/MS injection, the precursor ions fragmented from the initial inclusion list were added to the exclusion list to prevent further fragmentation, allowing for improved depth of MS/MS spectra. The separations for the LC- MS/MS analysis were the same as described above. The Orbitrap Fusion Lumos was operated in positive ion mode with a heated electrospray ionization source. The source parameters for all injections were spray voltage of 3.5 kilovolts, sheath gas 40 (arbitrary), auxiliary gas 10 (arbitrary), ion transfer tube temp of 300°C , and

vaporizer temperature of 300°C. The MS1, blank injection, and the initial pooled sample injection scans consisted of Orbitrap detection at 120,000 resolution using quadrupole isolation over a scan range of 75-375 m/z.

The profile MS1 scans had an ion population (AGC target) of 1E5, a maximum injection time 50 milliseconds, and an RF lens of 30%. The MSn scans of the pooled QC sample were performed with an initial MS1 scan using a standard AGC target. An intensity threshold filter of 2E4 was applied with dynamic exclusion settings of 2.5 seconds within ± 3 parts per million (ppm) of the precursor ion. Data dependent MSn scans were performed with Orbitrap detection in positive ion mode at 120,000 resolution using a 1.0 m/z quadrupole isolation window. The HCD or higher-energy collisional dissociation activation energy was stepped using 20, 50, and 85 collision energies with a maximum injection time of 22 milliseconds and an AGC target of 5xE4.

3.2.4.4 Data Processing and Analysis

Raw global metabolomics data (samples, pooled samples, and blanks) were searched using Compound Discoverer Software Suite (v 3.3, Thermo Fisher Scientific). Search settings were as follows: MS1 mass tolerance 5 ppm, minimum peak intensity 10,000, and signal to noise of 1.5 with a retention time tolerance of 1 minute. Fragmentation MS2 settings were 5 ppm mass tolerance, and mzCloud match threshold of 75. Tentative compound identification from mzVault, mzCloud, and mass list results were matched to the final feature list (105) and reported. Confident matches were shown in a spreadsheet with a name. These compounds are likely what was seen in the samples, so they are considered “tentatively identified.” For the metabolites that were not given names, the matches were less confident because they have issues such as multiple library matches, isomers, or a lot of extraneous or missing fragments that make the match less confident.

The results from the MS analyses were merged based on matching chemical formula. Chemicals were removed if they had fewer than two observations. Data were normalized and principal components analysis was performed using the R statistical programming language in Rstudio® (Posit Software, PBC, Boston MA).

3.3 Statistical analysis

The comparison of means for audiological data was performed by t-tests with a p-value accepted as less than 0.05.

4.0 RESULTS AND DISCUSSIONS

4.1 Demographic Data

The mean age of the F-15 group was significantly lower than CJ, CK, CM and T-4 (Table 6). The mean age of the F-16 group was also significantly lower than CK and CM. No significant differences were found among the other groups. Working experience as years in military service of the F-15 group was significantly lower than the other groups except for CJ and F-16. The average working experience as years in their career field was less than time in military service although for Kadena and Misawa they were closer than all other groups. The smoking rates were

higher in the jet fuel exposed groups (T-4, F-2, F-4, and F-15: 35.0 - 71.4%) with the exception of the F-16 group (16.7%) than in the control groups CJ, CK and CM (22.7 - 26.7%). The proportion of female subjects was higher in the control groups (20.6 - 50.0%) than in the jet fuel exposed groups (0 - 16.7%).

Table 6. Summary of demographic data for age, experience (years in service and career field), percent tobacco use, and percent female subjects

	Control			Jet fuel exposure					Statistically significant difference
	CJ	CK	CM	T-4	F-2	F-4	F-15	F-16	
Number	34	22	15	29	7	20	23	18	
Fuel	N/A			JetA1		JP-4	JP-8		
Age $\pm$ SEM* (years) *Average $\pm$ SEM (Standard error of the mean)	27.9 ± 1.0	29.6 ± 1.0	31.3 ± 1.3	26.9 ± 0.8	26.6 ± 1.7	25.2 ± 1.1	22.6 ± 0.6	23.9 ± 0.8	CJ > F-15 CK > F-15 CM > F-15 T-4 > F-15 CK > F-16 CM > F-16
Work experience $\pm$ SEM (years in service)	6.0 $\pm$ 1.2	8.3 $\pm$ 1.1	8.7 $\pm$ 1.2	7.2 $\pm$ 0.9	7.0 $\pm$ 1.8	6.5 $\pm$ 1.0	2.5 $\pm$ 0.4	3.9 $\pm$ 0.6	CK > F-15 CM > F-15 T-4 > F-15 F-2 > F-15 F-4 > F-15
Work experience $\pm$ SEM (years in career field)	4.9 $\pm$ 1.0	8.0 $\pm$ 0.9	5.8 $\pm$ 1.2	6.7 $\pm$ 0.9	6.4 $\pm$ 2.0	6.1 $\pm$ 1.1	2.4 $\pm$ 0.4	3.5 $\pm$ 0.4	
Smokers (%)	9 (26.5)	4 (18.2)	4 (26.7)	13 (44.8)	5 (71.4)	7 (35.0)	9 (39.1)	5 (27.7)	
Female (%)	7 (20.6)	11 (50.0)	6 (40.0)	7 (24.1)	0	0	1 (4.3)	3 (16.7)	

While the JASDF showed the demographic data by individual bases and aircraft (e.g., Table 6), this report shows demographic data by JASDF and USAF ABs (Tables 7-10). In addition, hobbies involving VOCs and caffeine intake standardized to 8-ounce (oz) cups of liquid were added to Tables 7 and 8.

Table 7. Summary of initial demographic data for age, experience (years in service, time in career field), gender, hobbies with VOCs, tobacco use, caffeine intake at JASDF ABs

Base / Subjects	Rank	Career Field by Name	Years of service	Age	Gender	Time in career field (Yrs)	Hobbies involving VOCs	Tobacco use	Caffeinated drinks (based on 8 oz)
Hyakuri Exposed	Airman = 12 SSgt = 7 TSgt = 1	Aircraft Maintenance	6.5 ± 1.0	25.2 ± 1.1	Male = 20	6.1 ± 1.1	0.2 ± 0.1	0.4 ± 0.1	1.4 ± 0.3
Hyakuri Controls*	Enlisted = 10 Officer = 7 civilian = 1	Medical Corps	9.0 ± 2.0	29.2 ± 1.7	Male = 14 Female = 4	6.9 ± 1.8	0	0.3 ± 0.1	1.9 ± 0.4
Matushima Exposed	AIC = 3 SSgt = 10 2nd LT = 1	Aircraft Maintenance	8.8 ± 1.1	28.1 ± 1.0	Male = 13 Female = 1	8.4 ± 1.2	0	0.4 ± 0.1	0.5 ± 0.2
Hamamatsu Exposed	AIC = 9 SSgt = 12 TSgt = 1	Aircraft Maintenance	6.2 ± 1.1	26.0 ± 1.0	Male = 16 Female = 6	5.8 ± 1.0	0.3 ± 0.2	0.5 ± 0.1	1.1 ± 0.2
Iruma Controls ⁺	Enlisted = 8 2nd LT = 4 civilian = 4	Medical Corps; Researcher; Pharmacist	2.7 ± 0.5	26.4 ± 0.8	Male = 13 Female = 3	2.7 ± 0.5	0.1 ± 0.1	0.2 ± 0.1	1.2 ± 0.3

Data as average ± SEM (standard error of the mean); *Hyakuri, Yokota, Iruma; +for Matushima & Hamamatsu; oz = ounce

Table 8. Summary of initial demographic data for age, experience (years in service and time in career field), gender, hobbies involving VOCs), tobacco use and caffeine intake by USAF ABs

Base / Subjects	Rank	Career Field by Name	Years of service	Age	Gender	Time in career field (Yrs)	Hobbies involving VOCs	Tobacco use	Caffeinated drinks (based on 8 oz)
Kadena Exposed	Airman = 2 AIC = 12 SRA = 8 SSgt = 1	Crew Chief = 18 Avionics = 4 elect & environ = 1	2.5 ± 0.4	22.6 ± 0.6	Male = 22 Female = 1	2.4 ± 0.4	0.1 ± 0.1	0.4 ± 0.1	1.1 ± 0.3
Kadena Control	Enlisted = 21 Officer = 1	BE Tech; Pub Health; Dental	8.3 ± 0.9	29.6 ± 4.7	Male = 11 Female = 11	8.0 ± 0.9	0.05 ± 0.2	0.05 ± 0.04	1.0 ± 0.2
Misawa Exposed	AIC = 6 SRA = 7 SSgt = 4 TSgt = 1	Crew Chief = 7 Armament = 3 AC Maint. = 3 AGE = 3 Specialist = 2	3.9 ± 0.6	23.9 ± 0.8	Male = 15 Female = 3	3.5 ± 0.4	0.06 ± 0.06	0.3 ± 0.1	1.7 ± 0.5
Misawa Control	Enlisted = 14 Officer = 1	Flight Medicine BE Tech	8.7 ± 1.2	31.3 ± 1.4	Male = 9 Female = 6	5.8 ± 1.2	0.07 ± 0.07	0.2 ± 0.1	1.4 ± 0.3

Data displayed as average ± SEM (standard error of the mean); oz = ounce

The questions that dealt with personal protective equipment and their use were summarized and shown in Tables 9 and 10, again by either JASDF or USAF ABs.

Table 9. Results of the hearing PPE questions from the Demographic Questionnaires for JASDF ABs

Base / Subjects	What hearing protection did you wear? - No (0); earplug (1) or earmuff (2) or both (3) or N/A (4) headset only (5)	Did you remove your hearing protection when speaking to others?	if yes for how long? Seconds (Mean $\pm$ SEM)	What types of noise were you exposed to during your shift today?	Were they continuous (0), impulsive (1), intermittent (2), or varying (3)	Were today's exposures typical for you?
Hyakuri Exposed	1 = 1; earmuff = 9; 3 = 1; 5 = 8	No = 17; Yes = 3	145 $\pm$ 80.6 (n=3)	jets and AGE	3 = 20	all Yes
Hyakuri Controls*	N/A = 18	N/A = 18	N/A	Office or clinic sounds	N/A = 18	all Yes
Matsushima Exposed	1 = 2; earmuff = 11; 3 = 1	Yes = 6; No = 8	1300 $\pm$ 335	jets with/without AGE	0 = 10; 2 = 4	all Yes
Hamamatsu Exposed	1 = 1; earmuff = 21	Yes = 18; No = 4	2360 $\pm$ 833 (39.3 minutes)	jets with/without AGE	0 = 16; 2 = 6	all Yes
Iruma Controls ⁺	N/A = 16	N/A	N/A	Office sounds only	N/A = 16	all Yes
*Hyakuri, Yokota, Iruma						
⁺ for Matsushima & Hamamatsu						

SEM (standard error of the mean); AGE = Aerospace Ground Equipment

Table 10. Results of the hearing PPE questions from the Demographic Questionnaires for USAF ABs

Base / Subjects	What hearing protection did you wear? - No (0); earplug (1) or earmuff (2) or both (3) or N/A (4)	Did you remove your hearing protection when speaking to others? Yes = 1; No = 0	if yes for how long? Seconds (Mean $\pm$ SEM)	What types of noise were you exposed to during your shift today?	Were they continuous (0), impulsive (1), intermittent (2), or varying (3)	Were today's exposures typical for you?
Kadena Exposed	earmuff = 13; both = 10	Yes = 4; No = 19	48.3 $\pm$ 11.7 (n=3)	jets with/without AGE; one panel removal	0 = 10; 2 = 3; 3 = 10	all Yes
Kadena Control	No = 3; earplug = 2; earmuff = 1; N/A = 16	Yes = 3; N/A = 18; 3 = noise w/o PPE	600 for 2	pneumatic chisel, pump, grinder, suction, air hose, phone; office & clinic sounds	0 = 3; 2 = 1; 4 = 1; N/A = 17	all Yes; 6 in Dental Clinic
Misawa Exposed	0 = 2; 1 = 1; 2 = 1; 3 = 12; 4 = 2	3 out of 18	10 $\pm$ 6	jets, AGE; forklift; music	0 = 14; 1 = 0; 2 = 1; 3 = 1; N/A = 2	Yes = 10 No = 8
Misawa Control	N/A = 15	N/A	N/A	Office or clinic sounds; 1 ambulance; 1 jet flew by while driving	N/A = 15	all Yes

SEM (standard error of the mean); AGE = Aerospace Ground Equipment

The post shift interview questions that corresponded to exposures on the flight line were more subjective and qualitative, so they were summarized for the entire study in Table 11. Quantitative concentrations of VOCs found in jet fuel were measured and analyzed by AML and can be found for air, blood and urine in the AML final report (Ohrui et al., 2023).

Table 11. Results of the qualitative exposure assessment from the post shift demographic questionnaire for both JASDF and USAF ABs

Time around refueling: 14.8 minutes average for all bases
Average time around running engines: 2.7 hours
Average time on flight line: 3.6 hours
Smelled exhaust (all but 6) or jet fuel (all but 10); out of 97 exposed subjects
Exposure to any spills: jet fuel, hydraulic fluid, engine oil
Exposures other than jet fuel: Hydraulic fluid, engine oil, isopropanol, hydrazine (F-16s)
Dermal exposures to jet fuel or aircraft fluids: Happens but more limited – 29.9% of subjects
Level of physical activity: Average was moderate

4.2 Sound level measurements

Noise dosimetry is reported by JASDF and USAF ABs in Table 12. The Hyakuri flight line was the loudest as they were still flying F-4 fighters at the time of the sampling. The next loudest aircraft were the F-15s. The actual TWA was calculated by the noise dosimeter based on the time of the shift which was the sampling time.

Table 12. Results for the sound level measurements at JASDF and USAF ABs

Subjects		Average (dB)	Average (dB)	Actual TWA for time of sampling	Actual TWA for time of sampling	Sampling time (hr)	Plane / Jet Fuel
		Meter 1	Meter 2	Meter 1	Meter 2		
Hyakuri Flight Line	Average	98.9	100.2	98.8	100.1	8.2	F-4
	SEM*	0.5	0.5	0.7	0.7	0.6	JP-4
Matsushima Flight Line	Average	98.0	91.0	94.4	85.1	3.6	F-2
	SEM	0.7	0.8	0.5	0.5	0.2	Jet A
Hamamatsu Flight Line	Average	97.5	92.5	92.4	84.1	2.5	T-4
	SEM	0.7	0.4	0.7	0.4	0.04	Jet A
JASDF Controls	Average	58.2	67.9	55.7	65.4	4.7	N/A
	SEM	1.8	1.6	1.8	1.6	0.2	
JASDF Controls-Iruma AB	Average	48.3	25.5	48.1	25.1	7.6	N/A
	SEM	1.4	6.9	1.4	6.9	0.1	
Kadena Flight Line	Average	102.2	101.9	103.0	102.6	9.7	F-15
	SEM	0.9	0.9	1.0	1.0	0.3	JP-8
Kadena Controls	Average	66.3	75.2	65.7	74.7	7.0	N/A
	SEM	1.8	2.1	1.8	2.1	0.1	
Misawa Flight Line	Average	90.7	93.6	90.6	93.5	7.8	F-16
	SEM	2.2	1.7	2.2	1.7	0.1	JP-8
Misawa Controls	Average	69.2	74.2	69.3	78.7	8.2	N/A
	SEM	1.6	5.1	1.6	1.5	0.2	

TWA = time weighted average

4.3 Audiological History

The audiological histories are summarized for JASDF ABs and USAF ABs in Table 13. Tinnitus was only reported for the F-4 flight line personnel for JASDF ABs but was also reported by one control subject at Iruma AB who had rare incidences of ringing. For USAF ABs tinnitus was reported in both exposed and control subjects but there were more exposed subjects than controls. Interestingly, there were more overall history items reported for controls than for exposed at Kadena AB.

Table 13. Results of the Audiological History questionnaires for JASDF and USAF ABs

Base / Subjects (Mean $\pm$ SEM)	n	Temporary Hearing loss	Chronic or bothersome tinnitus	Ear infections, drainage or surgeries	Spinning, dizziness, vertigo, or dizziness with head/neck movement	Ear pain or feeling of fullness	Family history of hearing loss	Chronic or recurrent medical condition	None of these	Taking herbal supplement	History of noise exposure off duty or recreationally (x out of n)
Hyakuri Exposed	20	0.1 $\pm$ 0.07	0.37 $\pm$ 0.11	0	0.15 $\pm$ 0.08	0.1 $\pm$ 0.07	0.05 $\pm$ 0.05	0	0.45 $\pm$ 0.11	0.1 $\pm$ 0.07	9
Hyakuri Controls*	18	0.11 $\pm$ 0.08	0	0.11 $\pm$ 0.08	0	0.06 $\pm$ 0.06	0	0	0.89 $\pm$ 0.08	0.28 $\pm$ 0.11	1
Kadena Exposed	23	0.04 $\pm$ 0.04	0.26 $\pm$ 0.09	0	0.04 $\pm$ 0.04	0.09 $\pm$ 0.06	0.17 $\pm$ 0.08	0	0.57 $\pm$ 0.11	0.09 $\pm$ 0.06	12
Kadena Control	22	0.09 $\pm$ 0.06	0.18 $\pm$ 0.08	0.14 $\pm$ 0.07	0.09 $\pm$ 0.06	0.05 $\pm$ 0.05	0	0.09 $\pm$ 0.06	0.64 $\pm$ 0.10	0.27 $\pm$ 0.10	6
Matushima Exposed	14	0	0	0	0	0	0.07 $\pm$ 0.07	0	0.93 $\pm$ 0.07	0.07 $\pm$ 0.07	3
Hamamatsu Exposed	22	0.05 $\pm$ 0.05	0	0	0.05 $\pm$ 0.05	0.05 $\pm$ 0.05	0.05 $\pm$ 0.05	0	0.86 $\pm$ 0.07	0.32 $\pm$ 0.10	3
Iruma Controls ⁺	16	0	0.06 $\pm$ 0.06	0.06 $\pm$ 0.06	0.06 $\pm$ 0.06	0	0	0	0.81 $\pm$ 0.10	0.19 $\pm$ 0.10	1
Misawa Exposed	18	0.11 $\pm$ 0.08	0.56 $\pm$ 0.12	0.11 $\pm$ 0.08	0.11 $\pm$ 0.08	0.11 $\pm$ 0.08	0	0	0.33 $\pm$ 0.11	0.22 $\pm$ 0.10	9
Misawa Control	15	0	0.33 $\pm$ 0.13	0.13 $\pm$ 0.09	0.07 $\pm$ 0.07	0.33 $\pm$ 0.13	0.13 $\pm$ 0.09	0.33 $\pm$ 0.13	0.27 $\pm$ 0.12	0.33 $\pm$ 0.13	8

Average $\pm$ SEM (standard error of the mean); *Hyakuri, Yokota, Iruma; +for Matushima & Hamamatsu

4.4 Audiological Tests

4.4.1 JASDF ABs

Test results are shown for T-4 flight line subjects in Tables 14 (Immittance), 15 (OAE), and 16 (ABR). Significant differences are shown as p values in bolded dark red. If a p value was 0.20 or less, it was bolded in black to indicate a potential trend. Overall, there is a trend toward hearing issues for flight line personnel working on T-4 aircraft.

Immittance data is measured as static compliance (SC) in cubic centimeters (cc); Peak pressure (P) in decapascals (daPa); equivalent ear canal volume (ECV) in cc; AR and Contra (C) AR in dB. Only SC was significantly increased in the immittance test for the right ear; left ears did show a trend towards significance. However, these differences are more of a statistical artifact than an indication of potential hearing loss. Also trending in the left ears were peak pressure which was still normal for controls and T-4 subjects and AR at contra 2000, true trend towards hearing loss.

Table 14. Immittance data for T-4 subjects

Subject										
Control		SC	Peak P	ECV	AR500-I	AR1000-I	AR2000-I	AR500-C	AR1000-C	AR2000-C
Right Ear	Mean	0.58	-5.21	1.29	86.1	87.3	88.3	95.2	94.0	94.1
all Iruma + HY + Yokota	STD	0.26	12.81	0.33	6.3	5.7	5.9	5.6	4.7	5.4
	SEM	0.04	2.20	0.06	1.2	1.1	1.1	1.1	0.9	1.0
	n	34	34	34	28	28	30	26	27	29
T-4	Mean	0.75	-2.57	1.24	87.8	88.6	89.0	94.8	94.8	95.9
Right Ear	STD	0.35	10.43	0.28	6.4	4.7	4.9	6.1	5.7	4.7
	SEM	0.07	1.97	0.05	1.2	0.9	0.9	1.3	1.2	1.0
	n	28	28	28	29	28	29	22	22	23
		p=0.03	p=0.38	p=0.53	p=0.32	p=0.36	p=0.62	p=0.81	p=0.59	p=0.21
Control	Mean	0.67	-8.00	1.27	85.5	87.0	88.9	92.4	93.8	92.7
Left Ear	STD	0.30	12.12	0.32	5.4	5.2	5.9	6.6	5.9	5.9
all Iruma + HY + Yokota	SEM	0.05	2.08	0.06	1.0	0.9	1.0	1.4	1.2	1.2
	n	34	34	34	32	32	32	21	24	24
T-4	Mean	0.82	-3.54	1.20	88.6	85.5	89.7	94.3	93.6	95.2
left ear	STD	0.49	8.17	0.30	7.1	15.8	5.2	5.1	4.8	4.2
	SEM	0.09	1.54	0.06	1.3	3.0	1.0	1.1	1.0	0.9
	n	28	28	28	28	28	29	21	21	22
		p=0.14	p=0.10	p=0.38	p=0.50	p=0.61	p=0.58	p=0.30	p=0.90	p=0.11

SC=static compliance (cc); Peak P=peak pressure (daPa); ECV=equivalent ear canal volume (cc); AR=acoustic reflex (dB); C=contra

For OAE, only 5666 Hz was significantly decreased in the right ears while 2920 Hz was significantly decreased in the left ear (Table 15). There were several trending decreases in amplitudes for T-4 subjects but no consistent differences across frequencies.

Table 15. OAE data for T-4 subjects with values in Hz

Control	Ear	2000	2198	2416	2656	2920	3210	3529	3880	4265	4688	5154	5666	6229	6847	7527	8275
Mean	Right	20.7	21.2	20.5	22.6	22.8	23.6	25.3	25.9	27.6	27.4	29.6	30.3	28.3	29.0	22.5	22.7
STD		10.0	6.9	5.5	5.8	5.7	5.9	6.3	6.6	5.7	6.0	7.0	7.6	8.9	8.4	7.3	7.1
SEM		1.8	1.2	1.0	1.0	1.0	1.0	1.1	1.2	1.0	1.1	1.2	1.3	1.6	1.5	1.3	1.2
n		32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
T-4																	
Mean	Right	21.9	20.7	19.4	21.2	22.1	21.4	22.8	24.7	26.4	27.0	26.4	26.8	26.9	26.0	23.4	22.9
STD		6.8	6.7	5.3	6.6	4.9	5.1	5.4	5.0	4.6	5.4	5.3	5.9	8.0	10.1	6.6	7.4
SEM		1.3	1.2	1.0	1.2	0.9	0.9	1.0	0.9	0.9	1.0	1.0	1.1	1.5	1.9	1.2	1.4
n		29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
		p=0.59	p=0.78	p=0.43	p=0.38	p=0.61	p=0.13	p=0.10	p=0.43	p=0.37	p=0.79	p=0.83	p=0.048	p=0.52	p=0.21	p=0.62	p=0.91
Control																	
Mean	Left	21.6	20.7	20.8	22.4	23.3	23.0	25.1	26.7	27.4	27.9	29.0	30.3	29.5	28.6	22.9	21.0
STD		5.8	5.3	5.7	6.1	5.9	6.9	5.8	5.9	5.8	6.3	7.1	6.9	6.9	7.8	7.2	7.1
SEM		1.0	0.9	1.0	1.1	1.0	1.2	1.0	1.0	1.0	1.1	1.2	1.2	1.2	1.4	1.3	1.3
n		32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
T-4																	
Mean	Left	20.0	19.9	19.6	19.7	19.9	21.7	23.4	26.0	26.6	28.2	28.0	29.0	27.9	27.9	23.9	23.0
STD		6.4	5.7	5.0	5.1	5.6	5.0	4.4	4.2	4.7	4.2	4.5	4.6	5.0	5.5	4.7	4.8
SEM		1.2	1.1	0.9	0.9	1.0	0.9	0.8	0.8	0.9	0.8	0.8	0.9	0.9	1.0	0.9	0.9
n		29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
		p=0.31	p=0.57	p=0.39	p=0.07	p=0.02	p=0.41	p=0.21	p=0.60	p=0.56	p=0.83	p=0.52	p=0.40	p=0.31	p=0.69	p=0.53	p=0.21

For slow rate ABR in the upper portion of Table 16, Wave I to Wave V latency was significantly decreased in right ears but not in the left. However, Wave I amplitude was significantly decreased in left ears as was the amplitude to latency ratio for Wave 1. For the fast rate ABR results in the lower portion of Table 18, Wave V latency was slightly slower in the right ears than the left in controls but was not different between T-4 subjects and controls. Otherwise, there were no differences between right and left ears for control or T-4 subjects. There was a strong trend for a greater fast rate Wave V latency in the left ears of T-4 subjects than controls but the Wave V amps and amplitude to latency ratios were not different. However, in the right ears the trend was the opposite with the Wave V amps and amplitude to latency ratios showing a slight trend for differences between controls and T-4 subjects and no difference for Wave V fast rate latency.

Table 16. ABR slow rate (upper portion) and fast rate (lower portion) results for T-4 subjects with values in milliseconds

Subject	Ear	Wave I Latency	Wave V Latency	I-III Latency	III-V Latency	I-V Latency	Wave I Amp	Wave V Amp	Wave V /Wave I amps	amplitude /latency* Wave I	amplitude /latency Wave V
Control											
Mean	Right Ear Slow	1.67	5.68	2.21	1.84	4.03	0.24	0.45	2.55	0.15	0.080
STD		0.21	0.24	0.27	0.12	0.20	0.14	0.16	1.62	0.094	0.030
SEM		0.036	0.041	0.046	0.021	0.035	0.023	0.027	0.28	0.016	0.005
n		34	34	34	34	34	34	34	34	34	34
Exposed:											
T-4											
Mean	Right Ear Slow	1.60	5.73	2.29	1.89	4.14	0.21	0.38	2.40	0.13	0.07
STD		0.093	0.18	0.27	0.09	0.16	0.091	0.16	2.06	0.061	0.029
SEM		0.018	0.034	0.051	0.017	0.031	0.017	0.030	0.39	0.012	0.005
n		28	28	28	28	28	28	28	28	28	28
		p=0.11	p=0.37	p=0.25	p=0.074	p=0.022	p=0.36	p=0.085	p=0.75	p=0.34	p=0.19
Control											
Mean	Left Ear Slow	1.58	5.70	2.22	1.87	4.09	0.26	0.43	2.13	0.17	0.08
STD		0.10	0.21	0.18	0.15	0.23	0.11	0.13	1.51	0.07	0.02
SEM		0.018	0.035	0.031	0.026	0.039	0.019	0.022	0.26	0.013	0.004
n		34	34	34	34	34	34	34	34	34	34
Exposed:											
T-4											
Mean	Left Ear Slow	1.59	5.73	2.29	1.88	4.14	0.19	0.39	2.26	0.12	0.07
STD		0.096	0.162	0.19	0.11	0.13	0.086	0.15	1.25	0.059	0.028
SEM		0.018	0.030	0.035	0.021	0.025	0.016	0.028	0.23	0.011	0.005
n		29	29	29	29	29	29	29	29	29	29
		p=0.69	p=0.53	p=0.14	p=0.77	p=0.30	p=0.0106	p=0.207	p=0.70	p=0.004	p=0.10

*amplitude/latency ratios ($\mu\text{V/ms}$; termed response magnitudes)

Subject	Ear	Wave V Latency	Wave V Amp	amplitude / latency Wave V	Ear	Wave V Latency	Wave V Amp	amplitude/ latency Wave V
Control	Right Ear fast				Left Ear fast			
Mean		6.21	0.27	0.043		6.14	0.27	0.044
STD		0.25	0.08	0.014		0.20	0.07	0.013
SEM		0.04	0.01	0.002		0.03	0.01	0.002
n		34	34	34		34	34	34
	right vs left	p=0.19	p=1.0	p=0.76				
T-4	Right Ear fast	6.25	0.24	0.038	Left Ear fast	6.25	0.25	0.040
Mean		0.28	0.09	0.015		0.26	0.11	0.019
STD		0.05	0.02	0.003		0.05	0.02	0.003
SEM		29	29	29		29	29	29
n	right vs left	p=1.0	p=0.71	p=0.66				
	Control vs T-4	p=0.55	p=0.17	p=0.188		p=0.067	p=0.39	p=0.35

Test results are shown for F-2 flight line subjects in Tables 17 (Immittance), 18 (OAE), and 19 (ABR). Overall, there is a trend toward hearing issues for flight line personnel working on F-2 aircraft.

Only AR threshold at 1000 Hz was significantly decreased in the immittance test for the left ear. There was a trend for a decrease in right ears at 500 but there was no trend towards significance

in left ears or at the other frequencies. However, the differences are the opposite effect with the exposed ears showing better function. This could be a statistical artifact.

Table 17. Immittance data for F-2 subjects

Subject		SC	Peak P	ECV	AR500-I	AR1000-I	AR2000-I	AR500-C	AR1000-C	AR2000-C
Control	Mean	0.58	-5.21	1.29	86.07	87.32	88.33	95.19	93.96	94.07
Right Ear	STD	0.26	12.81	0.33	6.3	5.7	5.9	5.6	4.7	5.4
all Iruma + HY	SEM	0.04	2.20	0.06	1.2	1.1	1.1	1.1	0.9	1.0
Yokota	n	34	34	34	28	28	30	26	27	29
F-2 Right	Mean	0.57	-1.14	1.25	86.43	86.67	91.43	91.00	93.00	94.17
Ear	STD	0.20	3.08	0.17	6.90	7.53	8.52	7.42	2.74	3.76
	SEM	0.077	1.16	0.066	2.61	2.85	3.22	3.32	1.22	1.54
	n	7	7	7	7	7	7	5	5	6
		p=0.92	p=0.41	p=0.76	p=0.90	p=0.80	p=0.26	p=0.16	p=0.66	p=0.97
Control	Mean	0.67	-8.00	1.27	85.5	87.0	88.9	92.4	93.8	92.7
Left Ear	STD	0.30	12.12	0.32	5.4	5.2	5.9	6.6	5.9	5.9
all Iruma + HY	SEM	0.05	2.08	0.06	1.0	0.9	1.0	1.4	1.2	1.2
Yokota	n	34	34	34	32	32	32	21	24	24
F-2 Left	Mean	0.61	-2.57	1.26	83.33	82.50	87.86	91.67	94.00	96.00
Ear	STD	0.22	4.50	0.17	5.16	4.18	6.99	7.64	8.94	4.18
	SEM	0.082	1.70	0.065	1.95	1.58	2.64	4.41	4.00	1.87
	n	7	7	7	7	7	7	3	5	5
		p=0.62	p=0.25	p=0.94	p=0.34	p=0.039	p=0.68	p=0.86	p=0.95	p=0.25

SC=static compliance (cc); Peak P=peak pressure (daPa); ECV=equivalent ear canal volume (cc); AR=acoustic reflex (dB); C=contra

For OAE there were multiple significant decreases for amplitudes in both the right and left ears indicating functional changes in the cochlea (Table 18). Not all differences were seen in both ears although there were trends in the left ear when the right ear average was significantly decreased. At 5154, 5666, and 6229 there were decreases in the left ear not seen in the right ears although only 5666 was significantly decreased in the left ear. This was the one test with the most significant hearing changes. However, with the small sample size of only 7 it still needs to be interpreted cautiously.

Table 18. OAE data for F-2 subjects with values in Hz

Subject		2000.0	2198.0	2416.0	2656.0	2920.0	3210.0	3529.0	3880.0	4265.0	4688.0	5154.0	5666.0	6229.0	6847.0	7527.0	8275.0
Control	Mean	20.7	21.2	20.5	22.6	22.8	23.6	25.3	25.9	27.6	27.4	29.6	30.3	28.3	29.0	22.5	22.7
Right Ear	STD	10.0	6.9	5.5	5.8	5.7	5.9	6.3	6.6	5.7	6.0	7.0	7.6	8.9	8.4	7.3	7.1
all Iruma +	SEM	1.77	1.21	0.98	1.03	1.02	1.05	1.11	1.16	1.01	1.06	1.23	1.35	1.58	1.48	1.29	1.25
HY + Yokota	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
F-2 Right	Mean	16.69	14.96	15.20	14.23	16.24	16.73	19.37	23.21	21.79	21.66	26.09	28.63	26.79	26.23	23.24	21.60
Ear	STD	5.51	4.70	4.24	4.12	5.42	5.58	5.20	5.43	4.50	5.81	3.65	3.16	4.10	5.29	5.46	4.22
	SEM	2.08	1.78	1.60	1.56	2.05	2.11	1.96	2.05	1.70	2.19	1.38	1.19	1.55	2.00	2.06	1.59
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
		p=0.31	p=0.029	p=0.022	p=0.0009	p=0.0085	p=0.0078	p=0.026	p=0.32	p=0.016	p=0.027	p=0.21	p=0.57	p=0.67	p=0.41	p=0.80	p=0.70
Control	Mean	21.61	20.70	20.79	22.35	23.31	23.01	25.06	26.72	27.41	27.94	28.98	30.31	29.49	28.58	22.86	21.01
Left Ear	STD	5.85	5.28	5.65	6.08	5.93	6.85	5.82	5.89	5.81	6.27	7.05	6.87	6.90	7.76	7.24	7.12
all Iruma +	SEM	1.03	0.93	1.00	1.08	1.05	1.21	1.03	1.04	1.03	1.11	1.25	1.21	1.22	1.37	1.28	1.26
HY + Yokota	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
F-2 Left	Mean	19.23	17.41	16.44	15.44	15.40	15.80	18.93	22.90	22.10	22.79	23.71	24.61	24.43	25.66	22.43	21.36
Ear	STD	5.14	4.24	4.04	5.12	4.65	3.10	5.48	5.71	6.02	5.74	5.25	4.22	5.40	4.13	6.46	6.24
	SEM	1.94	1.60	1.53	1.93	1.76	1.17	2.07	2.16	2.28	2.17	1.98	1.59	2.04	1.56	2.44	2.36
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
		p=0.33	p=0.13	p=0.062	p=0.008	p=0.002	p=0.01	p=0.015	p=0.13	p=0.036	p=0.054	p=0.07	p=0.043	p=0.08	p=0.34	p=0.89	p=0.91

There were no significant differences and only one slight trend in the Wave I amplitude to latency left ear ratio for ABR results in F-2 flight line personnel (Table 19).

Table 19. ABR results for F-2 subjects with values in milliseconds

Subject		Wave I Latency	Wave V Latency	I-III Latency	III-V Latency	I-V Latency	Wave I Amp	Wave V Amp	Wave V /Wave I amps	amplitude /latency* Wave I	amplitude /latency Wave V
Control	Mean	1.67	5.68	2.21	1.84	4.03	0.24	0.45	2.55	0.15	0.080
Right Ear Slow	STD	0.21	0.24	0.27	0.12	0.20	0.14	0.16	1.62	0.094	0.030
all Iruma +	SEM	0.036	0.041	0.046	0.021	0.035	0.023	0.027	0.28	0.016	0.005
HY + Yokota	n	34	34	34	34	34	34	34	34	34	34
F-2 Right	Mean	1.65	5.65	2.10	1.89	3.99	0.21	0.49	2.48	0.13	0.09
Ear Slow	STD	0.095	0.154	0.148	0.068	0.142	0.060	0.13	0.95	0.041	0.024
	SEM	0.036	0.058	0.056	0.026	0.054	0.023	0.049	0.36	0.016	0.009
	n	7	7	7	7	7	7	7	7	7	7
		p=0.81	p=0.75	p=0.31	p=0.30	p=0.62	p=0.58	p=0.54	p=0.91	p=0.59	p=0.41
Control	Mean	1.58	5.70	2.22	1.87	4.09	0.26	0.43	2.13	0.17	0.08
Left Ear Slow	STD	0.10	0.21	0.18	0.15	0.23	0.11	0.13	1.51	0.073	0.024
all Iruma +	SEM	0.018	0.035	0.031	0.026	0.039	0.019	0.022	0.26	0.013	0.004
HY + Yokota	n	34	34	34	34	34	34	34	34	34	34
F-2 Left	Mean	1.62	5.67	2.15	1.90	4.06	0.21	0.49	2.56	0.13	0.087
Ear Slow	STD	0.055	0.16	0.067	0.13	0.12	0.081	0.17	0.89	0.052	0.033
	SEM	0.021	0.062	0.025	0.048	0.046	0.031	0.066	0.34	0.020	0.013
	n	7	7	7	7	7	7	7	7	7	7
		p=0.31	p=0.72	p=0.32	p=0.63	p=0.74	p=0.26	p=0.30	p=0.47	p=0.18	p=0.51

*amplitude/latency ratios ($\mu\text{V}/\text{ms}$, termed magnitude responses)

Audiology test results are shown for F-4 flight line subjects in Tables 20 (Immittance), 21 (OAE), and 22 (ABR). Only the AR threshold at 2000 for the right ear showed a trend for elevation in the immittance test. There were no significant differences for the means of any frequencies for either ear (Table 20).

Table 20. Immittance data for Hyakuri AB F-4 subjects

Subject		SC	Peak P	ECV	AR500-I	AR1000-I	AR2000-I	AR500-C	AR1000-C	AR2000-C
Control	Mean	0.58	-5.21	1.29	86.07	87.32	88.33	95.19	93.96	94.07
Right Ear	STD	0.26	12.81	0.33	6.3	5.7	5.9	5.6	4.7	5.4
all Iruma + HY +	SEM	0.04	2.20	0.06	1.2	1.1	1.1	1.1	0.9	1.0
Yokota	n	34	34	34	28	28	30	26	27	29
F-4	Mean	0.66	-3.47	1.34	86.79	89.29	91.00	93.93	93.89	95.77
Right Ear	STD	0.33	11.23	0.49	5.04	5.14	6.04	8.13	5.46	4.94
	SEM	0.09	2.90	0.12	1.35	1.37	1.56	2.17	1.82	1.37
	n	15	15	16	14	14	15	14	9	13
		p=0.37	p=0.65	p=0.67	p=0.71	p=0.28	p=0.16	p=0.57	p=0.97	p=0.34
Conrol	Mean	0.67	-8.00	1.27	85.5	87.0	88.9	92.4	93.8	92.7
Left Ear	STD	0.30	12.12	0.32	5.4	5.2	5.9	6.6	5.9	5.9
all Iruma + HY +	SEM	0.05	2.08	0.06	1.0	0.9	1.0	1.4	1.2	1.2
Yokota	n	34	34	34	32	32	32	21	24	24
F-4	Mean	0.73	-7.00	1.30	86.33	87.67	90.31	91.11	95.00	93.57
Left Ear	STD	0.59	15.24	0.43	5.16	6.51	6.94	6.01	4.47	5.69
	SEM	0.15	3.94	0.11	1.33	1.68	1.74	2.00	1.35	1.52
	n	15	15	16	15	15	16	9	11	14
		p=0.64	p=0.81	p=0.78	p=0.62	p=0.71	p=0.47	p=0.62	p=0.55	p=0.66

SC=static compliance (cc); Peak P=peak pressure (daPa); ECV=equivalent ear canal volume (cc); AR=acoustic reflex (dB); C=contra

For OAE there were no significant differences in either the right or left ear means (Table 21). There were only two trends in the left ear, one for distortion product response at 2920 and one at 3529. This is in contrast to the T-4 and F-2 data which did have significant hearing changes for this test.

Table 21. OAE data for Hyakuri AB F-4 subjects with values in Hz

Subject		2000	2198	2416	2656	2920	3210	3529	3880	4265	4688	5154	5666	6229	6847	7527	8275
Control	Mean	20.7	21.2	20.5	22.6	22.8	23.6	25.3	25.9	27.6	27.4	29.6	30.3	28.3	29.0	22.5	22.7
Right Ear	STD	4.13	6.9	5.5	5.8	5.7	5.9	6.3	6.6	5.7	6.0	7.0	7.6	8.9	8.4	7.3	7.1
all Iruma +	SEM	1.77	1.21	0.98	1.03	1.02	1.05	1.11	1.16	1.01	1.06	1.23	1.35	1.58	1.48	1.29	1.25
HY + Yokota	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
F-4	Mean	20.75	21.24	20.79	20.86	22.95	24.17	25.24	26.55	27.15	28.60	29.31	30.05	29.64	29.06	22.58	21.52
Right Ear	STD	4.13	4.57	5.14	4.81	3.60	4.06	3.62	4.11	4.02	4.03	5.64	6.49	5.41	6.87	6.05	6.18
	SEM	1.00	1.11	1.25	1.17	0.87	0.98	0.88	1.00	0.98	0.98	1.37	1.57	1.31	1.67	1.47	1.50
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
		p=0.98	p=0.98	p=0.86	p=0.30	p=0.92	p=0.72	p=0.97	p=0.71	p=0.77	p=0.46	p=0.88	p=0.91	p=0.57	p=0.98	p=0.97	p=0.57
Conrol	Mean	21.61	20.70	20.79	22.35	23.31	23.01	25.06	26.72	27.41	27.94	28.98	30.31	29.49	28.58	22.86	21.01
Left Ear	STD	5.85	5.28	5.65	6.08	5.93	6.85	5.82	5.89	5.81	6.27	7.05	6.87	6.90	7.76	7.24	7.12
all Iruma +	SEM	1.03	0.93	1.00	1.08	1.05	1.21	1.03	1.04	1.03	1.11	1.25	1.21	1.22	1.37	1.28	1.26
HY + Yokota	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
F-4	Mean	20.94	19.01	19.56	21.02	20.29	22.28	22.49	25.93	26.61	27.22	28.77	30.26	28.77	27.74	23.46	23.24
Left Ear	STD	4.48	5.06	4.54	4.53	5.27	5.45	6.30	5.36	4.91	4.85	5.39	5.64	5.97	6.75	6.24	5.70
	SEM	1.09	1.23	1.10	1.10	1.28	1.32	1.53	1.30	1.19	1.18	1.31	1.37	1.45	1.64	1.51	1.38
	n	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
		p=0.68	p=0.28	p=0.44	p=0.43	p=0.085	p=0.71	p=0.16	p=0.65	p=0.63	p=0.68	p=0.92	p=0.98	p=0.72	p=0.71	p=0.77	p=0.27

Although there were no significant differences for the ABR endpoints in F-4 flight line personnel, there were 6 that showed a trend for differences between exposed and control subjects (Table 22). Wave V showed a trend in both ears. Wave I amplitude showed a trend in both ears and the amplitude/latency ratio for Wave I showed a trend in both ears.

Table 22. ABR results for Hyakuri AB F-4 subjects with values in milliseconds

Subject		Wave I Latency	Wave V Latency	I-III Latency	III-V Latency	I-V Latency	Wave I Amp	Wave V Amp	Wave V /Wave I amps	amplitude /latency* Wave I	amplitude /latency Wave V
Control	Mean	1.67	5.68	2.21	1.84	4.03	0.24	0.45	2.55	0.15	0.080
Right Ear Slow	STD	0.21	0.24	0.27	0.12	0.20	0.14	0.16	1.62	0.094	0.030
all Iruma +	SEM	0.036	0.041	0.046	0.021	0.035	0.023	0.027	0.28	0.016	0.005
HY + Yokota	n	34	34	34	34	34	34	34	34	34	34
F-4	Mean	1.57	5.59	2.25	1.82	3.97	1.09	0.51	2.56	0.63	0.091
Right Ear Slow	STD	0.14	0.19	0.29	0.09	0.21	2.66	0.10	2.14	1.47	0.020
	SEM	0.031	0.042	0.065	0.020	0.048	0.59	0.023	0.48	0.33	0.005
	n	20	20	20	20	20	20	20	20	20	20
		p=0.061	p=0.14	p=0.61	p=0.52	p=0.30	p=0.067	p=0.14	p=0.98	p=0.062	p=0.15
Control	Mean	1.58	5.70	2.22	1.87	4.09	0.26	0.43	2.13	0.17	0.077
Left Ear Slow	STD	0.10	0.21	0.18	0.15	0.23	0.11	0.13	1.51	0.073	0.024
all Iruma +	SEM	0.018	0.035	0.031	0.026	0.039	0.019	0.022	0.26	0.013	0.004
HY + Yokota	n	34	34	34	34	34	34	34	34	34	34
F-4	Mean	1.57	5.62	2.21	1.85	4.06	0.21	0.44	2.49	0.14	0.078
Left Ear Slow	STD	0.18	0.21	0.14	0.13	0.16	0.10	0.085	1.52	0.084	0.016
	SEM	0.039	0.046	0.031	0.029	0.036	0.023	0.019	0.34	0.019	0.004
	n	20	20	20	20	20	20	20	20	20	20
		p=0.79	p=0.18	p=0.83	p=0.62	p=0.61	p=0.10	p=0.76	p=0.40	p=0.17	p=0.87
*amplitude/latency ratios ($\mu\text{V/ms}$; termed response magnitudes)											

4.4.1.1. JASDF Audiogram data

Audiogram data for JASDF T-4 flight line subjects are shown in Table 23. Hearing thresholds up to and equal to 25 dB are considered normal. Values 26 to 40 are considered mild hearing loss. At 500 Hz it is much easier for background noises to impact results. Increases at 6000 Hz are an indicator of hearing loss.

Therefore, the audiogram data averages appear normal for the JASDF T-4 subjects. However, 5 out of 29 T-4 subjects had a threshold greater than 25 dB in the right ear: 2 at both 500 and 6000 Hz, 1 at 4000 and 6000 Hz, 1 at just 500 and 1 at just 6000 Hz (with a 25 dB at 500 Hz). An additional 3 subjects had 25 dB at 6000 Hz with one of them at 500 Hz as well. Eight more subjects had thresholds of 25 dB at 500 Hz. Even though the average was normal for all thresholds a total of 16 out of 29 T-4 subjects had a value at 25 dB or greater at 500 and/or 6000 Hz. Except for the one subject with a 30 dB at 4000 Hz all of the mid-range thresholds (1000 to 4000 Hz) for the right ears ranged from -10 to 20 dB.

For the left ear, 3 of the subjects with thresholds greater than 25 dB in the right ears at 6000 Hz also had thresholds greater than 25 dB at 6000 Hz with only 1 of them also having a 35 dB at 500 Hz. An additional subject had a threshold of 45 dB at 6000 Hz in the left ear but was normal at the other frequencies. An additional 5 subjects had a value of 25 dB at either 500 or 6000 Hz (only one at both). All of the mid-range thresholds (1000 to 4000 Hz) for the left ears ranged from -5 to 20 dB. The results for right and left ears of T-4 subjects indicates many of them are high normal with 6 showing mild hearing loss.

Table 23. Audiogram data for T-4 subjects measured in decibels Hearing Level (dB HL)

Subject	Ear	500 Hz	1000 Hz	2000 Hz	3000 Hz	4000 Hz	6000 Hz
T-4 Right	Mean	20.00	9.83	6.55	5.69	6.03	16.38
	STD	7.32	6.05	5.36	6.23	8.06	8.95
	SEM	1.36	1.12	1.00	1.16	1.50	1.66
	n	29	29	29	29	29	29
T-4 Left	Mean	15.52	9.14	7.59	6.55	5.52	16.72
	STD	7.83	5.99	5.11	6.28	7.72	12.34
	SEM	1.45	1.11	0.95	1.17	1.43	2.29
	n	29	29	29	29	29	29

Audiogram data for JASDF F-2 flight line subjects are shown in Table 24. The audiogram data averages appear normal for the JASDF F-2 subjects. Only one subject had a value of 30 dB at 6000 Hz in the right ear with a 25 dB at 500 Hz. Another subject had a value of 30 dB at 6000 Hz in the left ear with a 15 dB at 500 Hz. However, 6 out of 7 subjects had a value of 25 dB or greater at either 500 or 6000 Hz. All of the mid-range thresholds (1000 to 4000 Hz) for the right and left ears ranged from -5 to 20 dB. The results for right and left ears of F-2 subjects indicates 4 of them are high normal at one frequency (500 or 6000 Hz) with 2 showing mild hearing loss at 6000 Hz. Only one subject had a range of threshold values for both ears of 0 to 15 dB across all Hz.

Table 24. Audiogram data for F-2 subjects measured in decibels Hearing Level (dB HL)

Subject	Ear	500 Hz	1000 Hz	2000 Hz	3000 Hz	4000 Hz	6000 Hz
F-2 Right	Mean	17.86	7.14	7.14	1.43	7.86	16.43
	STD	4.88	4.88	5.67	4.76	10.35	6.90
	SEM	1.84	1.84	2.14	1.80	3.91	2.61
	n	7	7	7	7	7	7
F-2 Left	Mean	10.71	6.43	8.57	2.86	7.14	18.57
	STD	4.50	4.76	5.56	5.67	9.06	13.14
	SEM	1.70	1.80	2.10	2.14	3.43	4.97
	n	7	7	7	7	7	7

4.4.2 USAF ABs

Test results are shown for Misawa AB F-16 flight line subjects in Tables 25 (audiogram), 26 (Immittance), 27 (OAE), and 28 (ABR). Overall, there is a trend toward hearing issues for flight line personnel working on F-16 aircraft.

There were no significant differences between Misawa AB control and flight line subject's audiograms (Table 25). There was a trend towards audiometric threshold levels being higher in exposed personnel at 1000 Hz for both the right and left ears. There was a less pronounced trend for the right ear only at 4000 Hz in which exposed had a better threshold than controls. Only one subject had a threshold of 35 db for the left ear at 6000 Hz. The other frequencies for this subject were either 20 or 25 db. Other than this one subject the thresholds ranged from -5 to 20 db for the right ear. Another subject had a 25 db at 3000 Hz for the left ear, otherwise the range was -5 to 20 db for the other 16 F-16 subjects. Only one subject showed a mild hearing loss based on audiogram data.

Table 25. Audiogram data for F-16 subjects measured in decibels Hearing Level (dB HL)

Subject		500 Hz	1000 Hz	2000 Hz	3000 Hz	4000 Hz	6000 Hz
Control Right Ear	Mean	8.33	8.00	5.00	7.33	9.33	2.67
	STD	6.45	4.14	5.98	7.29	8.63	9.04
	SEM	1.67	1.07	1.54	1.88	2.23	2.33
	n	15	15	15	15	15	15
Exposed Right Ear	Mean	8.89	10.00	5.28	8.06	5.83	6.11
	STD	3.23	3.43	4.36	3.89	6.00	7.58
	SEM	0.76	0.81	1.03	0.92	1.41	1.79
	n	18	18	18	18	18	18
		p=0.75	p=0.14	p=0.88	p=0.72	p=0.18	p=0.24
Control Left Ear	Mean	5.67	5.33	7.00	7.33	9.33	4.33
	STD	4.17	4.81	6.49	7.53	8.63	6.51
	SEM	1.08	1.24	1.68	1.94	2.23	1.68
	n	15	15	15	15	15	15
Exposed Left Ear	Mean	7.50	8.61	5.56	9.72	7.22	4.44
	STD	6.24	5.89	6.16	7.17	7.52	9.38
	SEM	1.47	1.39	1.45	1.69	1.77	2.21
	n	18	18	18	18	18	18
		p=0.34	p=0.094	p=0.52	p=0.36	p=0.46	p=0.97

Acoustic reflexes at 500 and 1000 contra were significantly increased in the immittance test for the right and left ear but only at 1000 for the right ear (Table 26). A peak pressure difference was seen in the right ear but only showed a trend in the left ear. However, both sets of peak pressures are within the normal range. There was a slight trend towards significance in the left

ear at acoustic reflex 500 contra and at 2000 contra in the right ear. However, at 2000 contra there was a significant increase in the left ear.

Table 26. Immittance data for Misawa AB F-16 subjects

Subject		Static Compliance	Peak Pressure	ECV	Acoustic Reflex 500	Acoustic Reflex 1000	Acoustic Reflex 2000	AR 500 Contra	AR 1000 Contra	AR 2000 Contra
Control	Mean	0.82	-4.13	1.35	85.00	87.14	88.00	95.77	92.73	93.21
Right Ear	STD	0.58	7.91	0.30	4.77	5.79	6.21	3.44	2.61	4.21
	SEM	0.15	2.04	0.08	1.38	1.55	1.60	0.96	0.79	1.13
	n	15	15	15	12	14	15	13	11	14
Exposed	Mean	0.78	4.61	1.44	90.29	90.59	88.82	95.00	97.69	96.00
Right Ear	STD	0.27	10.71	0.28	4.50	3.00	4.85	2.50	2.59	4.71
	SEM	0.06	2.52	0.07	1.09	0.73	1.18	0.83	0.72	1.21
	n	18	18	18	17	17	17	9	13	15
		p=0.80	p=0.014	p=0.38	p=0.005	p=0.04	p=0.68	p=0.57	p=0.0001	p=0.11
Control	Mean	0.72	-4.80	1.22	85.38	86.54	88.21	92.08	93.57	91.15
Left Ear	STD	0.46	8.13	0.28	5.19	6.25	6.96	5.42	4.57	4.63
	SEM	0.12	2.10	0.07	1.44	1.73	1.86	1.56	1.22	1.29
	n	15	15	15	13	13	14	12	14	13
Exposed	Mean	0.71	-0.28	1.32	89.71	88.82	88.13	95.00	97.31	95.36
Left Ear	STD	0.28	7.89	0.26	5.72	4.52	4.03	5.00	4.39	3.65
	SEM	0.07	1.86	0.06	1.39	1.10	0.98	1.51	1.22	0.98
	n	18	18	18	17	17	17	11	13	14
		p=0.94	p=0.12	p=0.30	p=0.04	p=0.26	p=0.97	p=0.19	p=0.04	p=0.014

SC=static compliance (cc); Peak P=peak pressure (daPa); ECV=equivalent ear canal volume (cc); AR=acoustic reflex (dB); C=contra

For OAE, the only trend towards a decrease was 6847 in left ears (Table 27). This contrasts with the F-2 and less than the small number of changes seen for T-4.

Table 27. OAE data for Misawa AB F-16 subjects with values in Hz

Subject		2000	2198	2416	2656	2920	3210	3529	3880	4265	4688	5154	5666	6229	6847	7527	8275
Control	Mean	23.57	23.22	23.15	24.57	25.45	25.95	27.33	27.55	26.72	28.55	30.88	31.77	29.00	28.80	21.22	18.52
Right Ear	STD	4.15	4.83	3.55	3.14	3.66	4.09	3.68	5.37	5.67	5.06	3.44	4.84	6.30	7.58	5.28	6.92
	SEM	1.07	1.25	0.92	0.81	0.94	1.06	0.95	1.39	1.46	1.31	0.89	1.25	1.63	1.96	1.36	1.79
	n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Exposed	Mean	23.76	23.71	23.32	24.57	25.06	25.25	26.93	25.87	25.29	27.41	29.90	29.78	28.85	28.26	20.83	20.94
Right Ear	STD	7.13	8.19	7.77	7.07	6.24	5.97	6.57	5.94	6.24	4.49	6.88	6.48	7.31	6.46	3.87	5.10
	SEM	1.68	1.93	1.83	1.67	1.47	1.41	1.55	1.40	1.47	1.06	1.62	1.53	1.72	1.52	0.91	1.20
	n	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
		p=0.93	p=0.84	p=0.94	p=1.0	p=0.83	p=0.70	p=0.84	p=0.40	p=0.50	p=0.62	p=0.33	p=0.95	p=0.83	p=0.81	p=0.26	
Control	Mean	21.35	23.26	22.57	23.28	22.81	24.40	26.64	27.49	26.55	27.95	30.63	31.52	30.72	29.80	21.69	19.87
Left Ear	STD	5.48	3.45	3.31	4.13	3.59	4.47	5.20	5.22	8.06	6.96	6.79	8.78	6.56	7.14	6.60	5.42
	SEM	1.41	0.89	0.86	1.07	0.93	1.15	1.34	1.35	2.08	1.80	1.75	2.27	1.69	1.84	1.70	1.40
	n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Exposed	Mean	23.00	23.18	22.54	23.54	23.58	25.57	26.24	26.77	25.99	27.79	30.05	30.85	29.37	26.71	21.02	19.42
Left Ear	STD	7.48	7.48	6.11	5.97	5.84	5.91	5.36	5.82	7.34	6.26	5.59	5.47	5.10	5.80	3.95	4.45
	SEM	1.76	1.76	1.44	1.41	1.38	1.39	1.26	1.37	1.73	1.48	1.32	1.29	1.20	1.37	0.93	1.05
	n	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
		p=0.48	p=0.97	p=0.99	p=0.89	p=0.66	p=0.53	p=0.83	p=0.71	p=0.84	p=0.95	p=0.79	p=0.79	p=0.51	p=0.18	p=0.72	p=0.80

For the slow rate results in the upper portion of Table 28, only Wave I to III latency (significance in right ear; trend in the left) and Wave V amplitude (trends in both ears but almost significant in the left) had decreases in both ears. There were trends in the left ear only for Wave I to V latency and the amplitude latency ratio for Wave V. There were significant differences in the right ear for Wave III latency (decrease) and Wave III to V latency (increase) with no trend or significance for the left ear. However, the Wave III latency and the I-III latency show better performance in the exposed ears. The Wave III-V latency in the right ear is the only significant difference showing a decrease in performance. Although the left ears had a higher Wave III-V latency in exposed ears there was no trend to support the significance in the right ears. Wave III amplitudes were not recorded so no Wave III amplitude to latency ratios could be determined which may have provided more insight.

For the fast rate results for Wave V in the lower portion of Table 28, there were no differences between right and left ears for latencies or the amplitude/latency ratios. However, there was a significant decrease in amplitude in the right ears of F-16 subjects and a trend for significance in left ears. This was supported by strong trends in the amplitude/latency ratios for both right and left ears.

The significant differences and trends for ABR in F-16 flight line personnel are in contrast to F-2 flight line personnel with one slight trend. They are comparable to T-4 subject where there were the same number of changes.

Table 28. ABR slow rate (upper portion) and fast rate (lower portion) results for Misawa AB F-16 subjects with values in milliseconds

Subject		Wave I Latency	Wave III Latency	Wave V Latency	I-III Latency	III-V Latency	I-V Latency	Wave I Amp	Wave V Amp	Wave V /Wave I amps	amplitude /latency W 1	amplitude /latency W V
Control Right Ear	Mean	1.61	3.86	5.69	2.25	1.83	4.08	0.29	0.47	1.76	0.18	0.082
	STD	0.098	0.19	0.18	0.16	0.094	0.12	0.099	0.17	0.69	0.071	0.032
	SEM	0.025	0.048	0.045	0.041	0.024	0.032	0.026	0.044	0.18	0.018	0.008
	n	15	15	15	15	15	15	15	15	15	15	15
Exposed Right Ear	Mean	1.62	3.72	5.65	2.10	1.95	4.04	0.33	0.40	1.50	0.21	0.070
	STD	0.10	0.11	0.12	0.11	0.12	0.13	0.15	0.12	1.08	0.098	0.023
	SEM	0.024	0.027	0.028	0.026	0.028	0.032	0.035	0.029	0.254	0.023	0.005
	n	18	18	18	18	18	18	18	18	18	18	18
		p=0.77	p=0.013	p=0.45	p=0.003	p=0.004	p=0.37	p=0.38	p=0.18	p=0.43	p=0.33	p=0.22
Control Left Ear	Mean	1.59	3.86	5.70	2.27	1.84	4.11	0.28	0.48	2.18	0.18	0.086
	STD	0.091	0.18	0.21	0.16	0.14	0.17	0.11	0.22	1.79	0.076	0.042
	SEM	0.024	0.047	0.053	0.040	0.035	0.043	0.029	0.057	0.46	0.020	0.011
	n	15	15	15	15	15	15	15	15	15	15	15
Exposed Left Ear	Mean	1.62	3.80	5.66	2.17	1.87	4.03	0.31	0.36	1.56	0.19	0.063
	STD	0.096	0.13	0.10	0.15	0.17	0.13	0.15	0.13	1.23	0.094	0.023
	SEM	0.023	0.030	0.025	0.034	0.041	0.031	0.035	0.030	0.291	0.022	0.005
	n	18	18	18	18	18	18	18	18	18	18	18
		p=0.37	p=0.28	p=0.48	p=0.07	p=0.59	p=0.14	p=0.52	p=0.0504	p=0.25	p=0.74	p=0.059

Subject		Wave V Latency	Wave V Amp	amplitude /latency W V	Subject		Wave V Latency	Wave V Amp	amplitude /latency W V
Control Right Ear	Mean	6.14	0.28	0.05	Control Left Ear	Mean	6.12	0.30	0.05
	STD	0.24	0.08	0.01		STD	0.25	0.15	0.03
	SEM	0.06	0.02	0.00		SEM	0.07	0.04	0.01
	n	15	15	15		n	15	15	15
Control right vs left					p=0.82 p=0.65 p=1.0				
F-16 Right Ear	Mean	6.11	0.22	0.04	F-16 Left Ear	Mean	6.13	0.23	0.04
	STD	0.20	0.09	0.01		STD	0.22	0.09	0.02
	SEM	0.05	0.02	0.003		SEM	0.05	0.02	0.004
	n	18	18	18		n	18	18	18
Control vs F-16					p=0.90 p=0.08 p=0.08				
F-16 right vs left					p=0.78 p=0.74 p=0.77				

Test results are shown for Kadena AB F-15 flight line subjects in Table 29 (upper slow rate and lower Wave V fast rate). There were no significant differences for slow rate latencies between flight line and control subjects. However, there was a significant increase in the Wave V latency measure by fast rate for the right ears; there was no difference in the left ears. There were a number of trends for a difference between control and flight line personnel. There was a slight trend for an increase in Wave I latency in the right ears but no difference in left ears. This supports the fast rate Wave V latencies. In the left ears there were 3 stronger trends for differences between control and exposed subjects but the Wave I and V amplitudes and their ratio are higher in-flight line subjects which is the opposite of an expected impact on the auditory pathway. This trend was also seen in the fast rate Wave V amps. However, the right ear latency data for Wave I slow rate and Wave V fast rate does indicate a possible trend toward hearing issues for flight line personnel working on F-15 aircraft based on ABR tests.

Table 29. ABR slow rate (upper portion) and Wave V latencies and amps at fast rate (lower portion) results for Kadena AB F-15 subjects with values in milliseconds

Subject		Wave I Latency	Wave III Latency	Wave V Latency	I-III Latency	III-V Latency	I-V Latency	Wave I Amp	Wave V Amp	Wave V /Wave I amps	amplitude /latency W I	amplitude /latency W V
Control	Mean	1.52	3.72	5.57	2.18	1.86	4.05	0.24	0.47	4.26	0.16	0.09
F-15 Right	STD	0.16	0.12	0.28	0.18	0.23	0.26	0.14	0.22	6.31	0.09	0.04
Ear Slow	SEM	0.03	0.03	0.06	0.04	0.05	0.06	0.03	0.05	1.34	0.02	0.01
27.5/s	n	22	22	22	21	22	21	22	22	22	22	22
Exposed	Mean	1.57	3.74	5.53	2.17	1.79	3.96	0.24	0.49	3.13	0.15	0.09
F-15 Right	STD	0.08	0.15	0.27	0.15	0.22	0.25	0.13	0.21	2.93	0.09	0.04
Ear Slow	SEM	0.02	0.03	0.06	0.03	0.05	0.05	0.03	0.04	0.61	0.02	0.01
27.5/s	n	23	23	23	23	23	23	23	23	23	23	23
		p=0.19	p=0.62	p=0.63	p=0.84	p=0.30	p=0.25	p=1.0	p=0.76	p=0.44	p=0.71	p=1.0
Control	Mean	1.54	3.73	5.58	2.18	1.85	4.07	0.21	0.42	2.84	0.14	0.08
F-15 Left	STD	0.12	0.17	0.27	0.17	0.26	0.38	0.11	0.19	2.37	0.08	0.04
Ear Slow	SEM	0.03	0.04	0.06	0.04	0.06	0.08	0.02	0.04	0.51	0.02	0.01
27.5/s	n	22	22	22	22	22	22	22	22	22	22	22
Exposed	Mean	1.54	3.79	5.56	2.23	1.77	4.01	0.28	0.50	2.43	0.18	0.09
F-15 Left	STD	0.14	0.22	0.23	0.16	0.16	0.18	0.13	0.14	1.86	0.09	0.03
Ear Slow	SEM	0.03	0.05	0.05	0.03	0.03	0.04	0.03	0.03	0.39	0.02	0.01
27.5/s	n	23	23	23	23	23	23	23	23	23	23	23
		p=1.0	p=0.31	p=0.79	p=0.32	p=0.22	p=0.50	p=0.06	p=0.11	p=0.52	p=0.12	p=0.35

		Wave V Latency			Wave V Amps		
		Right Ear	Left Ear		Right Ear	Left Ear	
Subject	Mean	5.95	6.05	p=0.18	0.26	0.26	p=1.0
Controls	STD	0.19	0.29	R vs L	0.09	0.11	R vs L
Fast Rate	SEM	0.04	0.06		0.02	0.02	
85.9/s	n	22	22		22	22	
Subject	Mean	6.07	6.05	p=0.77	0.31	0.31	p=1.0
F-15	STD	0.23	0.24	R vs L	0.15	0.10	R vs L
Flight Line	SEM	0.05	0.05		0.03	0.02	
Fast Rate	n	23	23		23	23	
85.9/s		p=0.02	p=1.0		p=0.196	p=0.12	

4.5 miRNA analysis

4.5.1 2018 and 2019 Sampling Years

Differences were observed between the samples collected at Hyakuri AB in 2018 and the samples collected in 2019 at Kadena AB (Figures 3 and 4). Despite equal concentrations of barcoded cDNA libraries being loaded, the total number of reads of the samples collected in 2018 at Hyakuri AB were almost twice that of the samples collected in 2019 at Kadena AB. Usable reads of the samples collected in 2018 at Hyakuri AB were in the mid-40%, while usable reads of the samples collected in 2019 at Kadena AB were in the mid-20%. The number of mapped reads per sample ranged from 728,138 to 9,342,838 for the samples collected in 2018, and 151,460 to 1,209,270 for the samples collected in 2019 (for expression profiling, 100K–2M mapped reads per sample is generally an accepted range per Genohub, 2023). Ion Sphere Particles loading so that the entire surface was covered by the cDNA population and read length were similar for both sets of samples.

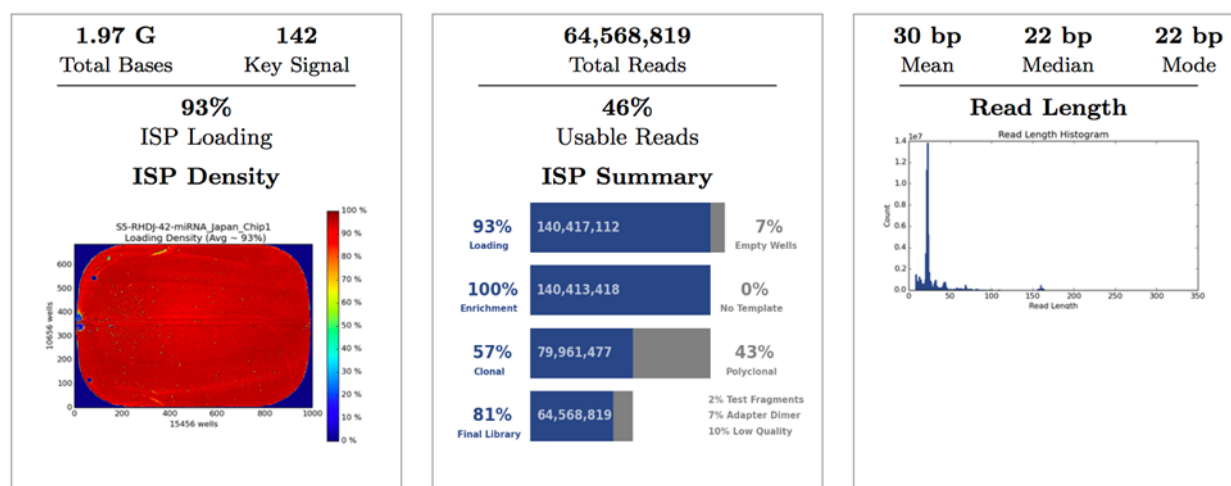


Figure 3. Example run summary from control and exposed samples collected in 2018 at Hyakuri, Iruma, and Yokota ABs

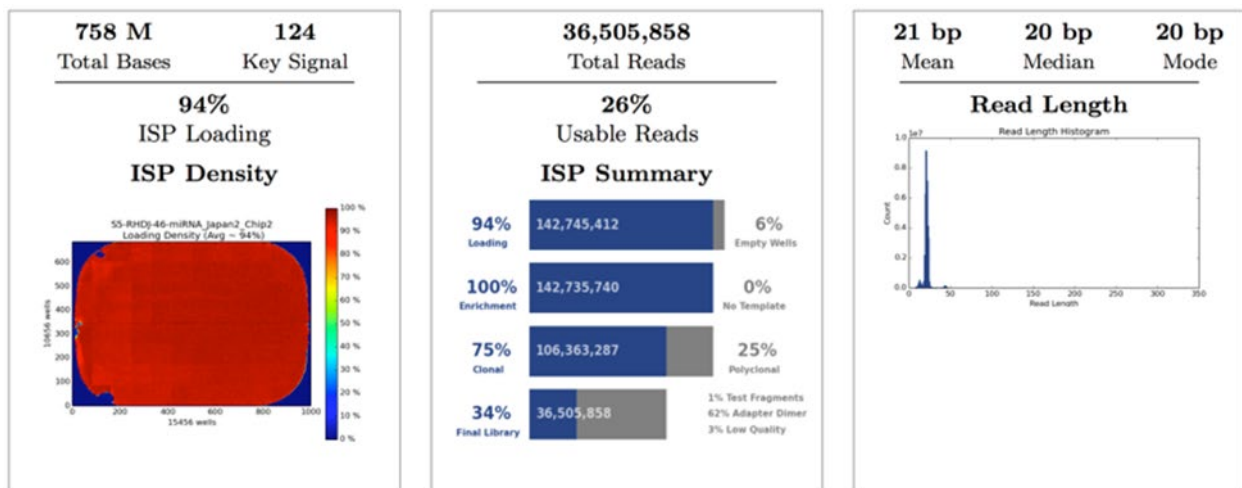


Figure 4. Example run summary from control and exposed samples collected in 2019 at Kadena AB

When comparing subjects at all ABs, exposure to the flight line resulted in minimal miRNA expression changes in the blood. Only 1 differentially expressed miRNA (Table 30) was identified when comparing control subjects to exposed subjects using fold change <-2 or >2 and $p\text{-value} < 0.05$ cut-offs. The principal components analysis plot (Figure 5) shows the differences between the control subjects and the exposed subjects prior to applying any filters. Control and exposed values for Japan subjects were different from U.S. subjects. Also control values for Japan subjects were mostly different from the exposed subjects at Hyakuri AB. This could indicate a greater effect of JP-4 exposure than JP-8. There was no difference between control and exposed subjects at Kadena AB. This suggests that exposure to JP-8 has little effect on miRNAs for exposure to this jet fuel.

Table 30. Differentially expressed miRNAs observed in blood after exposure to the flight line in subjects at Hykuri and Kadena ABs versus non-exposed controls at Hykuri, Iruma, Kadena, and Yokota ABs

miRNA ID	Total counts	P-value	FDR step up	Ratio	Fold change	LSMean(Control)	LSMean(Exposed)
rno-miR-15b-5p	2.59E+05	1.47E-05	1.08E-03	2.01E+00	2.01	6.06E+03	3.01E+03

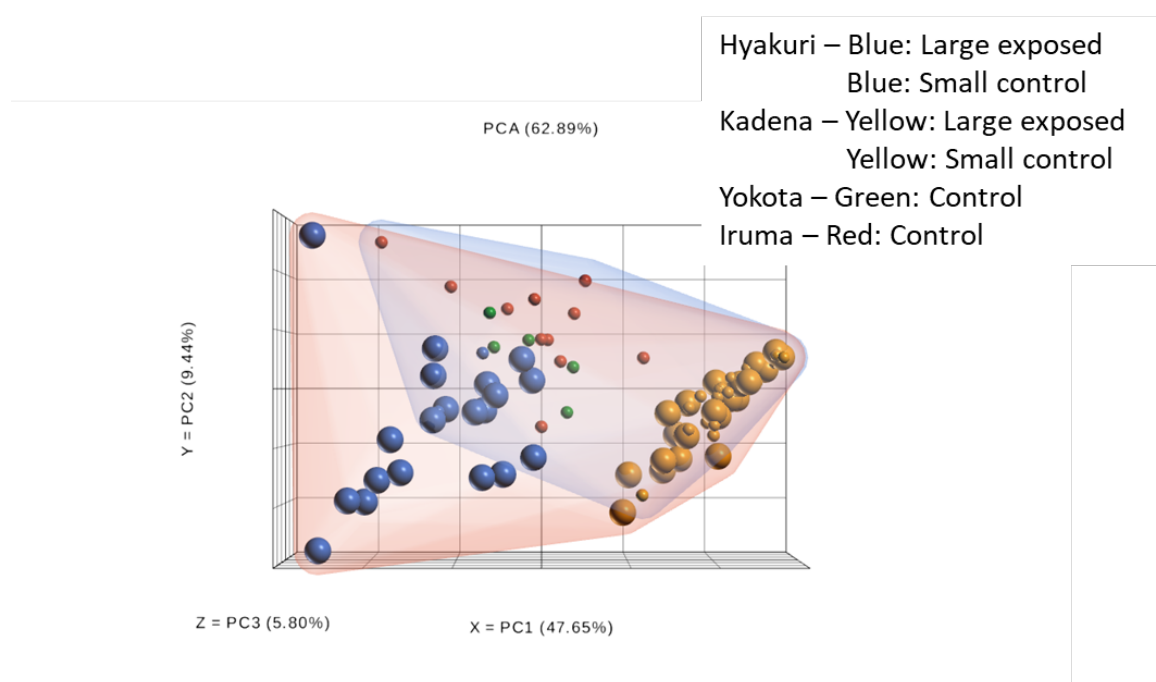


Figure 5. Principle components analysis plot of controls at Hykuri, Iruma, Kadena, and Yokota ABs versus exposed subjects at Hykuri and Kadena ABs

4.5.2 2021 to 2023 Sampling Years

4.5.2.1 Multidimensional Scaling (MDS) of miRNA Profiles

MDS ordination of samples were analyzed by exposure group (Figures 6-15). A 95% confidence interval is outlined by ellipse, with each group mean indicated as a larger dot within the ellipse. While the MDS have overlap in the 95% CI several of the conditions show some separation. The effect of increased within-group variation does reduce the overall statistical power of the differential expression test.

As seen with the MDS profile of all groups (Figure 6) there is not a clear separation of the exposed groups with control groups. There is an indication that the Iruma control group (I) shifts slightly from the grouping, but otherwise no separation is seen between jet fuel exposed groups compared to control groups. These profiles were individually delineated for clarity (Figures 7-15).

For the comparison of control groups, separation was seen in comparison of the two control groups, consisting of Iruma Airfield (IC) and from the Misawa Airfield (MC). This separation is

of interest, as the IC group consists of JASDF laboratory workers (all Japanese) whereas MC consists of USAF Medical workers (Figure 7, Table 31), a diverse group of Americans. We speculate that the lack of overlap and similarity of miRNA expression is possibly due to genetics, with the racial diversity between the Japanese and American military personnel. There could also be an impact from dietary contributions. While MDS comparisons were conducted combining JASDF/USAF personnel for the sake of completeness, it is clear that control data from JASDF and USAF cannot be combined for comparison and miRNA alteration identifications. These data also indicate care must be taken in construction and composition with regards to control groups.

In comparing JP-8 exposure alterations, for the profile comparison of MU (JP-8 Exposure, USAF personnel) and MC (control, USAF medical personnel) groups (Figure 8, Table 31) there is a large amount of overlap, indicating that there were not significant differences in blood miRNA expression between the two groups. This may indicate that some in the MU group had low to no JP-8 exposure and/or some in the MC group may have had exposures being on the same base. It may be that, in the JP-8 exposed group, proper PPE and protection mechanisms minimized exposure. However, it is unusual and concerning to see no miRNA changes – at all – between the exposure and control groups.

For JetA1 exposure alterations, comparing the IC (control, JASDF personnel) group to the MA (JetA1 exposure, JASDF) group (Figure 9) or the HO (JetA1 exposure, JASDF) group (Figure 10) there is almost no overlap (MA) or no overlap (HO), indicating significant differences in the miRNA profiles between the groups potentially due to exposures on the flight line. Combining the two exposure groups MA and HO (both JetA1, JASDF) versus the IC control group still demonstrates strong separation of the exposure group compared to controls (Figure 11). These changes were indicated in Figure 6 but are clearly seen in the individual comparisons.

For the profile comparisons MU (JP-8 Exposure) and HO, MA and the combined HO plus MA (JetA1) groups (Figures 12-14) minor differences were seen in the comparisons. The greatest shift was between MA and MU (Figure 12, Table 4, Table 31). However, due to the dissimilarity between control groups, it is possible that the changes seen in miRNA expression are not based on fuel exposure alone.

One area of concern for sampling and comparison between ABs is the potential for variability due to locale alone. However, comparing the HO (JetA1) and MU (JP-8) exposure groups in Figure 13, the MA and HO (JetA1) exposure groups and MU (JP-8) groups in Figure 14 and the two JASDF bases in Figure 15 does indicate that, while some differences are seen, the exposure groups do not seem to differ significantly in respect to miRNA profiling. The separation seen between MA and HO in Figure 15 might be due to the difference in aircraft at each base as well as location.

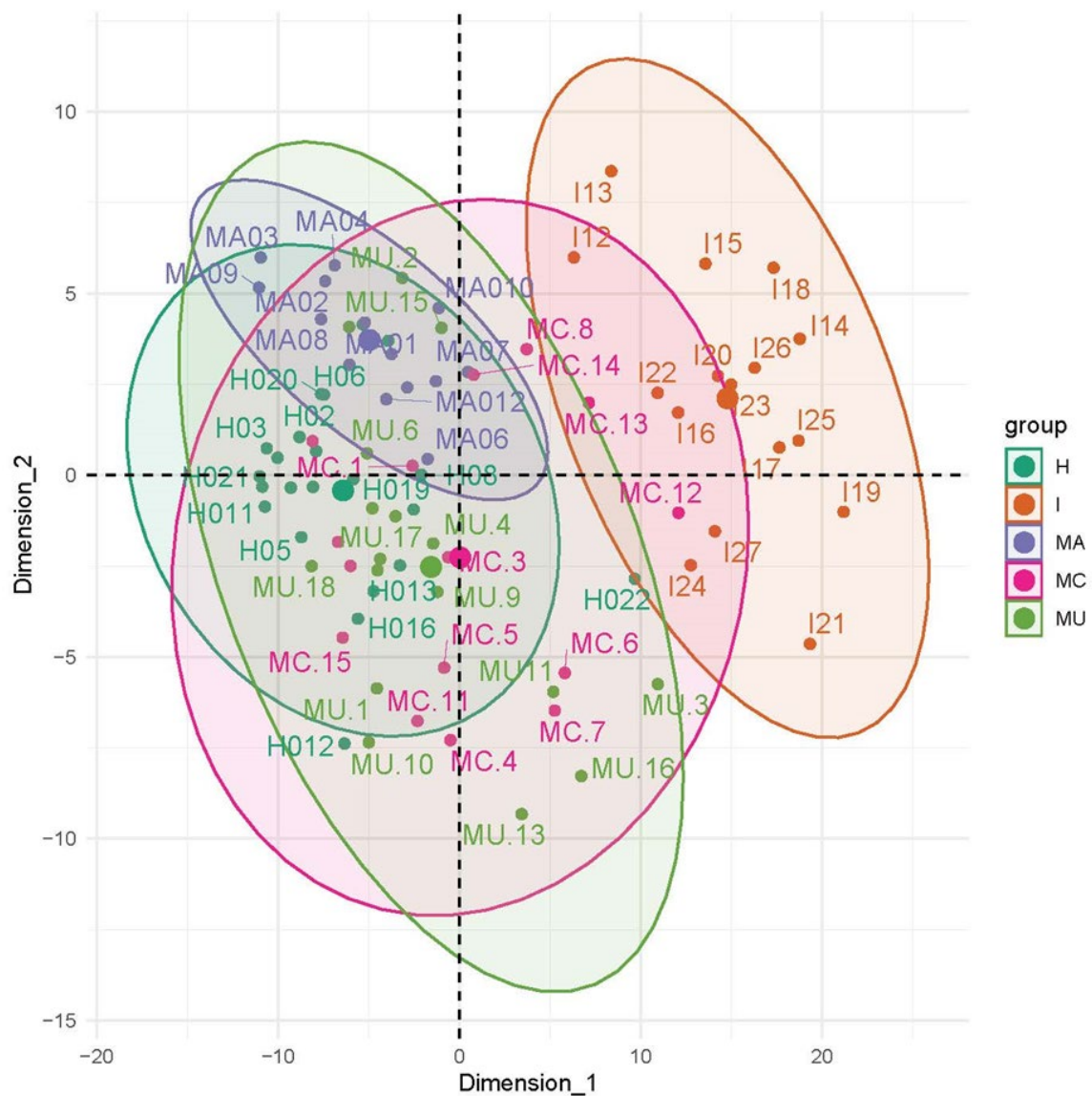


Figure 6. MDS Plot for all exposure and control groups

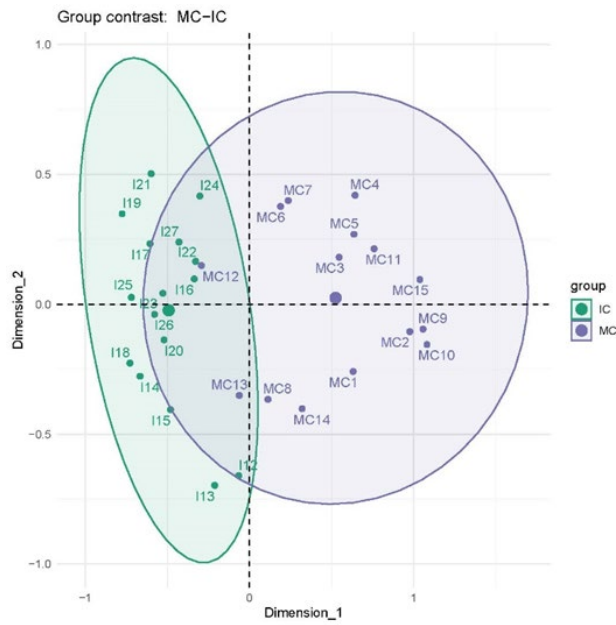


Figure 7. MDS Plot for IC (Control JASDF) and MC (control US Air Force) groups

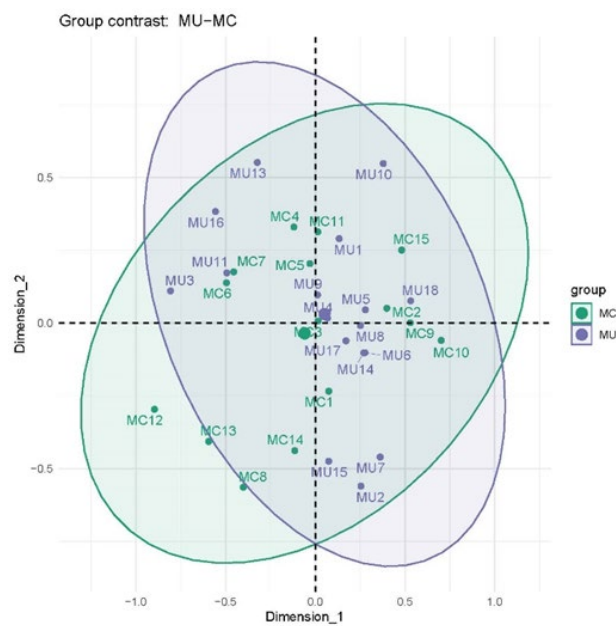


Figure 8. MDS Plot for MU (JP-8 Exposure) and MC (control) groups

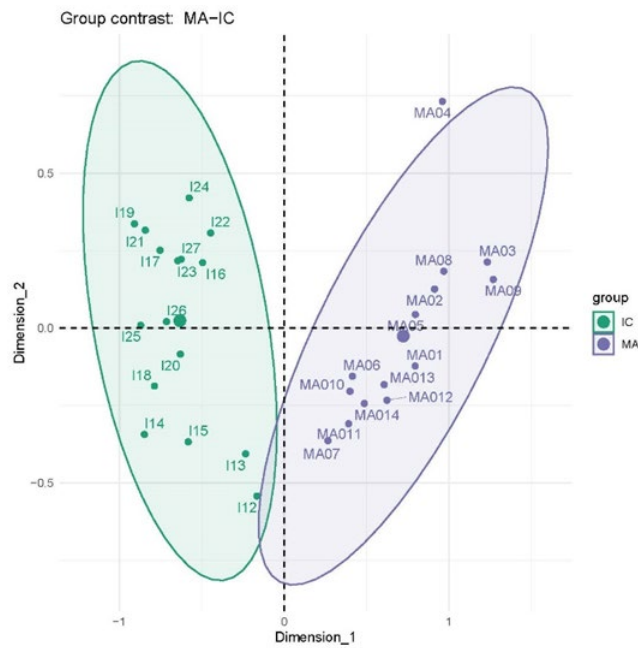


Figure 9. MDS Plot for MA (JetA1 Exposure) and IC (control) groups

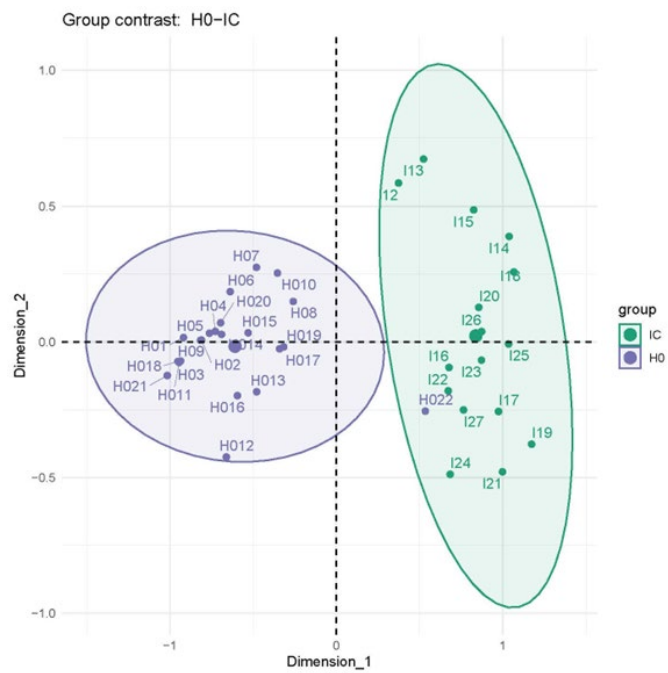


Figure 10. MDS Plot for HO (JetA1 Exposure) and IC (control) groups

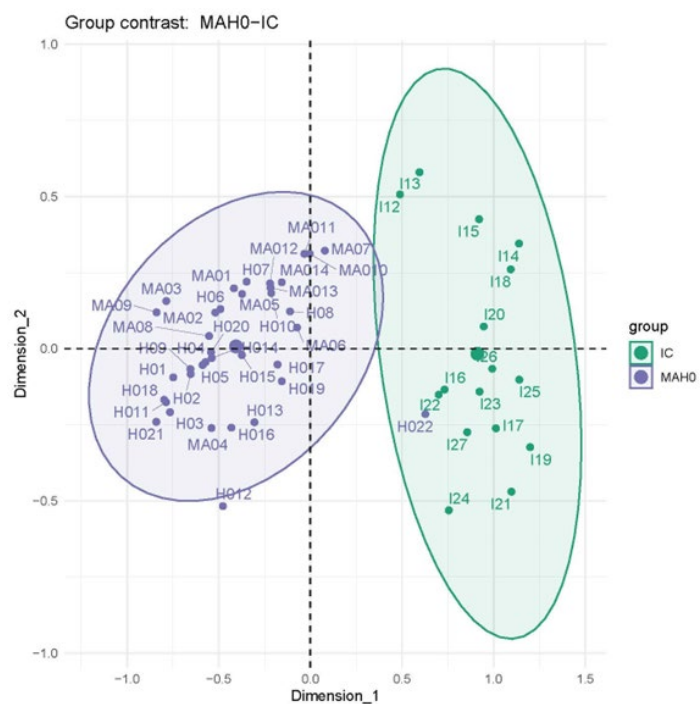


Figure 11. MDS Plot for MA+HO (JetA1 Exposure) and IC (control) groups

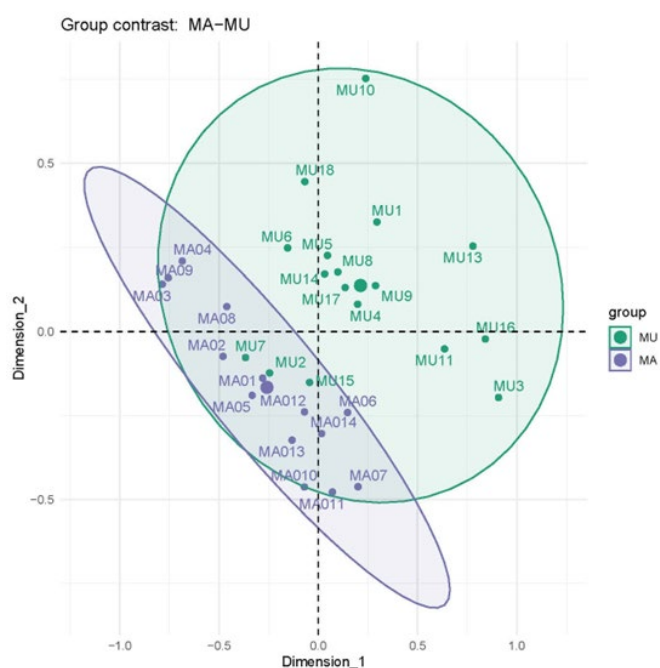


Figure 12. MDS Plot for MA (JetA1) and MU (JP-8) exposure groups

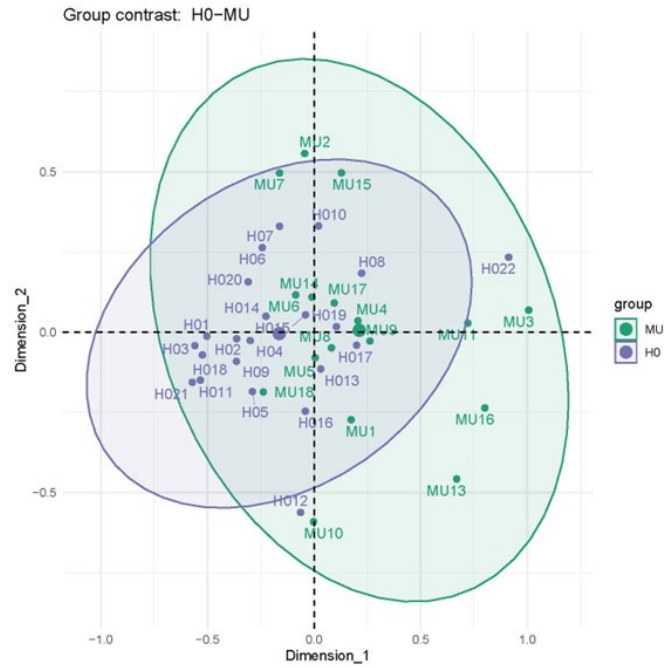


Figure 13. MDS Plot for HO (JetA1) and MU (JP-8) exposure groups

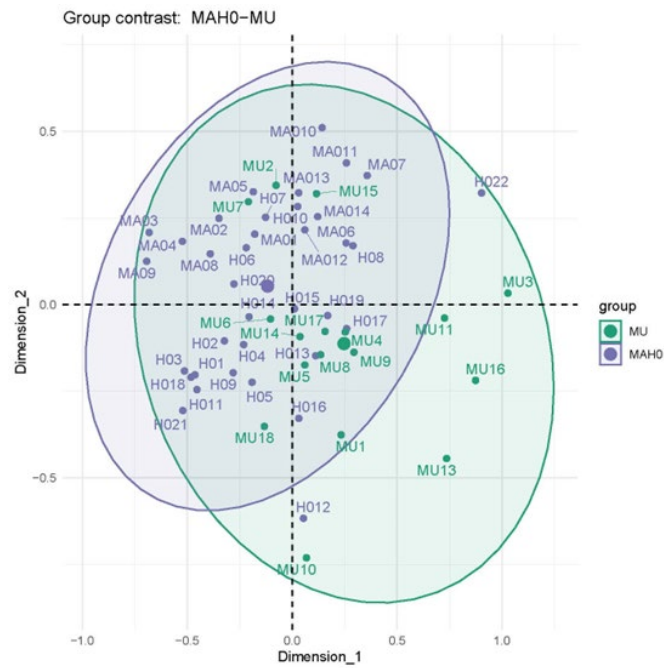


Figure 14. MDS Plot for MA and HO (JetA1) exposure groups and MU (JP-8) group

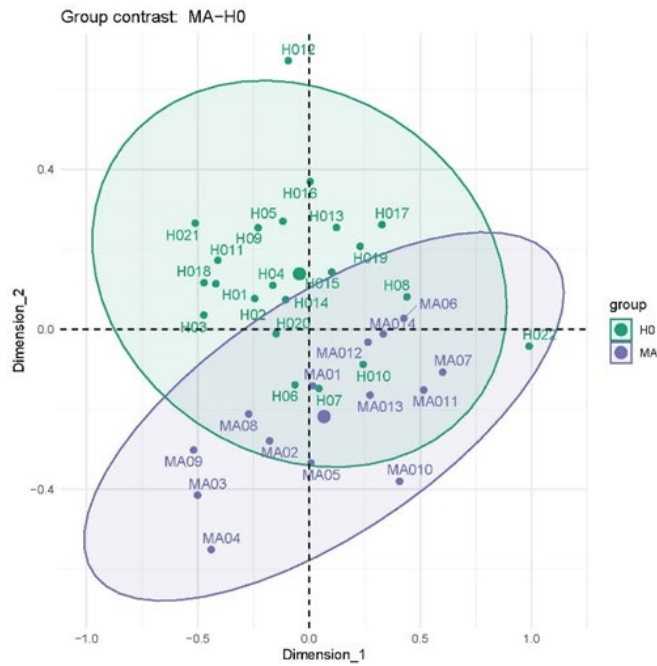


Figure 15. MDS Plot for MA (JetA1) and HO (JetA1) exposure groups

4.5.2.2 Variability in miRNA Expression in Exposed Groups

A Corrdiff plot heatmap of the top-50 most variable miRNAs indicates many miRNAs which show an altered expression (Figures 16 and 17). The criterion for "variable" is ranking variance of expression across all samples and sorting from most to least observed variation.

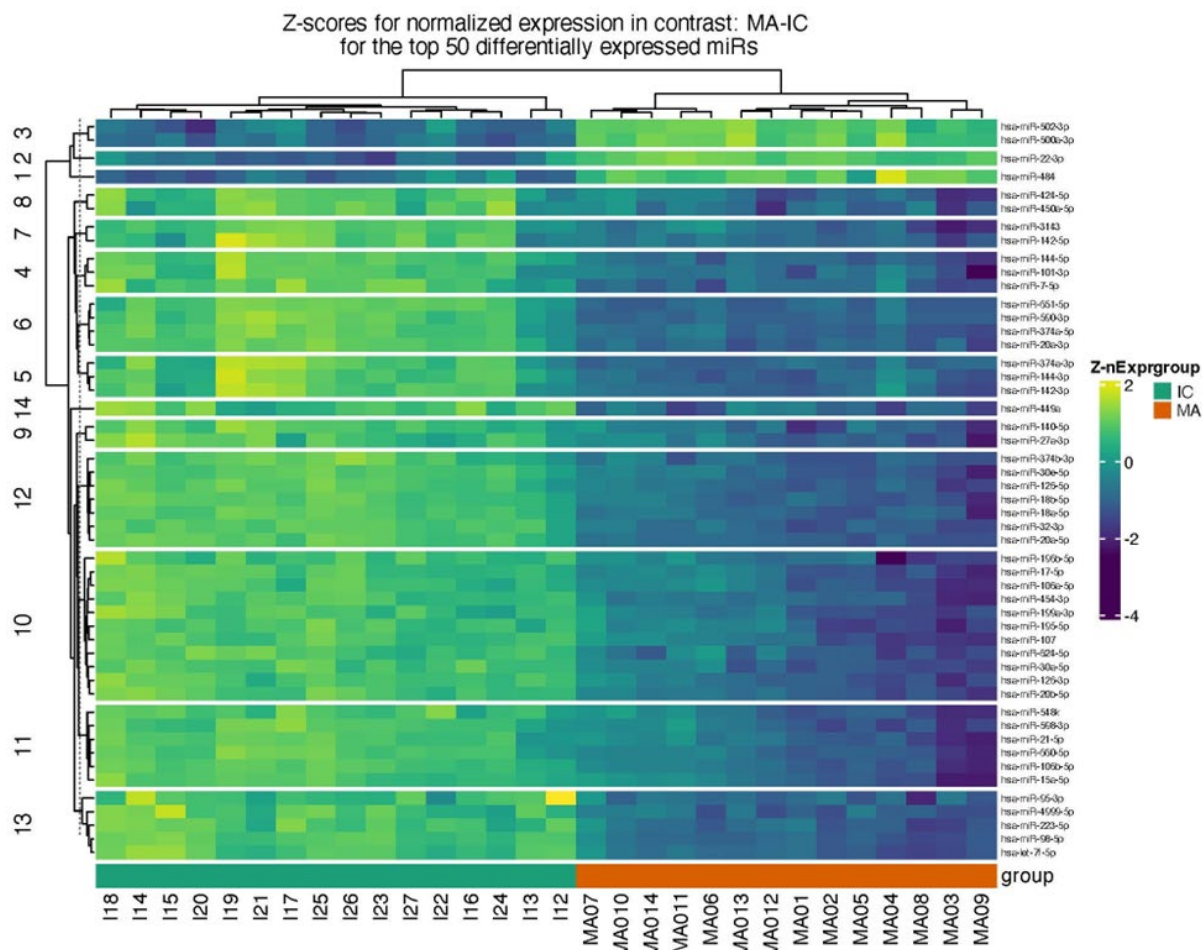


Figure 16. Expression Clustering of the top 50 differentially expressed miRNAs from MA JetA1 Exposure group compared to the IC Control group. All groups JASDF personnel.

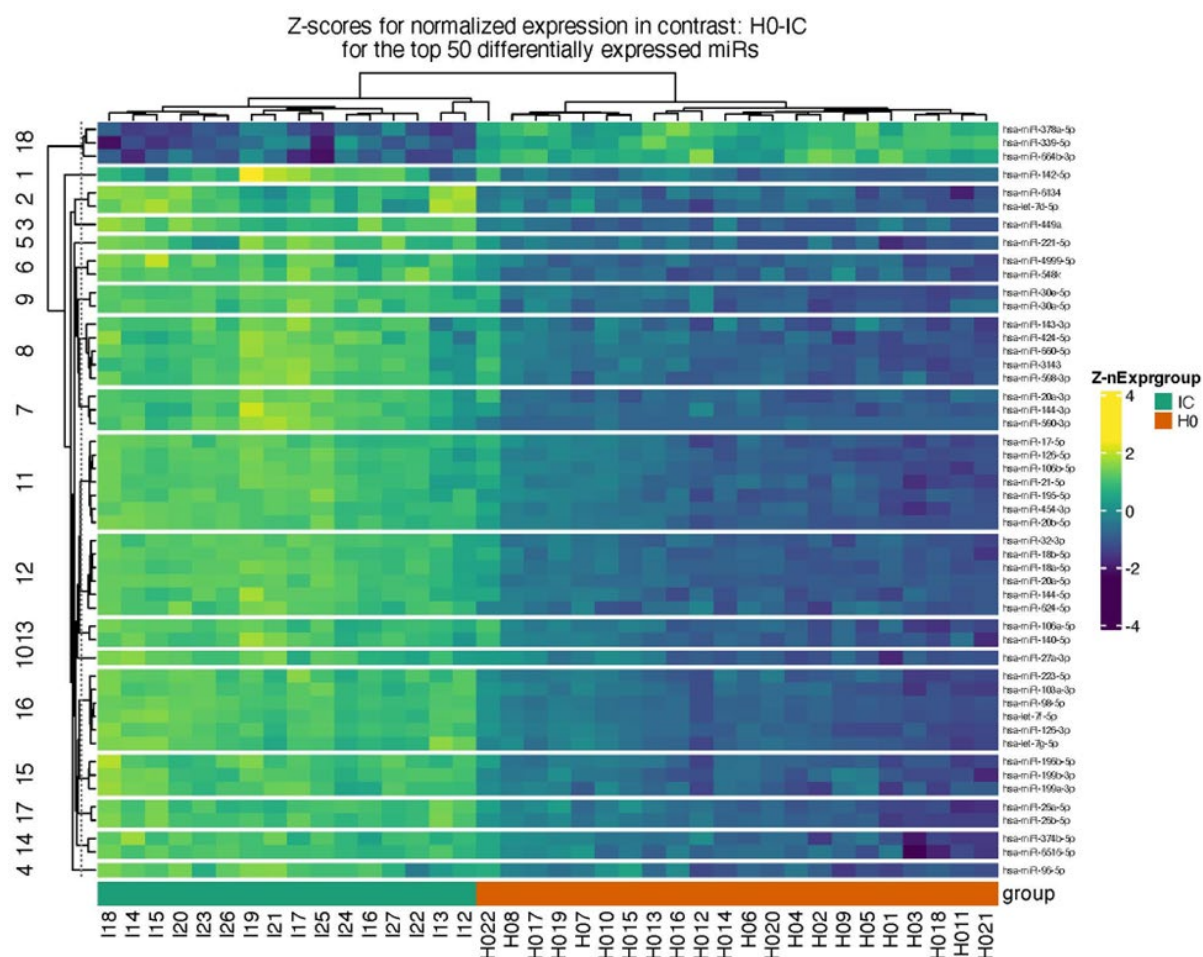


Figure 17. Expression Clustering of the top 50 differentially expressed miRNAs from HO JetA1 Exposure group compared to the IC Control group. All groups JASDF personnel.

4.5.2.3 Identification of Significantly Altered miRNAs

Interestingly, other than the JP-8 exposure comparisons which identified no statistically significant alterations, other comparisons identified large numbers of statistically altered miRNA species which demonstrated >2-fold change (either up or down) (Table 31).

Table 31. Summary of comparisons and number of altered miRNAs with statistically significant with > 2-fold change. Comparisons of interest marked in yellow

Groups	Controls & Fuels	Subjects	Expected miRNA Alterations	Total Significant miRNA Changes	Up Regulated	Down Regulated
MC vs. IC	Controls	USAF and JASDF	Variability between control groups comprised of JASDF or USAF. Indication of alterations due to lifestyles or other variable.	180	93	87
MU vs. MC	JP-8 Exposure	USAF	Alterations due to JP-8 fuel exposure to JP-8 in USAF personnel.	0	0	0
MU vs. MC+IC	JP-8 Exposure	USAF and JASDF	Alterations due to JP-8 Exposure in USAF compared to a combined Control data set (USAF and JASDF)	96	41	55
HO vs. MC+IC	JetA1 Exposure	USAF and JASDF	Alterations due to JetA1 Exposure in JASDF compared to combined Control data set (USAF and JASDF)	207	117	90
MA+HO vs. MC+IC	JetA1 Exposure	USAF and JASDF	Alterations due to combined JetA1 Exposure data (combined JASDF) compared to combined Control data set (USAF and JASDF)	211	116	95
MA vs. MU	JetA1 and JP-8 Exposure	USAF and JASDF	Alterations due to additives when comparing JetA1 (JASDF personnel) to JP-8 (USAF) exposures	142	70	72
HO vs. MU	JetA1 and JP-8 Exposure	USAF and JASDF	Alterations due to additives when comparing JetA1 (JASDF personnel) to JP-8 (USAF personnel) exposures	61	28	33
MA+HO vs. MU	JetA1 and JP-8 Exposure	USAF and JASDF	Alterations due to additives when comparing JetA1 Exposure (in JASDF personnel in two locales) to JP-8 exposures (USAF personnel)	118	68	50
MA vs. HO	JetA1 Exposure	JASDF	Alterations due to locale in Jet A1 exposure groups from JASDF	67	36	31
MA+HO vs. IC	JetA1 Exposure	JASDF	Alterations due to Jet A1, combined exposure groups from 2 ABs, (JASDF personnel)	309	183	126
HO vs. IC	JetA1 Exposure	JASDF	Alterations due to Jet A1 (JASDF personnel)	271	136	135
MA vs. IC	JetA1 Exposure	JASDF	Alterations due to Jet A1 (JASDF personnel)	260	129	131

Volcano plots were constructed to identify and visibly show the relationship between the fold change (FC) and statistical significance of altered miRNAs from each comparison in Table 31 (Figures 18-25) and consist of the FDR (x-axis) and FC (y-axis). Descriptions for each figure are

presented here before all of the figures in order to keep the size of each figure larger and more readable.

Figure 18 shows the wide range of down regulated to up regulated FC when comparing JASDF and USAF controls. This indicates that the control groups cannot be combined into one control group for all the Japan bases in this study.

Figure 19 confirms the lack of significant changes between Misawa AB exposed and controls by showing no red or blue dots. JP-8 exposure at Misawa AB either appears to have little effect on miRNA or the controls are equally exposed.

Figure 20 visibly indicates the significant differences between JASDF JetA1 exposure at Hamamatsu AB compared with JASDF controls while Figure 21 compares significant differences between Matsushima AB exposed subjects with JASDF controls. Low levels of JetA1 can still cause significant changes in miRNA in exposed personnel.

Figure 22 shows the differences between Matsushima AB JetA1 exposed subjects and Misawa AB JP-8 exposed personnel. Figure 23 depicts FC for Hamamatsu AB JetA1 exposed subjects compared with Misawa AB JP-8 exposed personnel. Figure 24 combines Matsushima and Hamamatsu AB JetA1 exposure groups and compares them to Misawa AB JP-8 exposures. There is a wide range of down regulated to up regulated FC when comparing JASDF JetA1 exposures and USAF JP-8 exposures. This is surprising since JetA1 and JP-8 are very similar in their hydrocarbon composition with the major difference being the low levels of additives found in JP-8.

Figure 25 compares significant FC for Matsushima AB JetA1 and Hamamatsu AB JetA1 exposure groups. There is a much smaller number of significant changes when comparing the two JASDF JetA1 exposed groups.

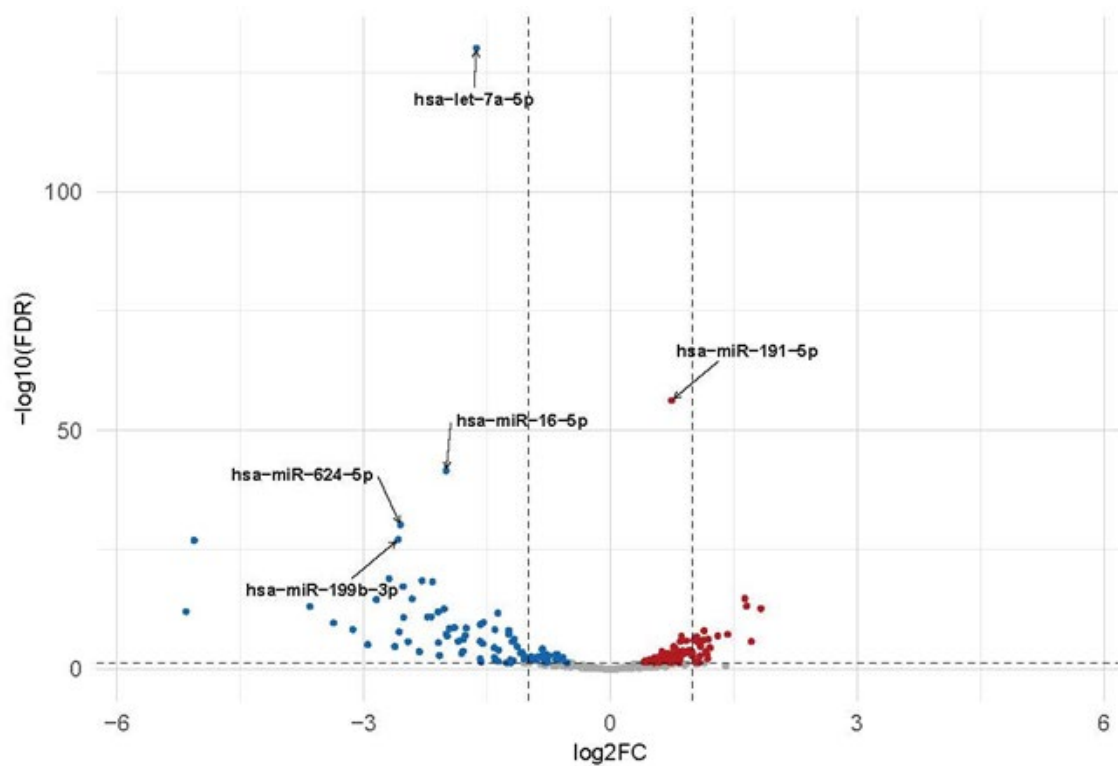


Figure 18. Volcano Plot (log fold changes) for IC (Control JASDF) and MC (control US Air Force) groups; *Up regulated indicated as red dots, down regulated indicated as blue dots*

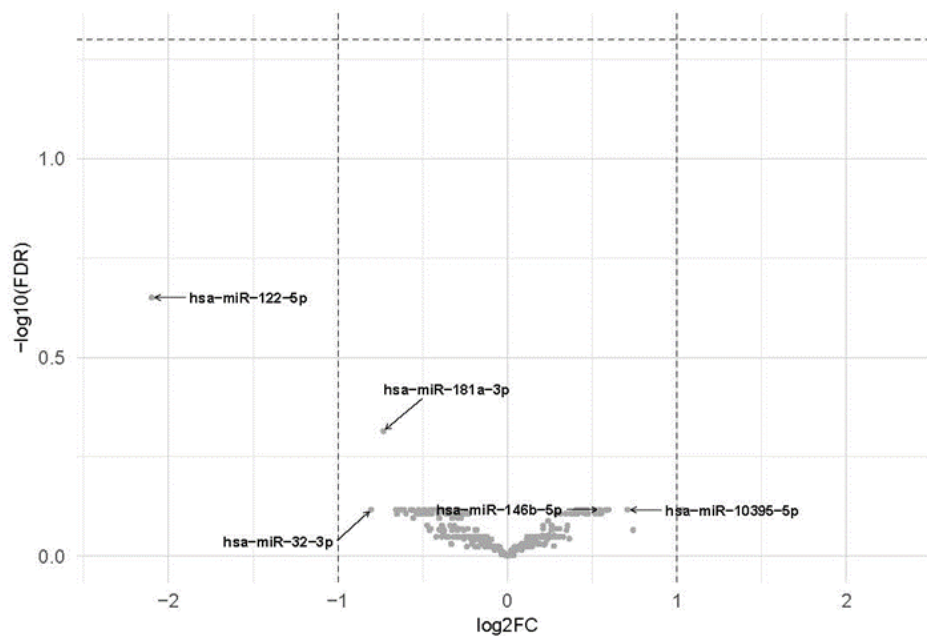


Figure 19. Volcano Plot (log fold changes) for MU (JP-8 Exposure) and MC (control) groups; *Up regulated indicated as red dots, down regulated indicated as blue dots*

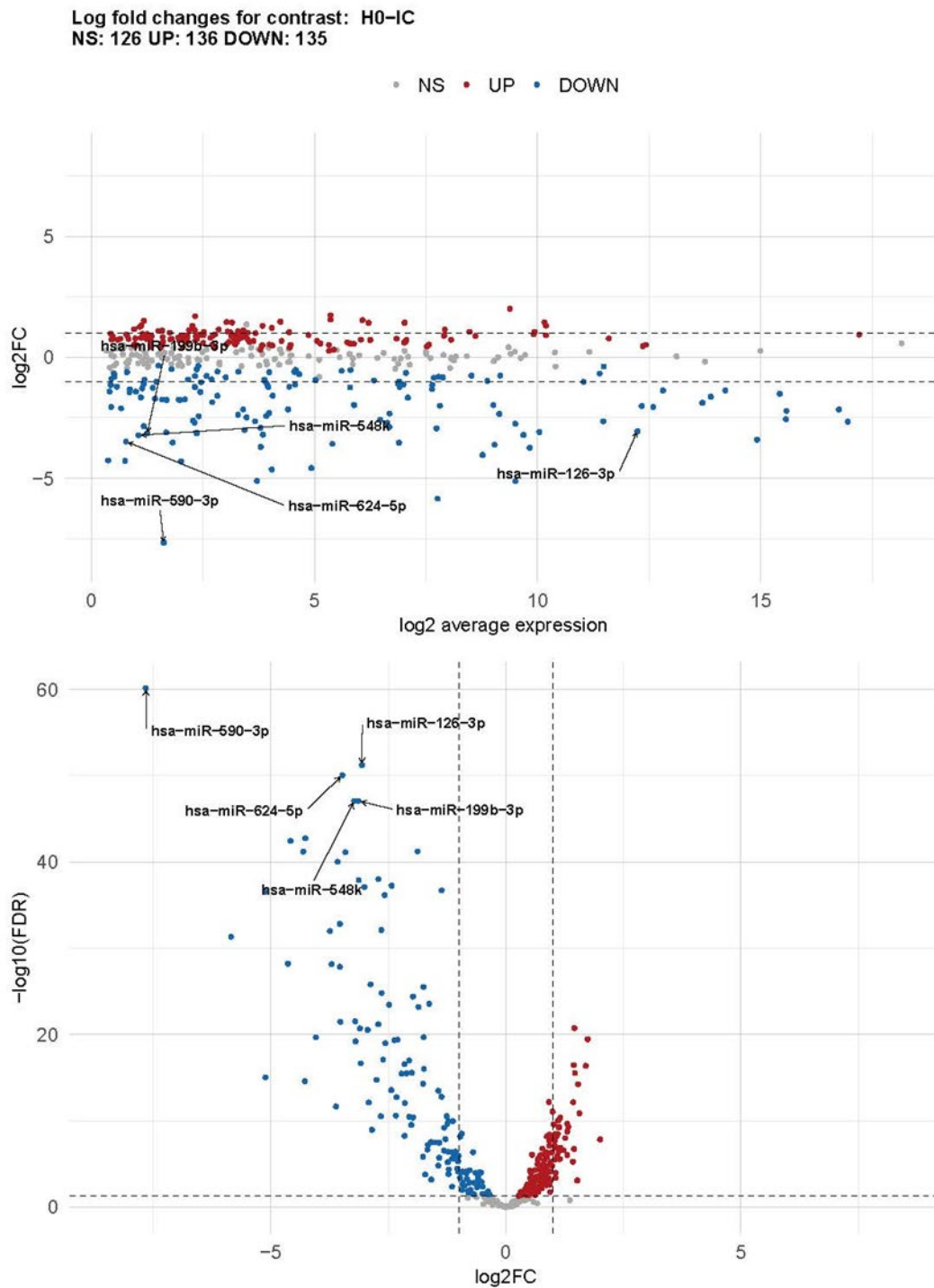


Figure 20. Volcano Plot (log fold changes) for HO (JASDF JetA1 Exposure) compared with IC (Control JASDF) groups; *Up regulated indicated as red dots, down regulated indicated as blue dots.*

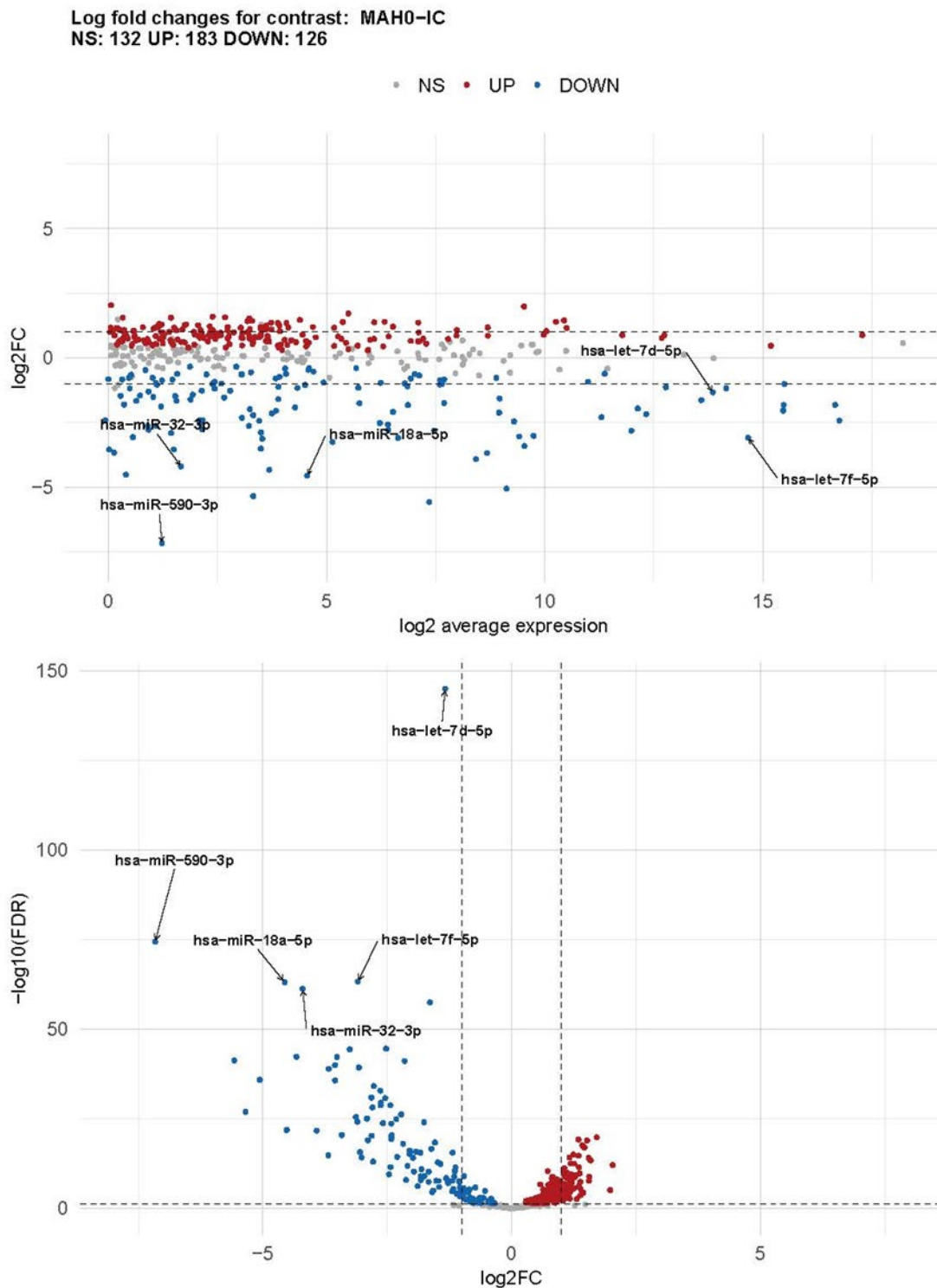


Figure 21. Volcano Plot (log fold changes) for MA (JASDF JetA1 Exposure) compared with IC (Control JASDF) groups; *Up regulated indicated as red dots, down regulated indicated as blue dots.*

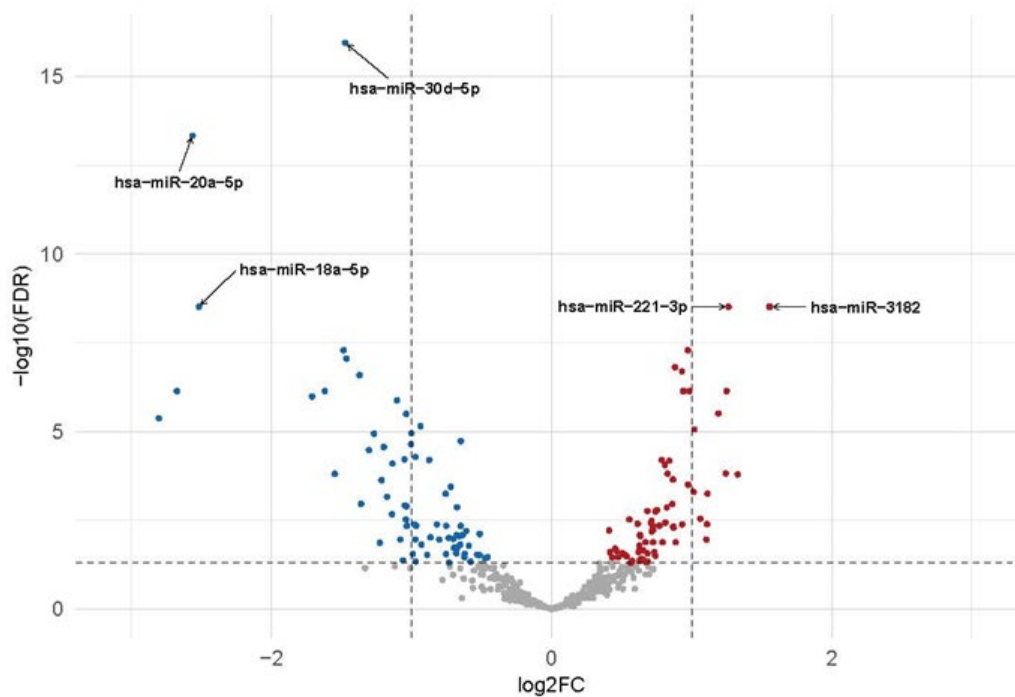


Figure 22. Volcano Plot (log fold changes) for MA (JetA1) and MU (JP-8) exposure groups; *Up regulated indicated as red dots, down regulated indicated as blue dots*

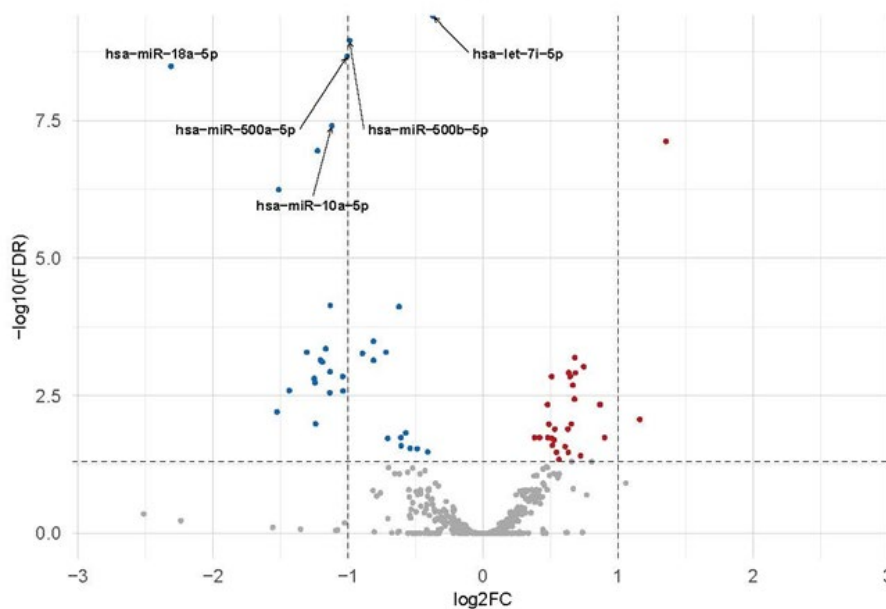


Figure 23. Volcano Plot (log fold changes) for HO (JetA1) and MU (JP-8) exposure groups; *Up regulated indicated as red dots, down regulated indicated as blue dots*

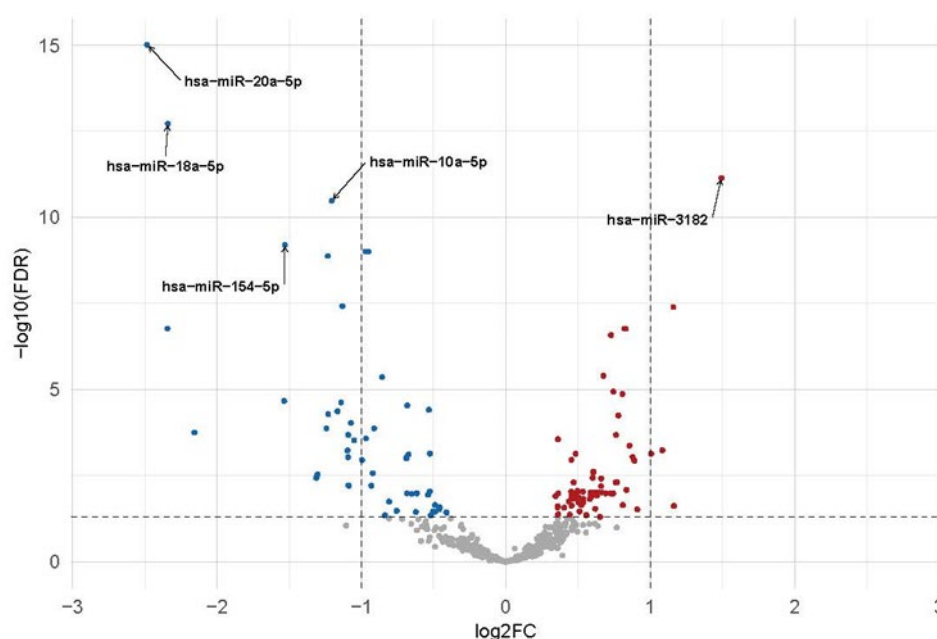


Figure 24. Volcano Plot (log fold changes) for MA and HO (JetA1) exposure groups and MU (JP-8) group; *Up regulated indicated as red dots, down regulated indicated as blue dots*

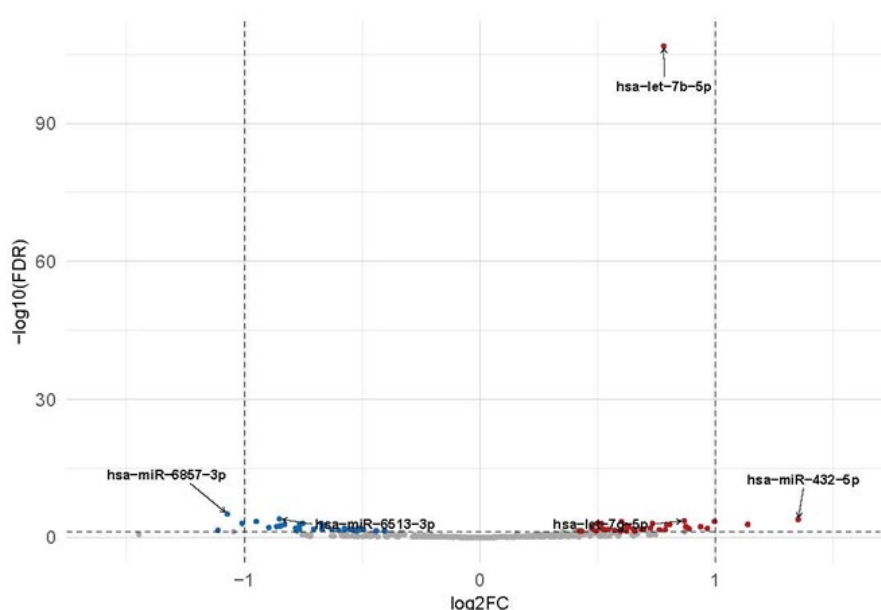


Figure 25. Volcano Plot (log fold changes) for MA (JetA1) and HO (JetA1) exposure groups; *Up regulated indicated as red dots, down regulated indicated as blue dots*

4.6 Metabolomic Analysis

Principle components analysis (PCA) of individuals in Figures 26 and 27 at Hyakuri AB, Kadena AB and Misawa AB show the comparison of exposure to JP-4 (Hyakuri AB) versus JP-8 (Kadena and Misawa ABs). The pre-shift PC1/PC2 shows that the metabolites for the Hyakuri

AB subjects overlap all but the Misawa controls so there is a large range which may be due to the extent of exposure to JP-4. The Yokota AB controls showed the largest range, but they are a smaller subset of controls than for all of the other bases. All of the metabolites for Misawa AB, both exposed and controls clustered in a very small range. This is in contrast to the Kadena AB personnel where the control and exposed subjects had a larger range of metabolites with a number of exposed personnel extending outside of the control range. The differences could be due to the aircraft at each base, F-16s versus F-15s, or the location, northern mainland Japan versus Okinawa an island south of mainland Japan.

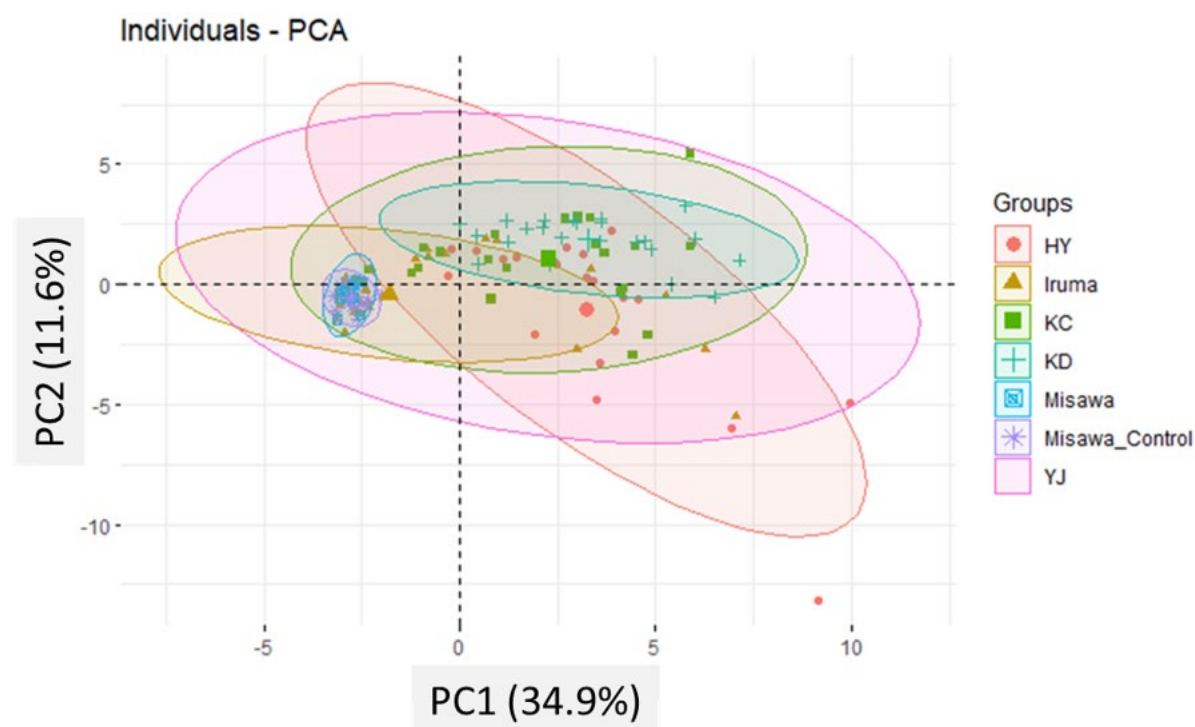


Figure 26. Pre-Shift PC1/PC2 for metabolites from flight line and control subjects at Hyakuri AB with JP-4 (HY), Kadena (KC) and Misawa ABs with JP-8 and for control subjects Iruma, Yokota (YJ), Kadena (KC) and Misawa ABs

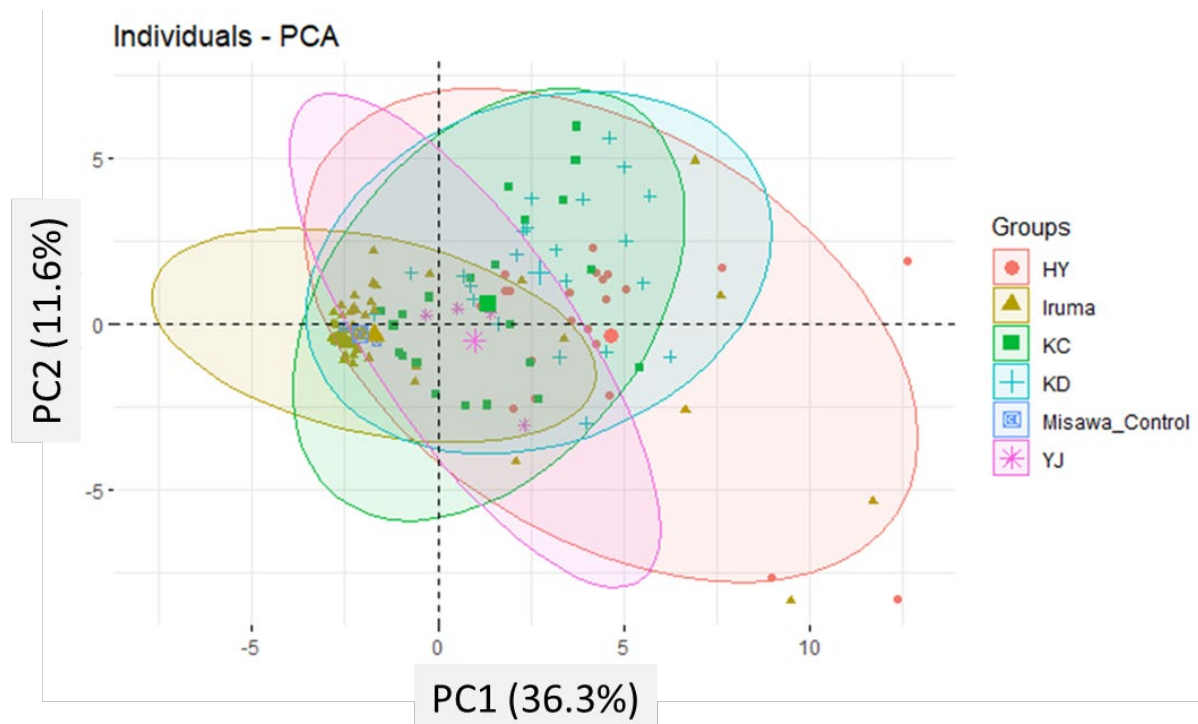


Figure 27. Post-Shift PC1/PC2 for metabolites from flight line and control subjects at Hyakuri AB with JP-4 (HY), Kadena (KC) and Misawa ABs with JP-8 and for control subjects Iruma, Yokota (YJ), Kadena (KC) and Misawa ABs

Figures 28 (pre-shift) and 29 (post-shift) show comparison of metabolites in flight line personnel by type of jet fuel versus controls combined from all bases with controls.

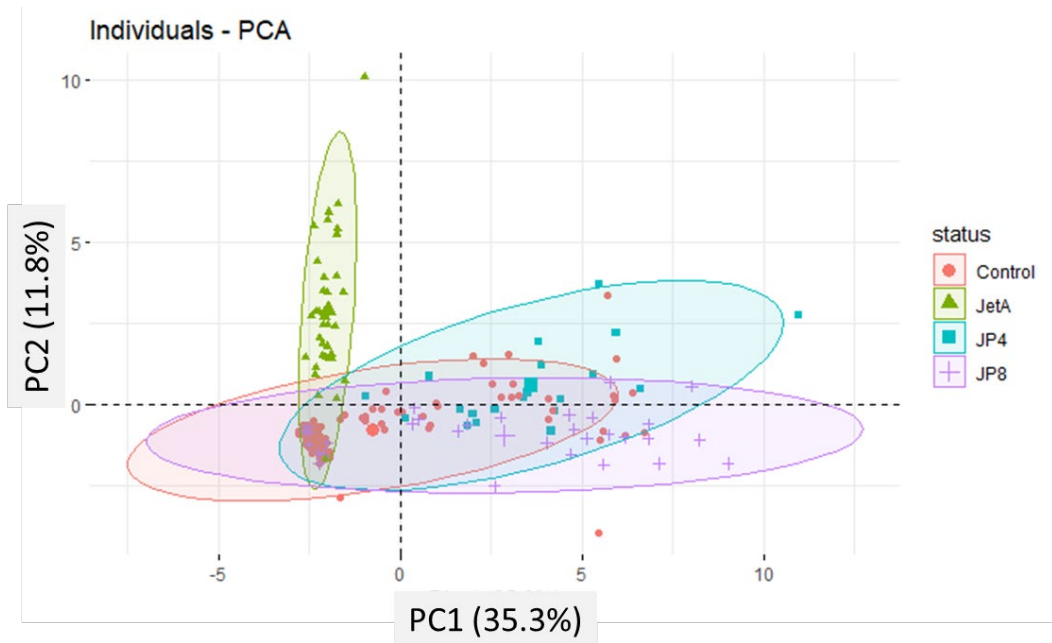


Figure 28. Pre-Shift PC1/PC2 for metabolites from flight line personnel by type of jet fuel and combined control subjects from all control bases

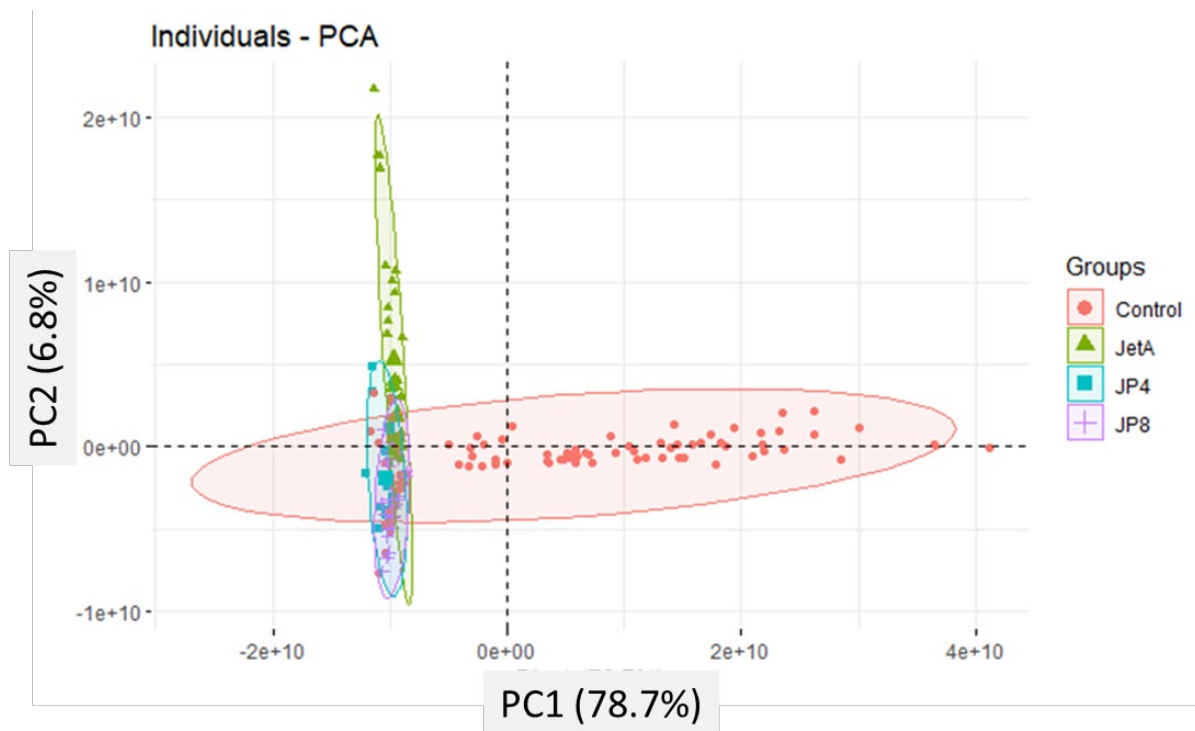


Figure 29. Post-Shift PC1/PC2 for metabolites from flight line personnel by type of jet fuel and combined control subjects from all control bases

5.0 CONCLUSIONS

Despite levels of VOCs below exposure limits and the use of hearing protection, a battery of hearing tests (audiograms, OAE, immittance and ABR) showed significant differences and trends towards potential hearing loss.

5.1 Noise and hearing loss

The noise level on flight lines was greater than the OSHA action level (85 dBA) for requiring hearing protection and was also greater than the 8-hour OSHA (90 dBA) and ACGIH (85dBA) exposure limits.

Audiological tests showed significant differences and trends between flight line personnel and control subjects for both T-4, F-2, F-4, F-15 and F-16 aircraft. The differences were not consistent between aircraft even though noise levels were comparable for all exposed subjects. Due to a COVID forced break in the study in 2020 the audiogram data was lost for F-4 and F-15 aircraft maintainers. Average audiogram values that were available for T-4, F-2 and F-16 maintainers were all normal so this test is not as reflective of potential hearing loss in this study. The COVID break also resulted in the loss of the F-15 immittance and OAE data. Making comparisons based on available data (number of trends and significances) showed that the F-2 had a greater potential for hearing loss, then the F-16 followed by T-4 then the F-4. If you use just the ABR data for ranking to include the F-15 data, then F-16 was greater than T-4 followed by F-15 then F-4 and finally F-2. Since the F-2 data went from first to last and audiogram data were normal, this shows the importance of using multiple tests to screen for hearing loss. Differences in the audiological data could be due to type of aircraft engine, operational tempo, layout of the AB, gender, and race (Japanese versus American Airmen).

Since the F-4 and JP-4 jet fuel are no longer operational in the JASDF and USAF, retiring both makes the current flight line environment safer based on noise levels and fuel.

5.2 miRNA

5.2.1 Lifestyle Contributions in Control Groups

The comparison of the two control groups (MC, with USAF personnel; IC, with JASDF personnel) resulted in a total of 180 statistically altered blood miRNA signatures, demonstrated that lifestyle or other factors could significantly alter miRNA expression. Due to this finding, care must be taking when interpreting comparisons utilizing different personnel groups.

5.2.2 Locale Contributions in JetA1 Exposure Groups

Interestingly, an examination of JetA1 exposures at two different locations in JASDF personnel identified a small number of statistically significant miRNA alterations between the two locales. As both JetA1 exposure groups consisted of JASDF personnel, lifestyle confounders are less likely to contribute to the differences observed.

5.2.3 Blood miRNA Signatures to JP-8 Exposure

The examination of miRNA alterations in a JP-8 USAF personnel exposure group at Kadena AB compared to a control group consisting of USAF medical personnel at the same base, did not identify any statistically significant alterations due to JP-8 exposures in the 2018-to-2019-time frame of this study. The more recent data set comparison (MU vs. MC) in the 2021-to-2023-time frame also utilized a control group on the same AB. There are some concerns with processing due to the failure to identify any significantly changed miRNAs (Table 31) which is unusual. However, examination of technical processes and bioinformatics executed in the effort have not identified any weakness or failure in sample analyses or data processing. This finding was similar to data from previous human study profiling – that is, no differentially expressed miRNAs were seen to correlate with JP-8 exposure (publication in preparation). Therefore, it appears these results can be trusted but additional data should be obtained.

5.2.4 Blood miRNA Signatures to JetA1 Exposure

Unlike the JP-8 analyses, comparisons of JetA1 exposed groups to control identified a high number of altered miRNAs with >2 fold change and adjusted $p > 0.05$ (Table 31). While some minor reservations should be made in interpretation, it is interesting that JetA1 exposures in JASDF personnel seem to provide the greatest exposure response. It is not clear if the strong response indicates a particular sensitivity to fuel exposures in JASDF personnel or different fuel handling processes; however, the differentially expressed miRNA profiles are compelling.

While pathway analyses are not yet been completed, some of the top miRNAs identified in the study indicate potential links to exposure. The hsa-let-7d-5p miRNA has been shown to be significantly upregulated in the blood from human exposures to particulate matter PM2.5 (Mancini et. al 2020). Has-miR-590-3p has been shown to be upregulated in blood from workers exposed to Cr (VI) (Jia et al., 2020). Interestingly the miRNA-18a family has been shown to regulate cellular responses to stress from chemical exposures, serving as an oncogene as well as a suppressor, with differential expression observed in cancer and several other diseases (Kolenda et al., 2020). Pathway analysis completion will aid in understanding the mechanistic changes seen in the human under relatively normal fuel exposure levels. Additionally, the identified modulated pathways will aid in identification of specific genes that could be altered by the differentially expressed miRNAs.

Lastly, the data generated in the Jet A1 samples with the high-level fold FC and FDR adjusted $p > 0.05$ justify follow-on efforts to validate the miRNA discovery data using qPCR of each miRNA in the remaining samples. If several of the top differentially expressed miRNAs validate, then prediction and verification of miRNA target genes should be conducted utilizing RNA from the existing samples.

5.2.5 Blood miRNA Signatures to Fuel Additives

Three comparisons (MA vs. MU; HO vs. MU; MA+HO vs. MU) were conducted using data from both JP-8 and JetA1 exposure groups to determine if specific miRNAs were altered in response to the corrosion inhibitor and anti-icing additives not found in JetA1. There were no representative JP-8 JASDF for the comparisons, therefore the data is only suggestive at best, and care must be taken in interpretation of miRNA alterations in this specific examination. Interestingly, almost twice the amount of statistically significant miRNAs are seen from samples

collected at the Matsushima AB MA (vs MU as JP-8 exposure) when compared to the Hamamatsu AB HO (compared to the same JP-8 data) (142 vs. 61, respectively). When the data from the two airfield exposure groups are combined (MA+HO vs. MU), miRNA levels are almost twice as high as from HO vs. MU alone (118 vs. 61 respectively). With the previous caveats mentioned regarding lifestyle confounders, further potential pathway analyses may provide the rigor needed to separate out miRNA signatures due to the additives alone.

5.3 Metabolomics

Overall, there was overlap between controls and flight line personnel when comparing individual bases. The degree of overlap varied by base and aircraft. When all three jet fuel metabolites were combined for the post shift data there was a high degree of separation between controls and flight line personnel. Additional analysis might show clearer results for the individual bases. There were differences seen between pre shift and post shift, but the most significant change was when all three jet fuels were combined to compare with all controls.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

AB	air base
ABR	auditory brain response
AGC	target ion population
AGE	Aerospace Ground Equipment
AML	Aeromedical Laboratory
AR	acoustic reflex
C	Contra
cc	cubic centimeters
CJ	Non-exposed control subjects in JASDF
CK	Non-exposed control subjects in Kadena
CM	Non-exposed control subjects in Misawa
Cr	creatinine
°C	degrees Centigrade
cDNA	Complimentary DNA
daPa	decapascals
dB	decibels
DNA	deoxyriboneucleic acid
DOEHRS-HC	Defense Occupational and Environmental Health Readiness System-Hearing Conservation
DPOAE	distortion product otoacoustic emissions
DoD	Department of Defense
ECV	equivalent ear canal volume
EI	electron ionization
F-2	JetA1 exposed F-2 flight line crews in Matsushima
F-4	JP-4 exposed F-4 flight line crews in Hyakuri
F-15	JP-8 exposed F-15 flight line crews in Kadena
F-16	JP-8 exposed F-16 flight line crews in Misawa.
FC	fold change
FDR	false discovery rate
GC-FID	Gas Chromatography-Flame Ionization Detector

GC-MSD	Gas Chromatography-Mass Spectrometer Detector
HILIC	hydrophilic interaction chromatography
Hz	hertz
JASDF	Japan Air Self-Defense Force
Jet A	jet fuel aviation
Jet A1	jet fuel aviation 1
JP-4	jet propulsion fuel 4
JP-8	jet propulsion fuel 8
JSOH	Japan Society for Occupational Health
LC-MS	liquid chromatography mass spectrometer
m/z	mass-to-charge ratio
mm	millimeters
mM	millimolar
mL	milliliters
MS	mass spectrometer
MS1	full scan injection
MSn	iterative pooled QC sample injections
OAE	otoacoustic emissions
OSHA	Occupational Safety and Health Administration
oz	ounce
%	percent
P	Peak pressure
PELs	permissible exposure limits
PCA	principal components analysis
ppm	parts per million
QA	quality assurance
QC	quality control
RF	radio frequency
RNA	ribonucleic acid
miRNA	microRNA
SC	static compliance
SEM	standard error of the mean

T-4	JetA1 exposed T-4 flight line crews in Matsushima and Hamamatsu
TWA	time weighted average
μL	microliters
USAF	US Air Force
VOCs	volatile organic compounds
x g	times gravity