Original Article

Health Profiles of Methyl Bromide Applicators in Greenhouses in Turkey

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Abstract

Introduction: Methyl bromide is a toxic substance that has hazardous effects on human health with acute and chronic exposure. Our previous study showed that methyl bromide applicators frequently use large amounts of methyl bromide haphazardly in greenhouses in the prefectures of Narlidere and Balcova in the Aegean city of Izmir. This study aims to evaluate the health conditions of these workers. Materials and Methods: Our previous study showed that there are 38 methyl bromide applicators in our study area. After the informed consent of methyl bromide applicators was obtained, a questionnaire was used for a survey of demography and symptoms. Each subject was examined before and after application of the compound. Blood and urine samples were collected and stored. Blood samples were analysed for methyl bromide and bromide ion, kidney and liver function tests and lipid profile. Results: The age range of subjects was 19 to 53 years (mean age: 41 ± 8.57). This study showed that methyl bromide applicators use large amounts of methyl bromide disregarding legal regulations and that some of them had non-specific complaints. Subjects had been working as methyl bromide applicators for approximately 9.7 ± 4.15 years. A total of 69.7% of methyl bromide applicators reported that they did not use protective equipment while 33.3% of them had a history of acute methyl bromide intoxication. A statistically significant relationship was found between the usage of protective equipment and the level of blood bromide ion in the blood (P <0.05). Conclusion: Usage of methyl bromide, training, screening and follow-up of applicators must be rigorously controlled in accordance with national legal arrangements and international protocols. Greater efforts are required in the implementation of controls to achieve the targets set by the legal regulations and to ensure continual improvement in the limitation of the risks of this environmental hazard.

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Introduction

Methyl bromide is primarily used as a fumigant for the control of nematodes, fungi and weeds in greenhouses, warehouses and mills. Today, around 50,000 tons of methyl bromide are used per year and about 80% of this are consumed in developed countries. The largest consumers are the United States, Italy, Japan, Israel and Spain. Developing countries with the largest annual consumption include Turkey, Brazil, China, Korean Republic, Mexico, Morocco, South Africa and Zimbabwe.[1-5]

Consumption of methyl bromide has been restricted because of its hazardous effects on human health and the environment. In accordance with the Montreal Protocol, the use of methyl bromide will be phased out by the year 2015. The concepts of this protocol, to define new standards, supervision and implication of methyl bromide usage have been codified by the regulations (The regulation for the limiting of agricultural use of methyl bromide on 23 June 2000) in our country.[6-10]

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In Turkey, although there is a high level of consumption and legal regulation of methyl bromide usage, no information is available on the health profile of methyl bromide applicators. In our previous study, we observed that methyl bromide applicators use methyl bromide haphazardly even without protective clothes.[11] This study aims to evaluate the health status of methyl bromide applicators in greenhouses in the prefectures of Narlidere and Balcova in the province of Izmir.

Materials and Methods

Ethics Committee

The proposal of the study was submitted to the Ethics Committee of Dokuz Eylul University School of Medicine. This Committee approved the project (document number 63/2001, dated 25 October 2001).

Subjects

Out of the 38 methyl bromide applicators included in our previous study,[11] 15 could be reached and agreed to be included in our current study. With the inclusion of 18 additional individuals who were working as methyl bromide applicators during the period of the current study, the total number of subjects became 33. The population from which subjects were recruited was the same as for our previous study.[11]

Physical Examination

Methyl bromide applicators were invited to our hospital. The subjects were provided with explanations regarding the study and written informed consents were obtained. A questionnaire was prepared to obtain data related with the demographic features, application habits and some complaints which may be relevant to their habits. During the face-to-face interview, each subject was supplied with the questionnaire.

Before the physical and neurological examinations, subjects' medical histories were taken and blood and urine samples collected and stored. All methyl bromide applicators were re-examined after methyl bromide application. Re-evaluation consisted of all the steps that had been undertaken in the first evaluation. Thirteen applicators could not attend the re-evaluation since some of them had quit their jobs and some of them had health problems. Therefore, only 20 (60%) applicators could be included in the re-evaluation steps.

Sampling

Collected blood samples were analysed for methyl bromide and bromide ion, kidney and liver function tests and lipid profile. Two blood samples were collected concurrently from each subject. Blood samples (8.6 ± 0.26g) were incubated for methyl bromide in pre-weighed 22-mL crimp-top sealed gas chromatography (GC) glass vials containing 2 g sodium chloride, capped, re-weighed and stored at -20°C until analysis.

Blood samples were drawn for serum bromide, Neuron Specific Enolase (NSE) and routine biochemical analysis after 12-hour fasting was done. Urine samples were collected and stored at -70°C until analysis. The urine samples were analysed biochemically.

Control Group

Twenty people were chosen for the control group. The individuals in the control group had different jobs and their ages and gender were similar to the people in the applicator group. Sampling and analysing procedures were done by the same methods and using the same parameters.

Analyses

Determination of serum bromide levels by a colorimetric method

Serum levels of bromide, a parameter for biomonitoring exposure to methyl bromide, was measured by a colorimetric method based on the oxidation of bromide to bromine, which reacts with phenolsulfonphtlein in the presence of chloramine-T to yield a coloured complex measured at 589 nm.[12] For calibration, 50 mM sodium bromide standard stock solution was diluted to contain 25; 50; 100; 200; 400 nmol bromides/100 μL, respectively. A serum pool was prepared using blood samples obtained from non-exposed healthy individuals and standard addition at different concentrations was applied in order to determine linearity and precision. The detection limit was 3.37 mg/L (41.1 nmoL/mL) or 5.28 mg/L (66.0 nmoL/mL) with 95% or 99% probability, respectively. The method was linear up to 56 mg/L (700 nmoL/mL). CV values for 12 mg/L (150 nmoL/mL) and 32 mg/L (400 nmoL/mL) were 3.05 (n = 20) and 1.49 (n = 20), respectively. The recovery was 89% for 12 mgL/L and 102% for 32 mg/L. The minimum limit that could be assessed for bromide ion was 3.4 mg/L, therefore the values below this level were also considered as 3.4 mg/L.

Determination of methyl bromide

1. Reagents
   Methyl bromide (99.5%) was obtained from Labor Dr. Ehrenstorfer-Schafers (Germany) and sodium chloride was purchased from Sigma-Aldrich Chemie, (Stteinheim, Germany).

2. Gas chromatographic conditions
   The amount of methyl bromide in blood samples was

determined by a headspace (Agilent 7694) GC/MS (Hewlet Packard 6890 GC, 5973 MSD). A J&W, DB-624 capillary column with dimensions of 30 m x 0.25 mm x 1.4 µm was used for the determination. The selected-ion monitoring (SIM) mode was chosen.

The samples were held for 12 minutes at 45°C in the headspace before the analysis. The injection port and detector temperatures were 250°C and 290°C, respectively. The temperature was held at 45°C for 3 minutes and adjusted to increase from 45°C up to 200°C with an increase rate of 20°C/min. For methyl bromide, m/z 94 was used for quantitative calculation, and m/z 96 as qualifier ion. The retention time for methyl bromide was 2.73 minutes. The concentration of methyl bromide in blood was calculated by dividing the amount by the weight of the blood sample. Staff performing the assay were blinded to the exposure of subjects to methyl bromide.

Table 1. Methyl Bromide Worker's Demographic Characteristics

	Subjects		Control group	
	Frequency	Percent	Frequency	Percent
Sex				
Male	28	84.8	15	75.0
Female	5	15.2	5	25.0
Age (y)				
19-39	12	36.4	12	60.0
40-59	21	63.6	8	40.0
Mean ± SD	41.0 ± 8.6		40 ± 8.6	
Median	43		40	
Minimum	19		27	
Maximum	53		55	
Education status				
Illiterate	1	3.0	-	
Primary school	18	54.5	-	
High school	11	33.3	2	10
University	3	9.1	18	90
Marital status				
Married	28	84.8	17	85
Single	5	15.2	3	15
Residence near greenhouse?				
Yes	17	51.5	-	0.0
No	16	48.5	20	100.0
Total	33	100.0	20	100.0

Table 2. Methyl Bromide Workers' Application Characteristics

	Frequency	Percent
Job experience		
0-10 years	11	33.3
11-20 years	22	66.7
Mean ± SD	9.8 ± 4.2	
Median	10.0	
Minimum	3.0	
Maximum	20.0	
Frequency of application		
Once a month	1	3.0
5-6 times of year	1	3.0
Once a year	24	72.7
Once two years	7	21.2
Amount of each application	33	100.0
0-5 gas cylinders	24	72.7
More than 6 gas cylinders	9	27.3
Mean ± SD	6.6 ± 7.2	
Median	5.0	
Minimum	1.0	
Maximum	30.0	
Application area within a year		
1-19 decare	26.00	78.8
20-40 decare	7.00	21.2
Mean ± SD	9.3 ± 11.3	
Median	5.0	
Minimum	1.0	
Maximum	40.0	
Usage of protective equipment		
Yes	10	30.3
No	23	69.7
History of suspected intoxication		
Yes	11	33.3
No	22	66.7
Total	33	100.0

3. Preparation of methyl bromide standards
   A calibration curve for the measurement of methyl bromide was formed by mixing bank blood samples with methyl bromide containing solution in a total volume of 2 mL to give the following concentrations of 0, 0.1, 0.2, 0.6, 0.8 and 1.0 µg/mL methyl bromide. The relationship between response and analyte concentration was linear in the considered calibration range (r^2=1.0).
   The limit of quantification of methyl bromide in blood

was 0.05 μg/mL. The intraday co-efficient of variation for methyl bromide concentration at 0.2, 0.4, 0.6 and 0.8 μg/mL was 4.9, 6.5, 7.1 and 13.9%, respectively (n = 4 for each concentration). The interday co-efficient of variation for methyl bromide solution at 0.3 and 0.7 μg/mL was 6.4 and 9.2% (n = 13, 12), respectively.

Blood and urine biochemical analyses were performed using Roche kits in a Hitachi auto analyser (Roche, Basel, Switzerland). Serum NSE levels were measured by the electrochemiluminescence method with Elecsys 2010 analyser (Roche, Basel, Switzerland).

Results

Methyl bromide workers' demographic characteristics are shown in Table 1. Methyl bromide workers' application characteristics are shown in Table 2. Our study showed that methyl bromide was being used by uncertificated people in our region. Out of 33 applicators, only 1 was an agricultural engineer and had the authority to apply methyl bromide. During the interviews, some applicators alleged that the application pressure and the dosage recommended by agricultural engineers were not appropriate. They prefer the way of application which they had learnt from their fathers.

Neurological, psychiatric, gastrointestinal and respiratory system complaints were the most common symptoms reported by 33 greenhouse workers. Of all the workers, 17 (51.5%) had psychiatric complaints (sleep disorders, behaviour disorder), 15 (45.5%) had neurological complaints (headache, dizziness), 12 (36.4%) had respiratory system complaints (dyspnoea, cough, phlegm) and 10 (30.3%) had gastrointestinal complaints (abdominal pain, burning stomach pain). Four subjects (8%) reported respiratory problems such as asthma and chronic bronchitis, while 3 (6%) mentioned gastric ulcers. Six workers suffered from chronic disorders which were under control with medications. Physical examinations revealed pharyngeal hyperemia in 9 applicators (18%).

Blood and urine biochemical test results were usually found to be normal. Only 1 applicator's blood glucose level was very high (pre-application [299 mg/dL] and post-application [121 mg/dL] levels). He was sent to the department of internal medicine.

Methyl bromide was not detectable in any of the blood samples (the limit of quantification was 0.05 μg/mL).

Serum bromide ion levels determined via biochemical methods were above the considered cut off value (3.4 mg/dL) in 2 of the 33 applicators. Since these 2 applicators did not apply methyl bromide, they did not attend the re-evaluation. The post-application bromide ion levels of 6 individuals were higher than their pre-application bromide ion levels, which had been below 3.4 mg/dL. Socio-demographical data and the information on the application, which are suggested to have a role in this increase, were investigated. No significant correlations with age, gender, residence, the frequency of application, the time of application, the amount of methyl bromide

Table 3. Probable Factors Influencing Serum Bromide Ion Levels (Categorical Variables)

	Increase in serum bromide ion levels					
	No		Yes			
Variables	Count	Percent	Count	Percent	Total	*P**
Sex						
Man	13	76.5	4	23.5	17	
Woman	1	33.3	2	66.7	3	0.202
Protective equipment						
Used	8	100.0	0	0.0	8	
Not used	6	50.0	6	50.0	12	0.042
Leakage during application						
Yes	3	60.0	2	40.0	5	
No	11	73.3	4	26.7	15	0.613
Location of residence						
Near greenhouse	6	75.0	2	25.0	8	
Far from greenhouse	8	66.7	4	33.3	12	0.545

* Fisher's Exact Test

Table 4. Probable Factors Influencing Serum Bromide Ion Levels (Numeric Variables)

		Percentiles				
	N	25th	Median	75th	Z**	*P*
Age	20	35.0	42.0	43.0	-0.083	0.934
The last time of application (Day)	20	3.0	4.5	14.5	-1.369	0.171
Frequency of application	20	1.3	3.0	7.5	-0.460	0.646
Amount of application (Gas cylinder)	20	2.0	3.0	5.8	-0.335	0.738
Area (Decare)	20	2.0	2.5	18.8	-0.254	0.800

** Mann-Whitney U test

Table 5. Serum Bromide Ion Levels of the Cases Before and After Application

		Before application (mg/L)	After application (mg/L)
N		20	20
Mean		3.40	5.08
Std. deviation		0.00	4.63
Minimum		3.40	3.40
Maximum		3.40	20.60
Percentiles	25th	3.40	3.40
	Median	3.40	3.40
	75th	3.40	3.62
Test statistics(*)		Z	*P*
Before application-After application		-2.201	0.028

* Wilcoxon Signed Ranks Test

applied, the area of application, direct exposure to methyl bromide due to leakage and the increase in serum bromide ion levels were found (Table 2). On the other hand, there was a significant correlation between the increase in serum bromide ion levels and protective equipment use (Fisher's Exact test, $P < 0.05$) (Tables 3 and 4).

Revealed serum bromide levels, before and after methyl bromide applications, were compared. Statistically, the difference between those levels was significant ($P < 0.05$) (Table 5).

Twenty applicators were compared to 17 controls according to blood bromide values before and after the application. There was no significant difference between the applicators and controls in blood bromide values (Table 6).

Mean serum NSE value was found to be 3.06 (±1.82) ng/mL for the first 33 people and 2.64 (±1.47) ng/mL for 20 people who came for the re-evaluation. All of the results were within the normal limits (<16.3 ng/mL).

Discussion

In accordance with the Montreal Protocol, "The regulation for the limiting of agricultural use of methyl bromide" was put into effect on 23 June 2000 in our country. According to this regulation, methyl bromide can only be applied by applicators who have received certificates after relevant education. In this study, uncertificated applicators preferred the way of application which they had learnt from their fathers. The reason for their preference was their feeling that traditional ways were likely to be much more effective. It was hypothesised that this heritage of application technique may have increased the hazardous effects.[6,7]

The findings of this study showed that people working in the greenhouses of our region apply a great amount of methyl bromide without using protective equipment and they do not have application certificates for the use of the substance. Various health complaints and findings were observed in methyl bromide applicators. In our previous

Table 6. Comparison of Bromide Levels of Cases and Controls Before and After Application

	Cases		
	Before application (mg/L)	**After application (mg/L)**	**Controls**
N	20	20	17
Mean	3.40	5.08	3.45
Std. deviation	0.00	4.63	0.19
Minimum	3.40	3.40	3.40
25	3.40	3.40	3.40
Median	3.40	3.40	3.40
75	3.40	3.62	3.40
Maximum	3.40	20.60	4.20
Test statistics (*)	Comparison of bromide levels of cases and controls before application	Comparison of bromide levels of cases and controls after application	
Mann-Whitney U	160.00	128.00	
Z	-1.085	-1.873	
P	0.278	0.061	

* Grouping Variable: Applicator and control

study, it was shown that pesticides other than methyl bromide were also used haphazardly and in enormous amounts.[11] We believe that it would be appropriate to investigate the damage of these haphazardly used chemical substances to the applicators and the environment, and necessary precautions should be taken.

As the methyl bromide application characteristics of greenhouse workers were analysed, it was observed that the duration of application of the given substance ranged between 3 and 20 years with an average of 10 years. Despite these long periods of usage, none of these people were aware of or anxious about the harmful effects on their and their relatives' health. Besides these, the residences of nearly half of the applicators are located in the vicinity of the greenhouses. Therefore, we believe that the members of these families should also be taken into consideration when health screening procedures for the applicators are planned.

It was observed that the level of protective equipment usage by our subjects was insufficient for the protection of their health. Only one-fourth of the applicators were using masks for the entire application. Proper protective equipment usage during application is likely an ideal behaviour that should be achieved worldwide. Thorough review of the literature showed that this was not the case in reality.[13-15] For example, a study from Japan reported that 20.7% of the applicators used masks, 4.7% used protective clothing and 23.3% used gloves.[16]

The applicators with increased serum bromide levels after methyl bromide application were also those not using protective equipment during application. The failure to use protective equipment suggests that methyl bromide applicators did not receive training for their profession, although this is legally obligatory in our country.[7]

In our study, neurological, psychiatric, gastrointestinal and respiratory system symptoms were observed in most of the applicators. In a similar study, it was mentioned that neurological system complaints were the most common complaints and these were found to be consistent with our study. Sleeping difficulties and disorders that are defined as psychiatric symptoms were also present in our study. Five out of 20 applicators (25%) stated that they experienced a burning sensation in the eye due to the leakage during the application. In acute exposure cases, symptoms such as irritation in the eyes and coughing were observed among the applicators.[16] In our study, 9.09% of the applicators stated that they had gastric ulcers. A similar incidence (8.9%) was reported by Kishi.[17]

The lack of a health monitoring system and health aid centre for emergency cases creates an unacceptable working environment for the applicators. The above observations of our study should be considered invaluable to the working safety of greenhouse workers.

Our study has some limitations in both the field study and laboratory investigation steps. The results obtained in laboratory investigations lead to limited discussions. Methyl bromide could not be detected in the blood samples of all workers. The probable reason for the absence of detectable methyl bromide in the samples may be its short half-life. It is well known that, after inhalation, methyl bromide is rapidly distributed and metabolised. A significant amount of methyl bromide is metabolised in the body into inorganic bromide, which is excreted in the urine.[1]

A significant difference was not determined between the serum bromide ion levels of the subject group and the control group. The half-life of bromide ion in the blood is 12 to 20 days. Blood sampling was not carried out until 10.3 ± 11.9 days after fumigation. Although the applicators were informed about the time of sample collection, the samples could not be obtained on the specified date and this was also considered a limitation of the study.[9,18]

We determined that there are few studies in the literature which evaluate the health status of methyl bromide applicators. In accordance with the Montreal Protocol, the signatory countries assume the responsibility of reducing the use of methyl bromide, ending the usage in the framework of a convenient programme and substitute its use with an alternative substance. However, our study showed that this toxic substance is haphazardly used by people who do not have the necessary application certificate in spite of legal arrangements. We think that the application of methyl bromide should be controlled more rigorously, the applicators should be educated and routine health controls should be performed.

REFERENCES

1. Hustinx WN, Van de Laar RT, van Huffelen AC, Verwey JC, Meulenbelt J, Savelkoul TJ. Systemic effects of inhalational methyl bromide poisoning: a study of nine cases occupationally exposed due to inadvertent spread during fumigation. J Ind M 1993;50:155-9.
2. Marracini JV, Thomas GE, Ongley JP, Pfaffenberger CD, Davis JH, Bernarczyk LR. Death and ınjury caused by methyl bromide, an ınsecticide fumigant. J Forensic Sci 1983;28:601-7.
3. De Haro L, Gastaut JL, Jouglard J, Renacco E. Central and peripheral neurotoxic effects of chronic methyl bromide ıntoxication. Clin Toxicol 1997;35:29-34.
4. Tanaka S, Abuku S, Seki Y, Imamiya S. Evaluation of methyl bromide exposure on the plant quarantine fumigators by environmental and biological monitoring. Ind Health 1991;29:11-21.
5. Workers Health Center. Available at: http://www.workershealth.com.au/facts009.html. Accessed 26 October 2006.
6. Available at: http://hq.unep.org/ozone/Montreal-Protocol/Montreal-Protocol2000.shtml. Accessed 26 October 2006.
7. Turkish Government Gazzette 23rd June, No. 24088 (2000) The regulation for the limiting of agricultural use of methyl bromide (in Turkish).
8. National Pesticide Telecommunications Network. Available at: http://npic.orst.edu/factsheets/MBgen.pdf. Accessed 26 October 2006
9. Norman CS. Potential impacts of imposing methyl bromide phaseout on US strawberry growers: a case study of a nomination for a critical use exemption under Montreal Protocol. J Environ Manage 2005;75: 167-76.
10. Lenhart SW, Gagnon YT. Health hazard evaluation of methyl bromide soil fumigations. Appl Occup Environ Hyg 1999;14:407-12.
11. Ergonen AT, Salacin S, Ozdemir MH. Pesticide use among greenhouse workers in Turkey. J Clin Forensic Med 2005;12:205-8.
12. Müller M, Reinhold P, Lange M, Zeise M, Jürgens U, Hallier E. Photometric determination of human serum bromide levels - a convenient biomonitoring parameter for methyl bromide exposure. Toxicol Lett 1999;107:155-9.
13. Burgess JL, Morrissey B, Keifer MC, Robertson WO. Fumigant-related ıllnesses: washington state's five-year experience. J Toxicol Clin Toxicol 2000;38:7-14.
14. Joyce S. Growing pains in South America. Environ Health Perspect 1997;105:794-9.
15. Available at: http://www.workershealth.com.au/facts009.html. Accessed 15 December 2006
16. Koda S, Kumagai S, Ohara H. Health effects of acute exposures to methyl bromide during soil fumigation ınside greenhouses. J Occup Health 2000;42:263-9.
17. Kishi R, Itoh I, Ishızu S, Harabuchi I, Miyake H. Symptoms among workers with long-term exposure to methyl bromide. Sangyo Igaku 1991;33:241-50.
18. Buchwald AL Müller M. Late confirmation of acute methyl bromide poisoning using S-methylcysteine adduct testing. Vet Hum Toxicol 2001;43:208-11.