Detailed Methods

Animals: Male Sprague Dawley rats (6 weeks age, from Hilltop Breeders, PA) were used for all experiments. Rats were housed on a 12:12 LD cycle (lights on 0600), with food and water available *ad libitum*, in an AAALAC International accredit animal facility at NIOSH, Morgantown. The average temperature of the animal facility and experimental room was 21.1-22.2 °C. Rats were acclimated to the facility for 1 week prior to being included in an experiment. In Experiment 1, rats (n = 4/group) were acclimated to restraint by placing them into Broome style restrainers every day for a week. After acclimation, rats were assigned to a cage control (no exposure or restraint), restraint control (restrained and placed into vibration exposure chambers) and vibrated (restrained and exposed to a single 4 h of tail vibration). Animals were exposed to a single bout of vibration or restraint and blood flow was measured immediately following the exposure. In Experiment 2, rats (n=6/group) were acclimated to restraint and then assigned to a restraint control or vibrated group. Because there were no significant differences between the cage and restraint control groups in Experiment 1, a cage control group was not used in Experiment 2. In Experiment 2, rats were exposed to vibration or restraint control conditions for 4 h/day, 5 days a week (M-F) for 20 days.

Exposure. Laser Doppler measurements were made using a Peri-flux system 5000 and PF 450 thermostatic small angle probe (Primed, Stockholm, Sweden). Prior to each reading the machine was calibrated by placing the probe into the calibration solution supplied by the manufacturer. Once calibrated, the probe was secured in a ring stand and positioned below the opening of a stainless steel, rectangular holder used to support the tail stable during recording. Measurements of blood flow and tail temperature were collected at 30 Hz, in an isolation chamber to prevent external noise or stimuli from affecting the measurements. In Experiment 1, blood flow (perfusion units) and tail temperature (°C) were collected for for at least 5 minutes immediately before and immediately following the exposure. A 5-minute collection period was chosen because studies in humans have demonstrated that blood flow returns to pre-exposure levels fairly quickly after an acute exposure

to vibration. To collect the blood flow measurement, each animal was placed into an isolation chamber, and their tail was placed into a specialized holder with the laser Doppler probe attached. PF 450 thermostatic small angle probe measured blood flow in a 1 cm area between the 15 and 16th vertebrate of the tail. The temperature sensor surrounding the laser simultaneously measured the temperature of the tail. After the blood flow measurement was completed, animals were returned to their home cage. In Experiment 2, rats were restrained, placed into the isolation chamber, and blood flow and tail temperature were collected for at least 15 minutes prior to vibration exposure on day 1 (pre-exposure), 5, 10, 15 and 20 of the exposure. Measurements were made prior to the exposure to determine if there were any effects of vibration on blood flow that were maintained and may be indicative of a longer-term problem in the functioning of the peripheral vascular system. Laser Doppler data were collected for 15 minutes because the goal of the study was to determine if there were vibration-induced changes in basal or resting blood flow. Collecting measurements for an extended period of time, provided more data that could be used to identify smaller changes that are associated with the exposure. Immediately following the blood flow measurements, rats were returned to their home cages.

Statistical Analyses: Data transferred from the Perimed program to Excel for analyses were converted to a 10 Hz signal by the Perimed program because some of the files were too large to transfer. To determine if vibration exposure had a significant effect on blood flow in Experiment 1, the average tail temperature and blood flow were calculated over the 5 minutes before and after the exposure and data were analyzed using 2-way mixed model ANOVAs (treatment (2) x pre vs post (2)) where animal served as a random factor. In Experiment 2, blood flow data average blood flow was calculated for the 15-minute collection period. Some animals moved during blood flow measurement, resulting in either very high readings of blood flow, or the inability to measure blood flow. The time over which these disruptions occurred was usually brief (usually less than 10 sec), but the fluctuations in blood flow measurements during these periods greatly affected the average blood flow calculation. To reduce variability in the mean due to motion, limits were set (blood flow less than 2 or greater than 100 perfusion units) based on previous data collected in the laboratory, and these regions of the

data were normalized by calculating running means. To calculate a running means the 20 measures prior to and the 20 measures following motion or loss of the blood flow reading were averaged, and this average (i.e., the running mean) was used to replace data lost to movement during the measurement[1]. Data were analyzed using a 2 (treatment) x 5 (days) mixed model ANOVA where animal served as a random variable. In addition, the average blood flow over each 3-minute interval was calculated and analyzed using the mixed model ANOVAs to determine if there were differences in blood flow over the testing interval. Fast Fourier transform (FFT) analyses were also performed to identify peaks in the Doppler signal. To perform these analyses, the data were divided into 25 sampling boxes for analyses. Therefore, the window length for Experiment 1 was 12 seconds/box and the window length for Experiment 2 was 36 seconds/box. Studies have shown that the higher frequency peak is representative of blood flow, and the lower frequency peak is usually indicative of the arterial pulsatile movement (Terada et al. 2007). Changes in the area of the curves obtained by FFT were analyzed using one way-ANOVAS to determine if these parameters changed as a result of vibration exposure. Statistical analyses were performed using Jmp 13.0.0 (SAS Institute, 2016). Differences with $p < 0.05$ were considered statistically significant.

Terada, K., Miyai, N., Maejima, Y., Sakaguchi, S., Tomura, T., Yoshimasu, K., Morioka, I., Miyashita, K. 2007. Laser Doppler imaging of skin blood flow for assessing peripheral vascular impairment in hand-arm vibration syndrome. *Ind Health* 45 (2):309-17.

[1] In a few files, there were not 20 points both before and after data that were out of range (less than 2 and greater than 100 perfusion units), so a running mean could not be calculated. These values appear as “0” in the data set, and were not used in the analyses.