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Heart Rate Variability Analysis in Workers Exposed to Methyl Bromide as a Quarantine Treatment

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Objective: To determine whether methyl bromide (MB) fumigation work for plants imported in Korea affects workers' health by assessing heart rate variability (HRV). **Methods:** We measured HRV indices (TP, VLF, LF, HF, HR, SDNN, pNN50 and HRV-index) and urinary bromide ion (Br^-) in 62 fumigators (study group) and 34 inspectors (the control group) before and after fumigation work. In addition, the relationship between Br^- concentration and HRV indices was analysed. **Results:** The fumigators' post-work HRV indices and Br^- level were changed compared with their pre-work values ($P < 0.001$). Conversely, inspectors' values were not shown a difference before and after work. The HRV indices in all subjects were negatively correlated with the Br^- levels ($P < 0.05$). **Conclusions:** Fumigators with high Br^- concentrations and low post-work HRV indices following MB fumigation work experienced adverse effects on their autonomic nervous systems.

Keywords: autonomic nervous system, fumigator, heart rate variability, methyl bromide, quarantine inspector, urinary bromide ion

Since methyl bromide (MB) efficacy on pests was first reported in 1930, MB has been used globally as a quarantine treatment for plants in trade and to disinfect insects or pathogens in structures or in soils.[1–3] However, MB was listed as an ozone-depleting substance under the Montreal Protocol of the United Nations Environment Programme (UNEP) in 1992.[4] Its use has rapidly decreased since then, and the replacement of MB has been ongoing worldwide.[4,5]

MB is a highly toxic pesticide that, when used as a gas, causes various toxicities to people working with it.[6–11] The primary intake of MB is via respiration. Inhaled MB is absorbed into the blood through the lungs. MB reactivity results in glutathione depletion in various organs such as liver, kidneys, lungs and brain.[12,13] Once absorbed into the human body, MB causes neurological disorders, lung damage, visual disturbance, genotoxicity, cytotoxicity, and various toxic activities.[6–8,14] Neurotoxicity in particular is known to be the most prevalent effect of exposure in animals as well as humans.[15] Interestingly, vomiting, nausea, tremors, convulsions and dizziness were included in the reported symptoms of MB intoxication; all these symptoms were associated with acetylcholinesterase, a neurotransmitter in the autonomic nervous system, which is inhibited by MB metabolites (eg, methyl phosphate) in the cells.[16]

Bromide ions in the blood, serum and urine have been used as biomarkers to determine whether workers are exposed to MB.[12,17,18] Among the biomarkers, the urinary bromide ion (Br^-) level in workers exposed to MB is detected to be higher than that in normal people, but the symptoms of poisoning are not always observed.[17,19] Why workers do not show symptoms of poisoning even when high Br^- concentrations are present in their urine is unclear. One possible reason is that the symptom onset differs between individuals.[19]

Heart rate variability (HRV) is defined as a change in the time interval between heartbeats. The HRV has been widely used as a non-invasive clinical prognostic method to diagnose autonomic dysfunction, ever since the standard method was developed in 1996 by an international professional committee composed of the members of the European Cardiology Society and the members of the North American Coordination and Electrophysiology Society.[20] HRV indices were primarily used to assess overall autonomic function responding to a variety of acute and chronic stress factors.[21] In particular, these indices feature distinct ageing trends that increase or decrease linearly with increasing age and are useful for assessing the functional degradation (weakness) of autonomic nerves.[22,23] This measurement can allow fumigation workers to assess the adverse effects on their health through the quantification of functional ageing (or deterioration) levels before and after work, even in the absence of disease.[24] The authors of this study have shown that the HRV indices of total power (TP), high frequency (HF), and low frequency (LF), which were measured with the same instruments and protocols as those used in this study, significantly increased after exercise, resulting in a positive effect.[25]

The most prevalent effects of MB exposure have long been known in neurotoxicity and include the functional degradation of the autonomic nervous system. The HRV indices reflect on the status of the autonomic nervous system. With this background measurement, the workers' HRVs were used to determine whether occupational exposure to MB affected the health of asymptotic workers. In addition, the relations between the Br^- levels, a traditional biomarker, and the HRV indices of the pre- and post-work day findings were assessed in all the subjects.

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The subjects provided written informed consent for participation and analysis of all collected data. The study was approved by the Dong-A University Institutional Review Board, Korea (IRB number: 2-1040709-AB-N-01-201806-BR-004-04).

All the data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Clinical significance: Dramatically reduced heart rate variability in fumigators exposed to methyl bromide implied that they were functionally aged, degenerated and possibly develop metabolic syndrome including higher levels of uric acid, non-HDL-cholesterol, body mass index or blood pressure. HRV indices could be useful for the early prevention of autonomic dysfunction by fumigation work

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METHODS

Subjects

The study group was involved in controlling pests with MB, all companies being registered with the Animal and Plant Quarantine Agency (APQA).[26] The control group consisted of quarantine inspectors who oversaw the MB work, all of whom were public officials in the Youngnam Regional Office (located in Busan, Korea) of the APQA.

Between February 2018 and August 2019, from a pool of 76 fumigators and 36 inspectors affiliated with Busan at the Youngnam Regional office of the APQA, a study group of 62, and a control group of 34 individuals were recruited. On the day of the MB fumigation work, the HRV indices were measured in one or two subjects per group. The measurements were performed from September to November in 2018 and from March to June in 2019. The two seasons in the fall of 2018 and the spring of 2019 were similar in temperatures and humidity, and both groups had the same working place and time. Prior to study participation, the written consent of all subjects was acquired. This observational study was conducted as part of the plant quarantine technology development programme of the APQA and approved by the Institutional Review Board (DONG-A UNIVERSITY INSTITUTIONAL REVIEW BOARD, IRB number: 2-1040709-AB-N-01-201806-BR-004-04). All participants were screened for HRV by registered clinical nurses, and the bromine ion concentration in urine was monitored before and after fumigation work.

Tasks of fumigation workers include; sealing containers, measuring MB concentrations, applying MB, and exhausting MB. Goods treated by MB include imported fruits in warehouses and wood in lumber yards. The quarantine inspectors were subject to similar working conditions as the fumigators; they monitored the MB fumigation and examined plants for pests that were imported or exported. However, since the inspectors only supervised the fumigation process and controlled the gas concentrations during the fumigation, they were less likely to be directly exposed to MB than the fumigation workers. Table S1, http://links.lww.com/JOM/A835 shows the work and HRV measurement processes of the subjects.

Heart Rate Variability Measurements

The HRV indices of all the subjects were measured in the morning before work and in the afternoon after work in an isolated area of Youngnam Regional office on the day of the MB fumigation work. All subjects were seated in a relaxed state, with their eyes open, and their HRVs were measured for 5 minutes by photoplethysmography (PPG). A PPG sensor (Model: ubpulse T1 (Pulse Analyzer, KFDA Certification No.11-1296)), LAXTHA Inc., Korea) was applied to the left index finger; PPG sensors are comparable to electrocardiograms in terms of the quality of their HRV signals.[27]

The hands attached to the sensors were placed on a table at heart level with rubber bands, and the subjects were cautioned to not take deep or abdominal breaths during the measurement to prevent respiratory sinus arrhythmia (RSA).[28] Using the device, the PPG, second derivative PPG (SDPPG), and pulse signals were measured simultaneously. The operator minimized data corruption by monitoring the signal and assured that it is not distorted by finger movements or shaking of the sensor. None of the relaxed-state, HRV data were rejected due to artifacts in this study.

Biomarkers and Calculations of Heart Rate Variability

The HRV indices used in this study were HR, SDNN, HRV-index, pNN50, VLF, HF, LF, and TP, and the analysis was conducted

TABLE 1. Description of HRV Biomarkers

Biomarkers	Full Name	Unit	Description	Reference Range*	Health Direction†
HF (log scale)	High frequency	ms^2	Power in the high frequency range (0.15-0.40 Hz) of normal-to-normal intervals as a relatively fast rhythm range. It reflects parasympathetic activity.	5.07 ± 0.96	↑
LF (log scale)	Low frequency	ms^2	Power in the low frequency range (0.04–0.15 Hz) of normal-to-normal intervals as a relatively low frequency range. It reflects sympathetic activity.	5.11 ± 1.00	↑
VLF (log scale)	Very low frequency	ms^2	Power in the very low frequency range (0.003–0.04 Hz) of NN intervals	5.73 ± 0.81	↑.
TP (log scale)	Total power	ms^2	Total power of NN intervals is the sum of the powers of all the regions of HF, LF and VLF	6.58 ± 0.78	↑
HR (bpm)	Heart Rate	bpm	Heart beats per minute	73.18 ± 8.66	The higher the HR, the stronger or weaker the health
HRV-Index	HRV triangular index	–	The geometric characteristics of the probability distributions from NN intervals. The integral of the density distribution (ie, the number of all NN intervals) divided by the maximum density distribution.	9.93 ± 3.05	↑
SDNN	Standard deviation of the NN interval	ms	Standard deviation of all normal-to-normal intervals	31.26 ± 11.29	↑
pNN50 (>50 ms)	Proportion of NN intervals greater than 50 ms	%	The percentage of the points having the distance between neighbouring points of 50 ms or more in the entire normal-to-normal intervals	24.55 ± 14.86	↑

*Reference ranges are expressed as the mean ± SD. From December 2007 to March 2008, a total of 300 healthy participants (male 150, female 150) aged 19–69 y were recruited at the Clinical Trial Center of Asan Medical Center (AMC) located in Seoul, Korea, and they were evaluated with the same instruments and protocols as those used in this study. The Institutional Review Board (ASAN MEDICAL CENTER INSTITUTIONAL REVIEW BOARD, IRB number: AMC-IRB-2007-0305) approved the study protocol. The details are shown in Figure S1, http://links.lww.com/JOM/A835 and Table S2, http://links.lww.com/JOM/A835 in supplementary materials.

†An arrow (↑) that points up means that the higher HRV indices, the younger and healthier was the autonomic nervous system.

based on international standard methods.[20] The descriptions of the HRV indices are summarized in Table 1.

Analysis of Bromide Ions in Urine

For the collection of the urine samples, all subjects had been familiarized with the urine collection procedure to prevent contamination. The procedure is as follows: after intentionally not collecting the first extracorporeal urine, more than 10 mL of intermediate urine is to be collected using a dedicated urine cup (Qorpak PLC-03701 natural polypropylene jar with 58–400 white polypropylene unlined cap 120 m) and stored at 4 °C to await transfer to a separate institution for testing. In preparation for urinary Br^- concentration analysis, five millilitres of each sample was allocated into labeled conical tubes (CELLTREAT 229412 centrifuge tube, 15 mL, polypropylene) and stored at −80 °C.

Br^- concentration analysis was performed utilizing a high-performance liquid chromatography/inductively coupled plasma mass spectrometer (HPLC/ICP-MS, HPLC; Agilent Technologies 1260 series/Agilent Technologies 7700 series, ICP-MS; Agilent Technologies, CA, USA). Technical details of the analyses are described in Park et al.[29]

Statistical Analysis

An independent *t* test or Fisher's exact test for continuous and categorical variables was utilized to investigate the differences in participants' baseline characteristics. A paired *t* test was performed to determine whether the HRV indices and Br^- concentrations, which are presented as the means ± SD of each group, were different before and after fumigation work within each of the groups. The HRV indices and urinary Br^- concentrations were analysed with linear mixed effects models to examine their trajectories depending on factors such as timing, group, age, gender, duration of work, gas mask use and interaction between timing and group.

Employing Pearson's correlations for all participants, the relationship between urinary Br^- concentrations and HRV indices was evaluated. Additionally, a partial correlation analysis was performed to assess the relationship between urinary Br^- concentrations and HRV indices in connection to age and gender. A statistical analysis of the data was performed using SPSS ver.23 (SPSS Inc., Chicago, IL, USA, 2009). The significance level was set to $\alpha = 0.05$ for all statistical tests (two-tailed).

RESULTS

Demographics

Table 2 summarizes the participants' demographic features and baseline values. The fumigators were predominantly male, older, and had a higher smoking rate when compared to the inspectors, and the gas mask use on either a test day or a typical day was also high. Other factors, such as the duration of work and alcohol consumption were not significantly different between both groups.

Comparison of HRV Indices and Urinary Br^- Before and After MB Work by Group

We summarized the differences in the change in HR indices over time by group, as shown in Fig. 1. All the fumigator HRV indices of TP, VLF, LF, HF, SDNN, pNN50, and HRV-index decreased significantly after work compared with the results before work. However, the inspectors did not display a difference between the levels before and after work. Figure 1 also shows urinary Br^- concentrations in fumigators and inspectors before and after the work. The concentrations of mean urinary Br^- in fumigators and inspectors before work were significantly different, at 7.13 and 3.77 μg/mg CRE, respectively. There was also a significant difference in the mean concentrations after work between the two groups (15.04 and 4.06 μg/mg CRE). That is, the concentration of mean urinary Br^- in the fumigators significantly increased after work compared with that before work ($P < 0.001$). Conversely, a significant difference was not shown in inspectors between the values before and after work.

According to the results of the linear mixed effect model analysis of HRV indices and Br^-, the effect of the timing (before and after work) on all the indices for all the subjects appeared significant ($P < 0.05$ for all indices). All indices except the VLF were significantly decreased by age ($-0.733 < \beta < -0.028$; $P < 0.01$ for all indices), but none of the indices except the HR ($P < 0.01$) were affected by group and the duration of work (data not shown). Effect estimates of TP, HF, SDNN, pNN50 and HRV-index in male subjects were significantly lower than in female subjects ($-9.456 < \beta < -0.403$; $P < 0.05$ for all indices). Smoking also negatively affected the TP, VLF, SDNN, and pNN50 in the subjects ($-6.510 < \beta < -0.300$; $P < 0.05$ for all indices). The group, alcohol, fumigation site, and gas mask use on the test day or a typical day did not affect any HRV indices except the HR (data not shown). There were interactive effects between the group and timing on all HRV indices except LF, HRV-index ($P < 0.05$ for all indices). The effects of the group, timing, and alcohol on Br^- were statistically significant, including the interaction between the group and the timing ($P < 0.01$ for all indices), but there was no significant effect of factors such as gender, smoking, gas mask use, age or duration of work. The details are shown in Table 3.

Correlation Between Br^- levels and HRV Indices in all Subjects

The relationship between the urinary Br^- concentrations and the HRV indices in all the subjects ($n = 96$) was assessed before and after work. Urinary Br^- levels and the HRV indices of TP, VLF, LF,

TABLE 2. Demographic Information

Demographic Variable	Fumigator	Inspector	*P* Value
Gender: Male	62 (100%)	21 (61.8%)	<0.001
Age (y)	42.27 ± 9.52	37.21 ± 10.61	0.019
Smoking: Yes	27 (43.5%)	8 (23.5%)	0.007
Alcohol: Yes	60 (96.8%)	32 (94.1%)	0.457
Duration of work (y)	10.32 ± 8.96	7.96 ± 8.48	0.176
Gas mask* use on test day: Yes	61 (98.4%)	21 (61.8%)	<0.001
Fumigation in a container on the test day: Yes	60 (96.8%)	30 (91.1%)	0.028
Always gas mask use on a typical day: Yes	24 (38.7%)	6 (17.6%)	0.003

The data are summarized as the means ± SDs for continuous variables (age and duration of work) and the frequencies and proportions for categorical variables (gender, smoking, alcohol, gas mask use on the test day and a typical day, and fumigation site). *P* values were derived from an independent *t* test for continuous variables or a Fisher's exact test for categorical variables.

*Gas masks that were used by subjects during MB fumigation work were attached with a canister for air purification.

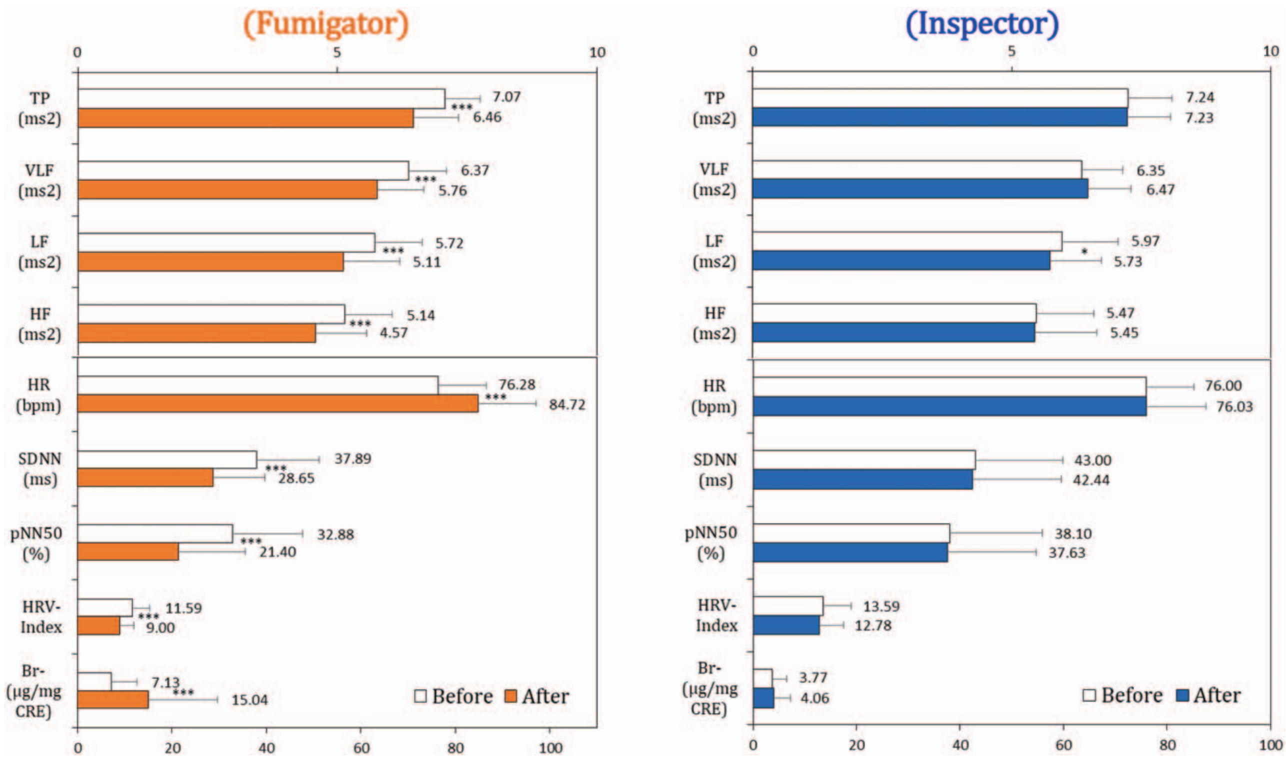

FIGURE 1. HRV indices and Br^- concentrations before and after fumigation work for fumigators and inspectors. A paired *t* test was performed to determine whether the HRV indices and Br^- were different before and after fumigation work within each of the groups. TP, VLF, LF, and HF were expressed as the log-scaled power (ms^2) of each frequency range; HR as beats per minute (bpm); SDNN as standard deviation (ms) of heart rate variability; pNN50 as the proportion (%) derived by dividing NN50 by the total number of NN intervals; HRV-index is unitless; and Br^- as bromide ion concentration in urine (μg/mg CRE). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

HF, SDNN, and pNN50 and the HRV-index—all indices except HR—showed negative linear correlations with *P* values < 0.05. Even though the *P* values obtained after controlling for age and gender were higher those obtained under the uncontrolled condition, the same tendency as above was also shown in the results of the partial correlation analysis after controlling for age and gender. The details regarding the correlation analysis are summarized in Table 4.

DISCUSSION

Ion chromatography,[18,30] gas chromatography,[19,30] and ICP-MS[31,32] have been used to analyse urinary Br^- concentrations as a biomarker of MB exposure. The HPLC/ICP-MS method, which was modified from the traditional ICP-MS method, was applied in this study. Some previous studies analysed Br^- concentrations in biological samples from MB fumigation workers. Tanaka showed that the concentration of mean urinary Br^- in 251 MB workers and 379 non-MB workers were different, at 9.1, 6.3 mg/L.[17] Yamano et al revealed median Br^- values of 13.0 μg/mg CRE in a synthesis group in a MB manufacturing facility over a 17-yr period, 11.9 μg/mg CRE in a filling group and 7.2 μg/mg CRE in an other group.[19] These findings show a similar tendency as the results of this study, which are the values of 15.04 μg/mg CRE in the 62 fumigators and 4.06 μg/mg CRE in the 34 quarantine inspectors were assessed. The authors in this study reported that the mean post-work urinary Br^- concentration in 44 fumigation workers was 18.311 μg/mg CRE, which is significantly increased from the pre-work value (7.390 μg/mg CRE).[29] Similarly, the workers' Br^- level (15.04 μg/mg CRE) after work increased by approximately two times compared to the level (7.13 μg/mg CRE) before work in this study.

Many studies have employed HRV analysis to quantitatively evaluate the parasympathetic and sympathetic control of the heart and quantify the balance of the autonomic nervous system.[20–24,28,33–38] In this study, the impact of MB on humans was measured using HRV analyses, which has not been performed to date. HRV was measured in working groups to determine whether MB fumigation work affected these groups.

The mean HRV indices after fumigation work on the study group decreased sharply compared to the indices from before fumigation work except HR (Fig. 1). According to observational study for age-trend analysis (Table S2, http://links.lww.com/JOM/A835), when the magnitude of decrease in the indices was compared, the difference depended on the reference range by age group (Fig. S1, http://links.lww.com/JOM/A835), and TP, LF, SDNN, pNN50, and HRV-index implied that fumigators were functionally aged from the 30s to 40s, with a VLF from the 20s to 40s and HF from the 40s to 50s. Symptoms of lung damage in the form of chemical pneumonitis or pulmonary oedema are observed after high levels of MB inhalation exposure, and signs of systemic poisoning, including headache, anorexia, nausea, vomiting and visual disturbance, may develop at lower levels of inhalation exposure.[13] The decrease in HRV indices may be milder than when there are signs of systemic poisoning, since the workers showed no symptoms; however, it would be clear that the autonomic nervous system suffers similar damage during MB fumigation as the function degradation by aging. It is because persons with lower HRV indices such as SDNN, TP, LF or HF, are likely to develop a metabolic syndrome, including higher levels of uric acid, high-sensitive C-reactive protein, non-HDL-cholesterol, body mass index or blood pressure.[24]

TABLE 3. Estimates of the HRV Indices and Br^- with Different Risk Factors

Indices	Effect Estimate (β)	Standard Error	P Value
TP (ms^2)			
Timing (before vs after)	0.009	0.171	0.004
Gender (male vs female)	−0.403	0.186	0.032
Smoking (yes vs no)	−0.300	0.114	0.009
Age (y)	−0.028	0.009	0.003
Group*timing	0.598	0.213	0.006
VLF (ms^2)			
Timing (before vs after)	−0.118	0.186	0.035
Smoking (yes vs no)	−0.460	0.124	<0.001
Group*timing	0.727	0.232	0.002
LF (ms^2)			
Timing (before vs after)	0.240	0.223	0.002
Age (y)	−0.034	0.012	0.005
HF (ms^2)			
Timing (before vs after)	0.023	0.205	0.022
Gender (male vs female)	−0.705	0.223	0.002
Age (y)	−0.049	0.011	<0.001
Group*timing	0.545	0.255	0.034
HR (bmp)			
Group (fumigator vs inspector)	11.136	2.498	0.001
Timing (before vs after)	−0.036	2.441	0.006
Smoking (yes vs no)	5.760	1.624	<0.001
Gas mask use on the test day (yes vs no)	−5.711	2.469	0.022
Age (y)	−0.733	0.129	<0.001
Duration of work (y)	0.594	0.144	<0.001
Group*timing	−8.406	3.038	0.006
SDNN (ms)			
Timing (before vs after)	0.561	3.031	0.010
Gender (male vs female)	−9.456	3.299	0.005
Smoking (yes vs no)	−4.419	2.017	0.030
Age (y)	−0.492	0.161	0.003
Group*timing	8.6934	3.771	0.023
pNN50			
Timing (before vs after)	0.471	3.351	0.005
Gender (male vs female)	−8.524	3.647	0.021
Smoking (yes vs no)	−6.510	2.230	0.004
Age (y)	−0.511	0.178	0.005
Group*timing	11.016	4.169	0.009
HRV-index			
Timing (before vs after)	0.814	0.879	0.002
Gender (male vs female)	−2.895	0.957	0.003
Age (y)	−0.144	0.047	0.002
Bromide ion (μg/mg CRE)			
Group (fumigator vs inspector)	11.289	2.173	<0.001
Timing (before vs after)	−0.299	2.124	0.002
Alcohol (yes vs no)	−10.556	3.290	0.002
Group*timing	−7.603	2.643	0.005

The estimates (β) were derived from linear mixed effect model on all factors in Table 2. The analysis also included timing, group, and the interaction between two variables. Factors such as timing and group were mainly selected in this study to investigate the effect of them on HRV indices. We selected factors that directly could affect HRV indices, such as age, smoking, and duration of work. Factors of gas mask use, fumigation site were also selected to evaluate the effect on MB exposure. There were 96 subjects before and after.

MB thus accelerates the aging process of neurological functions. This result could show a further deterioration of the nervous system of fumigators, in addition to the other negative effect on their central nervous system reported by Park et al.[29]

A further note is that the fumigators' HR showed a significant increase after the work. Indicators that reflect the variability of the HR (HF, LF, VLF, TP, HRV index, SDNN, pNN50) were all lowered, and at the same time, the increase in the HR can be said to result in a more negative state in terms of health following fumigation work.[39] This suggests that MB fumigant exposure was an excessive stressor on the body's autonomic nervous system to the extent that a 'lower HRV, higher HR' condition was induced. Circadian characteristics were not taken into account in this study, as all measurements of HRV metrics were made at a relatively constant time zone (8 AM to 6 PM).[40]

The timing (before and after the fumigation) was the significant factor affecting all indices (Table 3). These findings imply that MB fumigation work affects HRV and its related indices, which is supported by the study reported by Kakizaki.[41] The estimates of the HRV indices before work were higher than those after work ($P < 0.05$ for all estimates), indicating a similar tendency to the result that the fumigators' HRV indices fell after MB fumigation work (Fig. 1). Group did not appear as a main factor for any HRV indices except HR, possibly because the HRV indices of the two groups were not significantly different before MB work (Table S3, http://links.lww.com/JOM/A835) despite significant difference between two groups after the work (Table S4, http://links.lww.com/JOM/A835). There was an interactive effect between timing and group on most HRV indices, which can be attributed to the different magnitude in the effect of the two groups over time. Age, smoking and gender strongly affected the HRV indices, which would confirm the studies of Reardon, Voss or Dietrich.[22–24] Urinary Br^- levels were significantly affected by the group, timing, the interaction between the two variables, and alcohol ($P < 0.01$ for all estimates). Interestingly, the estimate of alcohol's effect on drinkers was −10.56, which may be assumed to be because alcohol promoted the excretion of MB through urine, which is the main elimination route of MB. However, further research is needed to reveal the exact effect of alcohol on MB.[16]

There was a negative correlation between the concentrations of urinary Br^- and the values of all the HRV indices, except the HR, in all subjects ($P < 0.05$ in all cases) (Table 4 and Figure S2, http://links.lww.com/JOM/A835). The subjects' urinary Br^- was correlated with all the HRV indices including HR even after controlling for age and gender (Table 4). Therefore, Br^- could be considered to affect HRV indices, and HRV indices could be useful for assessing autonomic functional degradation and the early prediction of disease risk by MB fumigation work.

The physical working conditions of fumigators were similar because they worked in the same geospatial areas and periods as

TABLE 4. Correlations of All Subjects' Urinary Br^- Levels and HRV Indices Before and After Work After Controlling for Age and Gender

Control Variables		TP	VLF	LF	HF	HR	SDNN	pNN50	HRV-Index
None*	Correlation	**−0.256**	**−0.239**	**−0.186**	**−0.198**	**0.191**	**−0.208**	**−0.237**	**−0.214**
	P value	**<0.001**	**0.001**	**0.010**	**0.006**	**0.008**	**0.004**	**0.001**	**0.003**
	df	190	190	190	190	190	190	190	190
Age and gender	Correlation	**−0.214**	**−0.203**	**−0.152**	**−0.145**	**0.188**	**−0.148**	**−0.186**	**−0.154**
	P value	**0.003**	**0.005**	**0.036**	**0.046**	**0.009**	**0.042**	**0.010**	**0.034**
	df	188	188	188	188	188	188	188	188

*Cells contain zero-order (Pearson) correlations. TP, VLF, LF, and HF are expressed as the log-scaled power (ms^2) of each frequency range; HR is expressed as beats per minute (bpm); SDNN is expressed as the log-scaled standard deviation (ms) of heart rate variability; pNN50 is expressed as the proportion (%) derived by dividing NN50 by the total number of NN intervals; and Br^- is expressed as bromide ion in urine (μg/mg CRE). The HRV-index is unitless but log-scaled value. The numbers of subjects were 96 before and after.

inspectors during the day, although the tasks involving sealing containers and removing tape from fumigation treatments were slightly more strenuous than inspection work (Table S1, http://links.lww.com/JOM/A835). Since intense physical activity raises HRV, fumigators' post-work HRV levels should have been higher than those of inspectors.[24] However, the indices were reduced (Fig. 1); this decrease does not seem to be due to physical activity.

Since this study involved people who work in ports where access is strictly restricted, there were many difficulties such as selecting subjects and a place for the measurements, resulting in the selection of the Yeongnam regional office as the measuring center, and inspectors were enrolled as a control group. An important achievement of this study is the enhanced understanding of the health effects on fumigators who show no symptoms of poisoning even after MB exposure. However, a limitation was that the sample size of the subjects was not enough because only 76 fumigators in the Busan port area were registered in the APQA, and a few fumigators did not participate in the health monitoring of this study. Finally, future research should include an identification of the causes of work exposure to MB, the chronic effect of MB on fumigators' health, and recovery patterns after neuronal degeneration or aging by MB. Despite limitations in this study, we showed that MB fumigation work negatively affected the workers' health by revealing higher MB concentration in the urine and lower post-work HRV indices in the fumigators. It is expected that the results of this study will be used to create a safer fumigation work environment, eliminate harmful factors, and protect workers' health rights by improving the fumigation work process or enhancing workers' safety awareness. Moreover, these results could serve as a basis for restricting the use of MB and promoting the development of MB alternatives.

CONCLUSION

All fumigators' post-work HRV indicators (TP, VLF, LF, HF, HR, SDNN, pNN50, and the HRV-index) were significantly changed towards a weakening of the subjects' autonomic nervous systems compared with the pre-work values ($P < 0.001$ for all indices). The mean pre- and post-work concentrations (7.13 and 15.04 μg/mg CRE) of urinary Br^- in the fumigator group were higher than those in the inspector group (3.76 and 4.06 μg/mg CRE), indicating that the post-work urinary Br^- concentrations in the fumigator group was significantly increased compared with the pre-work levels ($P < 0.001$). In contrast, there were no significant differences in the HRV indices and Br^- among the inspectors between pre- and post-work except in the LF. The concentrations of urinary Br^- in all subjects were negatively correlated with the HRV indices of TP, VLF, LF, HF, and SDNN and the HRV-index before work ($P < 0.05$ for all cases). To reduce this ''silent health risk'' for fumigators applying MB to plants and goods for trade, it is therefore necessary to make the fumigation process safer, including restricting the use of MB and expanding the use of alternative substances.

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