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Original Article

Exposure to diesel engine exhausts and increase of urinary 8-hydroxy-2’-deoxyguanosine among Male tank maintenance workers in the Republic of Korea Army

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Abstract: This study was conducted to evaluate the exposure of diesel engine exhaust (DEE) and oxidative stress among tank maintenance workers in the Republic of Korea Army. Airborne concentrations of elemental carbon (EC), polycyclic aromatic hydrocarbons (PAHs), and metals were measured at two units. Urine analysis for 1-hydroxypyrene and 8-hydroxy-2’-deoxyguanosine (8-OHdG) was performed for tank maintenance workers from one unit (n=17). To compare the level of 8-OHdG, the analysis was performed in 17 unexposed controls. The airborne EC concentration was 8.6–24.3 μg/m^3 in indoor unit. EC was not detected in the outdoor unit. As for the PAHs, trace –0.0004 mg/m^3 of naphthalene was detected. I_{TWA} for 26 metals was calculated to be 0.009–0.027. The geometric mean urinary 1-hydroxypyrene was 0.08 μg/g creatinine. The geometric mean of 8-OHdG was 1.04 μg/g for the maintenance workers, while 0.45 μg/g for controls. The level of urinary 8-OHdG was significantly higher among maintenance workers in multivariate analysis. In conclusion, tank maintenance workers are exposed to various by-products from diesel engine combustion during work, and their level of oxidative stress marker was increased. Countermeasures for reducing hazardous substances in the military workplace are necessary.

Key words: Diesel engine exhaust, DEE, Army, Military Worker, Oxidative stress, 8-OHdG

Introduction

Various types of work and training are conducted in the military. In the Republic of Korea (ROK) Army, there are special working environments including various the use of firearms and tracking devices, but research on industrial hygiene in the army is mainly limited to noise[1]. Recently,

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in the field of occupational health, much effort is being made to identify and manage substances that have chronic and irreversible effects on humans. The International Agency for Research on Cancer has been updating the list of carcinogens by reviewing the literature. In 2012, there was sufficient evidence for a relationship between diesel engine exhaust (DEE) and lung cancer[2)].

DEE contains hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), inorganic carbon, heavy metals, sulfur compounds, and volatile organic compounds (VOCs) in particulate and gaseous forms[3, 4)]. Because it is a mixture, it is difficult to measure quantitatively and qualitatively. Indices such as carbon dioxide, total dust, inhalable dust, nitrogen oxides, and black carbon have been used to estimate its exposure; however, recently, the airborne elemental carbon (EC) concentration has been used as an exposure index[5, 6)].

Various theories have been suggested regarding the mechanism of carcinogenicity of DEE, including inducing oxidative stress through activation of the reactive oxygen species system, formation of bulky DNA adducts, DNA damage, and oxidation by *in vivo* and *in vitro* studies[7–9)]. Markers for estimating oxidative stress in the body include 8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde, and the FOX-2 assay[10)]. 8-OHdG reflects DNA damage and can be easily collected and analyzed in human urine[11)]. The association between DEE exposure and 8-OHdG was confirmed in animal experiments[12)]. Studies on 8-OHdG and various health conditions, including lung cancer[13)], are still being actively conducted.

The army uses many tracked vehicles with diesel-based engines, such as tanks. Accordingly, workers might be exposed to DEE during driving and maintaining military vehicles. However, there have been few studies on the exposure to DEE in the army. Therefore, this study performed work environment measurements and biological exposure index monitoring related to DEE in the army's tracked vehicle maintenance unit. Urinary 8-OHdG was selected as a marker of oxidative stress and it was compared with a control group to investigate whether oxidative stress is affected by exposure to DEE.

Materials and Methods

Sampling Location

The study was conducted on two tank maintenance units in the ROK Army. There are several tank maintenance units that are large enough to perform tank engine maintenance and commissioning, and they perform similar tasks with tank engine indoors and outdoors, depending on the environment of the unit. Work was done indoor and outdoor in each unit. Air samples in workplace were collected three times from two units. Since it is necessary to relatively assess the exposure of composite materials arising from diesel engine emission rather than exposure to specific metals and substances, a control sample of outdoor air was collected in a military unit free of maintenance work to comparison.

Work environment sampling was permitted only when tank maintenance and commissioning were performed, therefore, sampling was performed only during each working hour. In indoor tank maintenance unit, one tank was under engine maintenance. In outdoor tank maintenance unit, since two tanks were under maintenance, two samples were gathered. According to the worker's interview, engine commissioning is not performed daily, besides engine commission was performed for up to 90 min per day. Therefore, the work environment was simulated as engine commissioning continued for all 90 min and additional sampling was performed. Additionally, short term sampling was done for 15 min to assess peak exposure of elemental carbon, immediately after engine starting ('cold start') which is expected to have the most particulate matter emitted. To minimize disruption to work, personal sample collection could only be performed once in indoor maintenance unit. Personal sample was sampled within 30 cm from the respirator. For area sampling, sampling devices were installed at a 3 m from the bottom of the engine outlet and 1 m from the floor. The detailed location and shape of the maintenance unit is not described as it violates military security. Sampling was conducted from September to October 2020.

Elemental Carbon

Airborne EC was sampled and analyzed as an exposure index of DEE. The sampling was performed using high-temperature treated quartz filters (SKC 225-401, SKC, USA) of 37 mm diameter, connected to calibrated high-volume air sampler (GilAir plus, Gillian, USA) at a flow rate of 2–4 l. All samples were shielded from the light until analysis. Sample analysis was performed at Korea Research Institute of Standards and Science (Republic of Korea, Daejeon). Analysis was performed using Lab OC-EC Aerosol Analyzer (Sunset Laboratory Inc., USA, OR), which is a thermo-optical analyzer (flame ionization detector). Analysis was performed according to NIOSH 5040 protocol[14)]. A method for separating organic carbon/elemental carbon using a light transmission method at a maximum of 870°C was performed. The quartz filter sampled in each workplace was punched with 1.2 cm^2 and injected into the analyzer for analysis.

Polycyclic Amoratic Hydrocarbons

The sampling and analysis were performed according to NIOSH 5515 protocol[15]. Calibrated high- volume air sampler (GilAir Plus, Gillian, USA) at a flow rate of 2–4 l was directly connected to 37 mm diameter, 2.0 µm pore size polytetrafluoroethylene filter (SKC 225-17-07, SKC, USA) and washed XAD-2 (100 mg/50 mg) sorbent tube (SKC 226-30-04, SKC, USA) for collecting gas and particulate phases PAHs. The samples were collected at a flow rate of 2–4 l/min. All samples were shielded from the light until analysis. Samples PAHs were analyzed at 'Korean Institute for Occupational Health' Corporation (Gyeonggi-do, Republic of Korea). The PAH standard used was TCL PAH mix 2,000 µg/ml polynuclear aromatic hydrocarbon mix stock solution with each component in methylene chloride: benzene (1:1) (CRM 48905, Supelco, USA). Samples were analyzed by Gas chromatography–Mass spectrometry (GC-MS) (Clarus 600, Perkin Elmer, USA) with 30 m × 250 µm × 25 µm column (HP-5ms, Agilent, USA). Injector temperature of GC-MS was 290°C. This study analyzed sixteen priority PAHs listed by the US Environmental Protection Agency (EPA); Acenaphthene, Acenaphthylene, Anthracene, Benz [a] anthracene, Benzo [a] pyrene, Benzo [b] fluoranthene, Benzo [ghi] perylene, Benzo [k] fluoranthene, Chrysene, Dibenz [a, h] anthracene, Fluoranthene, Fluorene, Indeno [1, 2, 3-cd] pyrene, Naphthalene, Phenanthrene, and Pyrene.

Metals

The metal samples from the air were collected using a mixed cellulose ester (MCE) filter (SKC 225-5, SKC, USA) with a pore size of 0.8 µm and a diameter of 37 mm, by calibrated high-volume air sampler (GilAir plus, Gillian, USA) in a flow rate of 2–4 l. Metals were analyzed at Smartive Corporation (Seoul, Republic of Korea). As a standard solution, add 100 µl of a metal mixed standard stock solution (10 mg/l) and 10 µl of individual standard solutions of Fe, Zn, Na and K (100 mg/l) in a 10 ml volumetric flask, and diluted with distilled water.

10 µg/ml of ICP-MS Multi-Element Solution 2 (SPEX Certiprep, USA) was used as standard solution of Vanadium, Chromium, Manganese, Cobald, Nickel, Copper, Arsenic, Selenium, Cadmium, Lead, Beryllium, Barium, Magnesium, Sodium, Aluminum, Calcium, Iron and Silver. 10 µg/ml ICP-MS Multi-Element Solution 3 (SPEX Certiprep, USA) for Antimony and Tin. 10 µg/ml of ICP-MS Multi-Element Solution 4 (SPEX Certiprep, USA) is used for Molybdenum, Titanium, Zirconium and Tungsten. 1,000 µg/ml Potassium (SPEX Certiprep, USA) and 1,000 µg/ml Zinc (SPEX Certiprep, USA) was used for each metal. The solution was used for analysis by preparing a standard material for a calibration curve at the following concentration using the serial dilution method. After transferring MCE filters to a 50 ml conical tube, 10 ml of 5% HNO_3 solution was filled. Samples was mixed with a shaker at 100 rpm for 1 hour, then diluted to 1 ml of sample in 4 ml of distilled water. Inductively Coupled Plasma-Mass Spectrometer (NexION200B, Perkin Elmer, USA) was used to analyze the sample solutions. The peak-hopping mode was used. Quantitative analysis was performed on twenty-six metals: Vanadium, Chromium, Manganese, Cobalt, Nickel, Copper, Arsenic, Selenium, Molybdenum, Cadmium, Antimony, Lead, Beryllium, Barium, Magnesium, Sodium, Aluminum, Potassium, Calcium, Titanium, Iron, Zinc, Zirconium, Silver, Tin and Tungsten.

For risk assessment and comparison at each measurement site considering threshold limit value of time-weighted average (TWA), an exposure index (I_{TWA}) for metals for which TWA is set was calculated and presented, as the formula recommended by the ACGIH ($I_{TWA}=C_1/TLV_1 + C_2/TLV_2 + ... + C_n/TLV_n$, with C_n the atmospheric concentration of component n and TLV_n its TLV-TWA).

Study Subjects

Urinary analysis was done for 17 tank maintenance workers in a unit with indoor maintenance works. To compare the level of oxidative stress marker with the non-exposed workers, urinary 8-OHdG was analyzed among the control group. The non-exposed control group consisted of 17 soldiers or army civilians from other military unit without experience in tank maintenance or tank driving, matched the age (± 5 yr) and smoking status.

Authors obtained approval from the Armed Force Medical Command Institutional Review Board (AFMC IRB) (IRB No. AFMC-2007-IRB-20-007). Written informed consent was obtained from all participants. This study was conducted in accordance with the ethical standards of the institutional research committee and/or national research committee with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Urine Sampling and Analysis

The urine samples of the workers and control group were collected in a 15 ml conical tube at the end of the morning shift on Thursday morning. The collected urine was immediately shielded from light, then stored in lower than −20°C until analysis. Baseline characteristics were obtained using questionnaires.

Urinary creatinine was measured to correct the urine concentration since the analysis was performed using spot urine instead of 24-hour urine. Creatinine was measured using Kinetic colorimetric assay, according to Jaffe's method with Cobas 8000 C702 (Roche, Germany). CRE2 (Roche, Germany) was used for reagent.

1-hydroxypyrene (1-OHP) was evaluated as the biological exposure index of PAHs. The analysis was performed at Smartive Corporation (Seoul, Republic of Korea). The standard solution used was 98% 1-hydroxypyrene solution (Aldrich-361518, Sigma-Aldrich, USA). The internal standard (20 ul), 1 mL of sodium acetate buffer and 0.5 M and 20 µl of β-glucuronidase enzyme were added to 5,000 µl urine and the mixture was incubated at 37°C for 16 h. Then, 4,000 µl of n-hexane was added. The solution of hydrolyzed urine and n-hexane was mixed at 180 rpm for 30 min. The mixture was centrifuged at room temperature (3,000 rpm) for 20 min to separate the metabolite. The task was repeated once more. The incubated mixture was evaporated under nitrogen, then re-dissolved in 100 µl N, O - Bis (trimethylsilyl) trifluoroacetamide. It was vortexed for 15 sec and incubated for 45 min at 90°C and cooled for 5 min. Finally, 2 µl of the final supernatant was injected into GC-MS (Clarus 680 T, PerkinElmer, USA). The injection was conducted in the splitless mode with 280°C injector temperature. 30 m x 0.25 mm x 250 µg column (HP-5MS, Agilent, USA) was used. Helium gas was used as the carrier gas with the constant flow rate of 1.1 ml/min. For the mass spectrometry, electron impact ionization mode with 70 eV was used.

8-OHdG was analyzed at H Lab Plus Corporation (Seoul, Republic of Korea). Urinary 8-OHdG was measured using a competitive enzyme-linked immunosorbent assay (ELISA) kit (IM-KOGHS 040914E, Japan Institute for the Control of Aging, Japan), and the procedure was conducted according to instructions ("Highly Sensitive 8-OHdG Check") given by the manufacturer. The amount of 8-OHdG in each subject was calculated, by comparing with the standard curve produced by 0.125–10 ng/ml of 8-OHdG. The limit of detection (LOD) of 8-OHdG was 0.1 ng/ml.

Statistical analysis

Demographic characteristics of subjects and the results of urine analysis were described. Each characteristic was compared statistically. Mann-Whitney U test and Fisher's exact test was performed for continuous and categorical values to compare baseline characteristics of both groups. Univariate analysis for urinary creatinine and urinary 8-OHdG, for both non-standardized and standardized by creatinine, was performed to compare exposed and control groups by Mann-Whitney U test. Multivariate analysis was performed to comparing two groups with adjusting confounders. Multiple linear regression was performed. To analyze the right-skewed data, the natural log transformation was done for 8-OHdG. Values below the LOD were replaced by LOD/2. For the first model (Model 1), *ln 8-OHdG* was selected as the dependent variable, and independent variables was selected by a stepwise method. In Model 2, variables known to be related to 8-OHdG in previous studies were added. Alcohol consumption and age was added as covariate. One maintenance worker who did not respond to the alcohol drinking questionnaire was replaced with 'yes', which was more frequent in the alcohol consumption variable in the worker group. For one missing data of drinking questionnaire, substitution of both 'yes' and 'no' were applied, but the effect on the coefficient of 'working status' variable or adjusted R square was negligible. The statistical significance level was defined as $p<0.05$. R project version 3.6.3 was used.

Results

Elemental Carbon

EC was not detected in the general environment and the unit where maintenance was done outdoors. EC was detected in the unit where the maintenance was performed indoors. EC levels of the indoor maintenance unit were 8.6 µg/m^3 for area samples and 9.1 µg/m^3 for personal samples. In the simulation of 90 min engine commissioning, measured on the other day, the levels of airborne EC were 18.8 µg/m^3. The airborne EC concentration for 15 minutes immediately after engine start was 24.3 µg/m3 (Table 1).

Polycyclic Aromatic Hydrocarbons

PAHs were not detected in samples collected in a general atmospheric environment. In the PAHs' measurement of the maintenance unit, only naphthalene was detected as trace in outdoor maintenance unit and indoor maintenance unit. In a simulated situation, 0.0004 mg/m^3 of naphthalene was detected. Other PAHs were not detected (Table 1).

Metals

The airborne concentrations of metals measured in the working are shown in Table 2. For comparison, airborne concentrations of metals in general environment are also

Table 1. Airborne elemental carbon and polycyclic aromatic hydrocarbons concentrations for each working environment

				Elemental carbon			Polycyclic Aromatic Hydrocarbons		
Sample	Temperature (°C)	Relative Humidity (%)	Description	Air volume (l)	Sampling time (Minutes)	Airborne Elemental carbon (μg/m^3)	Air volume (l)	Sampling time (Minutes)	Airborne Polycyclic Aromatic Hydrocarbons (mg/m^3)
Control sample (general atmosphere)	15.1	29.4	Area sample	234.8	60	N.D.	450.0	150	N.D.
Indoor maintenance unit	22.7	55.8	Area sample	278.7	140	8.6	284.5	140	Naphthalene: trace
Indoor maintenance unit			Personal breathing zone	282.0	140	9.1	284.3	140	N.D.
Outdoor maintenance unit	17.2	41.9	Area sample (Tank 1)	479.9	120	N.D.	480.8	120	Naphthalene: trace
Outdoor maintenance unit			Area sample (Tank 2)	481.9	120	N.D.	475.2	120	Naphthalene: trace
Indoor maintenance unit, simulated	18.3	19.1	Area sample	360.0	90	18.8	270.1	90	Naphthalene: 0.0004
Indoor maintenance unit, simulated			Area sample (Short term sampling for immediately after engine starting)	59.9	15	24.3	-	-	-

N.D. = Not detected
Note 1: The value present calculated airborne concentration of each sample.
Note 2: In the simulated situation, sampling was performed with engine commissioning lasting 90 min.

Table 2. Airborne concentrations of metals for each working environment

	General environment (area)	Indoor maintenance unit (area)	Outdoor maintenance unit (area)	Indoor maintenance unit, simulated (area)	TLV-TWA
Temperature (°C)	15.1	22.7	17.2	18.3	
Relative humidity (%)	29.4	55.8	41.9	19.1	
Air volume (l)	611.1	290.2	486.7	364.10	-
Sampling time (min)	150	140	120	90	-
Be (mg/m^3)	N.D.	0.00002	0.00000	0.00000	0.002
Ba (mg/m^3)	0.00001	N.D.	0.00004	0.00042	0.5
Mg (mg/m^3)	0.00008	N.D.	0.00012	0.00257	10
Na (mg/m^3)	0.00109	N.D.	0.00041	0.00628	C2
Al (mg/m^3)	0.00003	N.D.	0.00008	0.00146	10
K (mg/m^3)	0.00080	N.D.	0.00033	0.00377	C2
Ca (mg/m^3)	0.00021	0.00006	0.00030	0.00873	2
Ti (mg/m^3)	N.D.	0.00000	N.D.	0.00014	10
V (mg/m^3)	0.00000	N.D.	0.00000	0.00001	0.05
Cr (mg/m^3)	0.00000	N.D.	N.D.	0.00001	0.5
Mn (mg/m^3)	0.00001	0.00001	0.00005	0.00021	1
Fe (mg/m^3)	N.D.	0.00007	N.D.	0.00069	1
Co (mg/m^3)	0.00000	N.D.	0.00000	0.00001	0.02
Ni (mg/m^3)	0.00001	N.D.	0.00000	0.00000	1
Cu (mg/m^3)	0.00006	0.00102	0.00036	0.00046	1
Zn (mg/m^3)	0.00005	N.D.	0.00015	0.00685	5
As (mg/m^3)	0.00000	N.D.	0.00000	0.00000	0.01
Mo (mg/m^3)	0.00000	N.D.	0.00000	0.00007	10
Se (mg/m^3)	N.D.	N.D.	N.D.	N.D.	0.2
Zr (mg/m^3)	0.00000	N.D.	0.00000	0.00000	5
Ag (mg/m^3)	0.00000	N.D.	0.00000	0.00000	0.1
Cd (mg/m^3)	0.00000	0.00000	0.00000	0.00010	0.01
Sn (mg/m^3)	0.00000	0.00000	0.00000	0.00011	2
Sb (mg/m^3)	0.00001	0.00011	0.00006	0.00008	0.5
W (mg/m^3)	N.D.	N.D.	N.D.	0.00000	1
Pb (mg/m^3)	0.00009	0.00027	0.00041	0.00041	0.05
I_{TWA}	0.00200	0.01700	0.00900	0.02700	1

N.D. = Not detected.

Note: $I_{TWA} = C1/TLV1 + C2/TLV2 + \ldots + Cn/TLV_n$, *with* C_n *the atmospheric concentration of component n and* TLV_n *its TLV-TWA.*

Note 2: In the simulated situation, sampling was performed with engine commissioning lasting 90 min.

presented. The exposure index considering the exposure standard was in the order of outdoor maintenance unit (0.009), indoor maintenance unit (0.017), and indoor unit with engine commissioning simulation (0.027).

Table 3. The general characteristics of study subjects

		Tank maintenance workers (n=17)	Non-exposed control group (n=17)	*p*-value
Age (yr)		42.0 ± 10.2	41.2 ± 11.4	0.68
Job class	Soldier	5 (29.4%)	10 (58.8%)	0.08
	Army civilian	12 (70.6%)	7 (41.2%)	
Tank maintenance experience (yr)	None	0 (0.0%)	17 (100%)	< 0.001
	<1	0 (0.0%)	0 (0.0%)	
	1–3	3 (17.7%)	0 (0.0%)	
	4–5	1 (5.9%)	0 (0.0%)	
	6–9	2 (11.8%)	0 (0.0%)	
	>9	11 (64.7%)	0 (0.0%)	
Smoking status	Yes	3 (17.7%)	3 (17.7%)	1.00
	No	14 (82.4%)	14 (82.4%)	
Alcohol consumption	Yes	10 (58.8%)	8 (47.1%)	0.40
	No	6 (35.3%)	9 (52.9%)	
	Non-responder	1 (5.9%)	0 (0.0%)	

Note: A continuous value is presented as mean ± SD, compared by Mann-Whitney U test. Categorical values are compared by Fisher's exact test.

Demographic Characteristics of Study Subjects

Analysis was conducted on 17 workers in the unit performing indoor maintenance work and 17 controls matched by age and smoking status. All subjects including controls were male. The mean age is 42.0 ± 10.2 yr for the maintenance workers and 41.2 ± 11.4 yr for controls (p=0.68). Maintenance worker group consists of 5 soldiers (29.4%) and 12 army civilians (70.6%). Control group includes 10 soldiers (58.8%) and 7 army civilians (41.2%) (p=0.08). Three (17.7%) in each group were smokers (p=1.00). Ten (58.8%) were drink alcohols regularly in tank maintenance workers while 8 (47.1%) in control group (p<0.40) (Table 3).

Urine Sample Analysis

The geometric mean of urinary 1-OHP was 0.08 µg/g creatinine in maintenance workers. The geometric mean of urinary 8-OHdG 0.91 µg/l in maintenance workers and 0.58 µg/l in non-exposed control group (p=0.43). 8-OHdG, standardized by creatinine, was 1.04 µg/g in maintenance workers and 0.45 µg/g in control group (p=0.12). The geometric mean of urinary creatinine was 86.98mg/dl in maintenance workers and 129.67 mg/dl in control group (p=0.14) (Table 4).

Multivariate Analysis

The multivariate analysis of 8-OHdG is described in Table 5. In Model 1, a multiple linear regression was performed with ln 8-OHdG as a dependent variable, and creatinine and smoking status were adjusted as covariate in addition to working status. Maintenance workers showed significantly elevated urinary 8-OHdG/creatinine compared to control group (p<0.01). In Model 2, multiple linear regression model with creatinine, smoking status, age, alcohol consumption as independent variables in addition to working status. Maintenance workers showed significant elevation of urinary 8-OHdG compared to control group (p<0.05).

Table 4. Result of urinary biomarkers among the maintenance workers and unexposed control group

	Total (n=34)	Tank maintenance workers (n=17)	Non-exposed control group (n=17)	*p*-value
Urinary 8-OHdG (μg/l)	0.73 (7.97)	0.91 (8.69)	0.58 (7.62)	0.43
Urinary 8-OHdG/creatinine (μg/g creatinine)	0.68 (5.55)	1.04 (5.98)	0.45 (4.82)	0.12
Urinary creatinine (mg/dl)	106.20 (1.92)	86.98 (2.17)	129.67 (1.55)	0.14
Urinary 1-OHP (μg/g creatinine)	-	0.08 (1.89)	-	-

Note: The values are presented as geometric mean (geometric SD). Mann-Whitney U test was performed to compare continuous variables.

Table 5. Multiple linear regression of urinary 8-hydroxy-2'-deoxyguanosine among participants

	Independent variables included in regression model	Regression coefficient (95% CI)	Adjusted r^2
Model 1	Tank maintenance workers (compared to controls)	1.2 (0.2, 2.2) *	0.56
	Creatinine (mg/dl)	0.0 (0.0, 0.0) **	
	Smoking	−1.0 (−2.2, 0.3)	
	Intercept	−3.9 (−5.3, −2.5) **	
Model 2	Tank maintenance workers (compared to controls)	1.2 (0.2, 2.3) *	0.54
	Creatinine (mg/dl)	0.0 (0.0, 0.0) **	
	Smoking	−1.0 (−2.4, 0.3)	
	Alcohol drinking	−0.0 (−1.1, 1.1)	
	Age	0.0 (-0.0, 0.1)	
	Intercept	−4.7 (−7.1, −2.2) **	

*: $p<0.05$; **: $p<0.001$

Note 1: In both Model, ln 8-OHdG (μg/l) was entered as dependent variable.

Note 2: Regression coefficient and confidence interval represent the mean change of natural log transformed dependent variable per 1 unit of the increased/decreased corresponding covariate; ln 8-OHdG/creatinine (μg/g) and ln 8-OHdG (μ/l) need to be exponentiated to get 8-OHdG/creatinine (μg/g) and 8-OHdG (μg/l) for each model.

Discussion

EC was detected at 8.6–9.1 μg/m^3 in indoor, but not outdoor, units. In a simulation where engine commissioning was continuously performed for 90 min, EC was detected at 18.8 μg/m^3 and 24.3 μg/m^3 in two area samples. PAHs and metals were detected at low concentrations compared with the threshold limit values. The I_{TWA} of metals was in the order of simulation (0.027) > indoor maintenance (0.017) > outdoor maintenance (0.009). The geometric mean of urinary 1-OHP from tank maintenance workers was 0.08 μg/g creatinine. Multivariate analysis showed a significant elevation of urinary 8-OHdG among maintenance workers compared with the control group.

Due to their high efficiency and output, diesel engines are used for the operation of various vehicles within the military, and thus staff are continuously exposed to them when operating and maintaining the vehicles. As a specific substance detected only in diesel exhaust emissions, guidelines for the sampling and assessment of exposure using EC are widely used. Other studies using airborne EC as a DEE exposure indicator reported levels of 7.9 μg/m^3 in a forklift workplace[16)] and 15.5 μg/m^3 in a bus maintenance workplace[17)]. Mining is exposed to relatively high DEE, and previous studies have reported EC concentrations of 148–637 μg/m^3 [2)]. The airborne EC concentration measured in the indoor tank maintenance unit was 8.6–24.3 μg/m^3 depending on the work situation, which is comparable to the exposure level during forklift and bus maintenance work, and lower than the TWA exposure limit suggested by the European Union (50 μg/m^3)[18)]. The 8-hour time-weighted concentration would be lower, assuming an 8-hour working hour, including the time without engine commissioning because the work and sampling were performed for less than 8 hours in this study. However, because it is difficult to identify safe cut-off points and thresholds due to carcinogens, several organizations have recently suggested that a lower TWA threshold limit should be applied. In Canada's CAREX report, it was suggested that the air EC level should be lowered to 5 μg/m^3 in workplaces[19)]. Conversely, when working outdoors, the concentration of EC is below the detection limit, suggesting the risk to workers is relatively low. To minimize effects on the health of workers, measures to reduce dust exposure such as improved ventilation in the workplace should be continuously considered, with a special focus on indoor maintenance units.

DEE contains various carcinogens including VOC, PAHs, and metals[20)]. Among these components, the theory that PAHs, which have been identified as carcinogenic, play an important role in carcinogenicity is widely accepted[21)]. Nevertheless, Venkataraman *et al.*[22)] showed that the concentration of PAHs collected was less than 1% of the EC. Lee[23)] reported no PAHs were detected in a quantitative and qualitative analysis of diesel engine combustion particles. In this study, the sampling of EC and PAHs (gaseous and particulate forms) was performed simultaneously, but only naphthalene was detected for tracing –0.004 mg/m^3. According to studies conducted in the Republic of Korea, the mean urinary 1-OHP levels of workers exposed to relatively high PAHs were 0.28 μg/g creatinine among miners[24)], 0.36 μg/g creatinine among shipyard workers[25)], and 9.85 μg/g creatinine among workers using coal tar paint[26)]. The geometric mean of urinary 1-OHP levels in tank maintenance workers was 0.08 μg/g creatinine, comparable with that of the non-exposed control group in another study in Korea (0.10 μg/g)[27)]. These results suggest that the concentration of PAHs co-exposed with DEE in maintenance units cannot be considered high. Further research on the association of PAHs with DEE is needed.

Metals contained in catalysts and engine oils can be discharged during combustion. Metals are also present in DEE particles[20, 28, 29)]. In this study, metals, such as Cu and Zn, discharged during engine combustion through engine oils and catalysts were higher than those in atmospheric samples, and the I_{TWA} of the metals in the sample were in the order of the highest concentration of EC. Furthermore, exposure to engine combustion products can also affect metal exposure. Compared with TLV-TWA, it is a maximum level of 2.7%; therefore, the levels of metals are unlikely to cause acute poisoning. However, it was reported that heavy metals, even those in the air, continuously accumulate in the body inducing chronic diseases such as diabetes[30)] and cardiovascular diseases[31)]. Considering it is recommended to minimize exposure to heavy metals, even in daily life, it is necessary to investigate whether trace amounts of heavy metals can affect health, and to establish measures to reduce exposure.

Lung cancer is a disease in which DEE is a causal factor; however, the prevalence of malignant diseases is not high enough to assess the risk of carcinogen exposure in specific small groups. In this study, 8-OHdG, a marker that can identify oxidative stress, which plays an important role in the onset of carcinogenesis, was analyzed in urine samples to assess its effects on the health of tank maintenance workers. Duan *et al.*[32)] reported that the level of 8-OHdG between diesel engine testers and the control group was not statistically different. Conversely, Lee *et al.*[33)] reported that diesel engine inspectors exposed to diesel engines had higher 8-OHdG levels than the control group. In this study, soldiers and army civilians of similar age and smoking status were selected as the non-exposed control group to minimize confounding effects, including health worker effects, and statistically significant differences were shown in the multivariate models adjusted for confounding variables.

For the multivariate analysis, *ln 8-OHdG* was selected as a dependent variable instead of performing the standardization of urine concentration by dividing creatinine. Therefore, creatinine was added as a covariate of the statistical model with a non-standardized-dependent variable. This method is widely used to correct concentrations while statistically analyzing urine biomarkers, which have a relationship with creatinine, including 8-OHdG[34–36)]. In a previous study, 8-OHdG and creatinine were affected by muscle exercise[37)]. In Model 2, potent variables that can affect 8-OHdG were selected as independent variables including age[38)] and alcohol consumption[39)]. As a result, the biomarker levels of oxidative stress in tank maintenance workers were significantly higher than that of the non-exposed group in both linear regression models, indicating exposure to DEE contributes to oxidative stress.

In this study, we obtained information on smoking status, alcohol consumption, and age of subjects, which might correlate with 8-OHdG levels, which can be affected by numerous factors including body mass index[40)], cardiovascular disease[41)], and allergic disease[42)]. The lack of collecting and adjusting for anthropometric data such as body mass index (BMI), medical history, and other laboratory data was a study limitation when clarifying the elevation of 8-OHdG levels in tank maintenance workers. Moreover, biomarker analysis was performed in a specific unit and the sample size was relatively small; therefore, the result does not represent all tank maintenance workers in the military.

Because tank maintenance consists of various atypical and irregular tasks, another limitation was the inability to collect airborne and human derivative samples from various situations. Although several factors, such as engine aging and ventilation status of the unit, affect the emission and exposure of DEE, sampling was not performed sufficiently to assess diverse work environments due to the low accessibility of military units. Furthermore, it was difficult to present and compare the 8-hour time weighted average airborne concentrations of EC, PAHs, and metals due to irregular working hours and tasks, which were related to the inconsistency of sampling time and volume.

Although not described in the results, NO_2 was measured at an indoor maintenance facility once with a gas detection tube (No. 9L, GASTEC, Japan). The level was about 0.5 ppm during engine commissioning, which was considerably higher than that of the general atmosphere or indoor air. Another study limitation was the lack of a detailed and accurate exposure evaluation for NO2 using sensors or collection. Additional exposure assessments of NO_2, SO_2, and particulate matter (mass and size distribution) that can be emitted in diesel engine emissions, which are related to human DNA oxidative stress, might help interpret the 8-OHdG data.

Despite the increasing interest in DEE, studies on exposure to DEE and PAHs within the military, especially among tank maintenance workers, are uncommon because access to military units is low for security reasons. The assessment of tank maintenance workers' exposure to DEE, PAHs, and metals is rare but essential for the risk assessment of military workers. Regarding biomarkers, various studies have reported a wide range of standard values for 8-OHdG due to the different methods used[40)], and the specific nature of groups consisting of male army members; therefore, direct comparisons with reference values presented from previous studies if difficult. It is meaningful that the control group was recruited from the army and was matched by age and smoking status, and that the exposed group showed a significant increase in oxidative stress markers compared with the control group, even when a relatively small sample size was used.

In conclusion, the exposure status of EC, PAHs, and metals was assessed and confirmed in tank maintenance unit workers. The level of urinary 8-OHdG in maintenance workers exposed to carcinogenic DEE was significantly higher compared with the control group. This suggests tank maintenance workers are exposed to various substances related to DNA oxidative damage. The risk and carcinogenicity of various substances, including DEE, that workers are exposed to during vehicle maintenance should be continuously assessed in the army.

Disclaimer

The opinions or assertions contained here are the private views of the authors and are not to be interpreted as official views of the organization.

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Conflicts of Interest

All authors have no conflict of interest including financial or consultant, institutional and other relationships in this study.

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