

## Case Study

# A Cluster of Neurological Signs and Symptoms in Soil Fumigators

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Methyl bromide (CH<sub>3</sub>Br) is a practically odourless, highly toxic and penetrating volatile aliphatic hydrocarbon derivative that has been widely used in agriculture as a fumigant against rodents, insects, mites, and a range of pathogenic organisms in soil, compost, and timber. According to the Montreal Protocol on Substances that Deplete the Ozone Layer, it should have been phased-out of from use in developed countries by 1 January 2005. However, since an effective substitute does not exist, and its elimination would cause many problems in agricultural industries that are still heavily reliant on its use<sup>1,2)</sup>, it is still being employed for many purposes.

Methyl bromide exposure can cause skin, kidney, and respiratory, liver and neurological damage in workers. Fatal and non-fatal poisoning accidents have been reported since 1948<sup>3)</sup> and are still common both in developed<sup>4,5)</sup> and in developing countries<sup>6,7)</sup>. Long-lasting neurological consequences resulting from occupational accidents are also well known and <sup>8–10)</sup>. To our knowledge, however, no long-term prospective study of exposed workers has ever been published, and the effects of prolonged, low-dose occupational exposure are seldom reported.

Regular medical examination of exposed workers has been obligatory in Italy since 1956, and exposure assessment and biological monitoring of workers in compliance with European directives, have been required only since 2002. These regulations are not, however, always fully enforced in agriculture.

Here we report a cluster of cases of neurological signs and symptoms of varying severity, in a sample of farm workers from the same company who were employed as fumigators to vaporising CH<sub>3</sub>Br in greenhouses or open fields.

## Case Descriptions

Six male methyl bromide fumigators were observed at

the outpatient unit of Occupational Medicine. These workers, whose age ranged from 38 to 47 yr (mean 41.8 ± 3.5 yr), had been exposed to methyl bromide for a period ranging from 7 to 21 yr (mean 16.2 ± 5.3 yr). They had been employed in the same facility for many years with short-term contracts. Five of them were currently employed and one had been exposed to methyl bromide until one year prior to admission. These workers sought an examination in order to obtain a diagnosis of occupational disease to support an application for compensation.

Their jobs were principally to apply polyethylene films, remove films after fumigation and control the vaporiser. They all reported frequent eye-watering and coughing while removing films or on returning to a fumigated unit, and identified single episodes of significant exposure during their employment.

Patients were examined by an occupational health physician and a neurologist. Further investigation included: a) blood sampling for routine haematology, biochemical tests and measurement of serum bromide level; b) urine analysis, including immune-electrophoresis of urinary protein; c) electroencephalography; d) electromyography; e) motor and sensory evoked potentials; f) audiometric testing; and g) neurobehavioural testing according to the Italian battery of tests<sup>11)</sup>.

Neuropsychological assessment included a wide range of tests to quantify the subjects' performance not only in memory tasks but also in other cognitive skills such as language, reasoning, visuospatial ability and attention. The following tests were administered: Mini Mental State Examination (MMSE), Wechsler Adult Intelligence Scale, Rey's 15 word learning task (immediate recall and 15-min. delayed recall), Digit Span and Spatial Span, Street Completion Test, Copy of Rey's Fig. A, Facial Recognition, and Raven's Progressive matrices '47.

Patient 1 was a male, 38-yr-old former carpenter, who had been working for 18 yr as a fumigator and tractor driver in a firm specialized in the methyl bromide treatment of soil and greenhouses. He had a 23-yr-old son, but had repeatedly failed to have another child (his wife had 4 spontaneous abortions in 10 yr). Three sperm counts had previously revealed evidence of abnormal features: reduced motility (40–45% of immotile sperm), increased frequency of structural defects (44–53% of atypical forms) and increased sensitivity to the acridine orange test (55% resistant sperm, normal values >70%). The subject was worried, and wondered if occupational exposure could play a role in such phenomena.

During fumigations, the worker generally used a self-contained breathing apparatus, probably without a good seal, and admitted working frequently without a mask and wearing no clothing other than a pair of shorts to make the heat less unbearable during the summer, or in greenhouses.

For 2 yr prior to examination, he had experienced

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erectile dysfunction, tiredness, weakness, tingling or numbness of the extremities with painful feet dysaesthesia, headache, and myoclonic contractions of the lower limbs especially at night during sleep. He had also noted memory deficit and difficulty in concentrating.

Patient 2, a 42-yr-old man, had suffered seizure attacks since an occupational accident (malfunctioning gas valves) that occurred when he was 38 yr old. Immediately after the accident, he reported dermal burns and vesicles on the upper and lower limbs, heavy-headedness, drowsiness and vomiting. Subsequently he developed muscle fatigue, paresthesia, unsteady gait, myoclonic jerks of the face and finally suffered seizure attacks. He continued doing his job and reported other over-exposures to CH<sub>3</sub>Br, which he had never reported to the competent authorities. He had recurrent seizures and began to suffer from memory deficit, changes of mood, apathy, headache, leg cramps, and reduction of sexual potency.

Patient 3 reported some acute exposures caused by leakage from a CH<sub>3</sub>Br compressed gas cylinder. One of these accidents, with face involvement, was followed by cough, vomiting and temporary amaurosis; another accident, involving the hands, caused prolonged numbness of the extremities. None of these incidents was reported to the National Institute for Work Injury and Disease Prevention (INAIL). The patient had signs of eye and hand irritation, memory deficit, headache, tiredness, ataxia, hearing impairment and dizziness; sleep was disturbed by myoclonic jerks.

Other workers had similar case histories, signs and symptoms. Although patient 4 had not been exposed to methyl bromide for one year, signs of neurological impairment were still present. He reported memory deficit, difficulty in concentrating, tiredness and a reduction in physical strength. Patients 5 and 6 complained of peripheral paresthesiae, headache, reduction of physical strength and impairment of memory and concentration. They also reported symptoms of eye, nose, mouth and skin irritation, cough and headache.

All workers reported reduction of sexual potency, with normal libido.

Myoclonus was present in 3 out of 6 workers. The occurrence of myoclonic jerks often disturbed nocturnal sleep and caused insomnia. All exposed workers had hearing impairment and ringing in the ears. Audiometric testing confirmed mild to severe sensory-neural impairment in all exposed workers.

Signs of peripheral nervous involvement were also present in exposed workers; tingling or numbness of the extremities, or unsteady gait in more severe cases. However, electromyography and motor- and sensory-evoked potentials were normal.

Electroencephalography was normal in 5 patients, while in patient 2, bursts of slow antero-medial waves were observed on the left side. Brain MR revealed T1-

weighted hypointensities and T2 hyperintensities in the left frontal-temporal region.

Serum bromide levels were within the normal range in 5 out of 6 exposed men. A slightly increased bromide level was observed in patient 3 (6 µg/ml; normal level <5 µg/ml).

Neurobehavioural assessment in these workers showed slight to moderate memory impairment (patients 1,3,4,5,6), deficit of attention and learning (pts.1,2,4,5,6), and reduced ability to reproduce a complex drawing (pts.1,6).

## Discussion

The relation of symptoms to exposure is frequently a critical issue in occupational medicine. In order to define cases, there must be a high correlation between exposure and resulting symptoms, generally accompanied by physical evidence of exposure and objective medical observation of effects. According to CDC criteria<sup>12</sup>, a case was termed 'suspected' when the potentially exposed person was being evaluated for poisoning by methyl bromide, but no specific, credible threat existed. We termed "probable" a clinically compatible case in which a credible patient history regarding location and time existed for methyl bromide exposure, while a "confirmed" case was a clinically compatible case in which laboratory tests on environmental samples were confirmatory. However, a case was confirmed, even in the absence of laboratory testing, provided there was a predominant amount of clinical evidence.

In this study, a correlation between exposure and symptoms is highly probable. Clustering evidence increases the probability of association. Moreover, the evaluation of occupational histories in this group of workers provided evidence of relevant exposure. We therefore confirmed all cases.

To disinfect soil in greenhouses, methyl bromide can be applied to the soil by injection or by fumigation on the surface. A mixture of 97.51% methyl bromide, and 2.49% picryl chloride or chloropicrin (trichloronitromethane, Cl<sub>3</sub>NO<sub>2</sub>) is warmed up and brought under pressure from outside by a vaporiser and blown over to the ground under a polyethylene cover. Being three times heavier than air, the gas easily penetrates the ground. Studies have shown that polyethylene films are permeable to methyl bromide and other fumigant compounds<sup>13</sup>. Depending on local ventilation, a considerable amount of gas evaporates into the surrounding atmosphere. This emission is especially serious during the fumigation procedure and removal of the plastic cover. Mechanical injection of cold gas into the ground in open field areas is also routinely performed; during this operation, at least four workers, equipped with only a canister respirator, are simultaneously exposed to a gas concentration frequently exceeding the TLV-TWA

**Table 1.** Characteristics of the observed cluster

	Patient					
	1	2	3	4	5	6
Age (yr)	38	42	38	47	44	42
Cumulated exposure (yr)	18	13	21	7	18	20
Current exposure	+	+	+		+	+
History of over-exposure		+	+			
Symptoms or signs						
Irritated or burning eye, sore throat, cough, runny nose	+	+	+		+	+
Hand or leg dermatitis		+				
Memory deficit, difficulty concentrating	+		+	+	+	+
Insomnia or trouble sleeping at night	+		+	+	+	+
Headache	+	+	+	+	+	+
Fatigue, physical strength impairment	+		+	+	+	+
Tingling or numbness at the extremities	+	+	+	+	+	+
Myoclonus	+	+	+			
Epilepsy		+				
Ataxy		+	+			+
Hearing impairment, ringing in the ears	+	+	+	+	+	+
Dizziness		+	+	+		+
Erectile dysfunction	+	+	+	+	+	+
Wife's recurrent abortion	+					
Analyses						
Serum bromide level (microg/ml)	2.2	4.1	6.0	2.0	0.8	0.9
Electroencephalographic changes		+				
Electromyographic changes						
Reduction of motor and sensory evoked potentials						
Impairment at audiometric testing	+	+	+	+	+	+
Neurobehavioral testing:						
Memory impairment	+		+	+	+	+
Deficit of attention and learning	+	+		+	+	+
Reduced ability to reproduce a complex drawing	+					+
Brain MR changes		+				

level (ACGIH) of 5 ppm (19 mg/m<sup>3</sup>), sometimes as high as 200 ppm<sup>14, 15</sup>. Even exposures in the field during removal of plastic sheets after injection of methyl bromide into the soil may result in intoxication<sup>16</sup>.

Methyl bromide poisoning is difficult to confirm because routine laboratory testing is not always reliable. Measurable levels of the agent are rapidly reduced, probably as a result of direct tissue chemical reaction. Blood sampling with the specific purpose of detecting CH<sub>3</sub>Br should be performed within a few hours after acute exposure. Bromide ions have a biological half-life of 10–12 days; for this reason serum bromide levels have been used as an indirect measure of exposure and/or toxicity, but they correlate poorly with clinical symptoms and outcome<sup>17, 18</sup>, and are of little use in chronically exposed workers. Recently special testing has shown that protein adducts formed after exposure to methyl

bromide may be a better measure of significant exposure<sup>19</sup>.

After accidental over-exposure, cases of acute methyl bromide poisoning present symptoms such as headache, vomiting, clouding of consciousness, ataxia, slurred speech, and confusion. More serious effects caused by neurological lesions, such as myoclonus and generalised tonic-clonic convulsions, insidiously do not appear until after an interval of a few hours to several days<sup>17, 20–22</sup>. A myoclonus state, i.e. subintractant generalized synchronous symmetrical myoclonus, uncontrolled by therapeutics, may still be observed over weeks or months after exposure<sup>4, 23</sup>.

Neuropathological examinations of fatal cases reveal changes resembling Wernicke's encephalopathy. Similar lesions may be seen *in vivo* by MR of the brain<sup>24–26</sup>. Neurotoxicological studies of exposed workers have

demonstrated that even the low levels of methyl bromide currently observed in fumigation today may produce slight neurotoxic effects<sup>27)</sup>.

Few field studies have been performed on exposed workers without signs of acute poisoning. Most of these occupational surveys have shown that the prevalence of neurological symptoms in chronically exposed workers is significantly higher than in controls. Kishi *et al.*<sup>28)</sup> observed an increased occurrence of dizziness, numbness, paresthesia and weakness of extremities, nightmares, fatigue and dry and scaly skin among workers compared to controls. Also reports of irritation symptoms such as itching, bullae or reddish swollen hands and runny noses with nasal irritation were significantly more frequent in the exposed groups. After evaluating prospectively 15 male middle-aged workers before and after a fumigation period with methyl bromide, Acuna *et al.*<sup>29)</sup> confirmed higher frequencies of insomnia, headache, paresthesiae, mood changes, loss of memory and concentration, increased vibratory threshold, and strength reduction in these workers compared with controls. On the other hand, peripheral nerve effects observed in fumigation workers in south Florida were attributed to occupational ergonomic stress<sup>30)</sup>.

In evaluating the association between occupational exposure to methyl bromide and clinical condition, we considered some key points, i.e. whether: 1) symptoms and physical signs were appropriate for the exposure being considered; 2) all coworkers had the same pattern of disease; 3) the timing of the exposure and the onset of health problems were related; and 4) history of activity with direct dermal contact or inhalation exposure to methyl bromide confirmed occupational exposure to the pesticide. Given the exposure scenario, the biological plausibility of the resulting health effects was high. Furthermore, it was possible to rule out other non-agent exposures or pre-existing health problems.

Unfortunately, other conditions could not be satisfied since records of environmental monitoring data (e.g. bulk sampling, air, soil or water tests) were lacking, and no biological monitoring record was available.

Our study is only a "final-point" observation. By observing workers at the time they were submitted to our medical examination, we cannot distinguish the effects of remote and recent exposures to a toxic substance. We cannot say if the observed changes are long lasting sequelae of acute exposures, or represent chronic effects.

We concluded that, due to repeated sub-acute poisoning, or chronic low-dose occupational exposure to CH<sub>3</sub>Br, our patients had been affected by insidious neurological alterations, accompanied by cognitive and emotional impairment.

The adverse health effects reported in our study are consistent with the known toxicity of methyl bromide. Chloropicrin, that is added as an odorant, probably

increases irritation symptoms, and activates olfactory or trigeminal pathways. According to the hypothesis put forward by Gilbert<sup>31)</sup>, low-level chemical exposure may induce sub-convulsive chemical kindling in the olfactory bulb, the pyriform cortex, the amygdala, and hippocampus, thus providing a neurobiological mechanism that serves to amplify reactivity. In animal models, hyper excitability of focal limbic circuits may be induced by a number of chemicals and pesticides. The most common symptoms are myoclonic jerks, and clonic movements of forelimbs. Behavioural sequelae are known to persist well beyond the ictal episodes, and deficits in learning and memory are well evident during seizure-free periods, as well as disturbances of affect and emotions<sup>31)</sup>. These animal findings strictly resemble clinical phenomena occurring in humans.

Prior to our observation, these workers had been periodically submitted to medical examinations at the workplace every 3rd month, since the beginning of their activity. However, the physician was not a specialist in occupational medicine, and medical examinations were not integrated by biological monitoring or specific diagnostic tests for occupational exposure. Fearing loss of employment, workers generally under-reported occupational accidents and work-related symptoms. None of the workers had ever been removed from occupational exposure to CH<sub>3</sub>Br, nor were preventive measures implemented, although an important activity associated with the medical surveillance of workers should be the rapid identification and investigation of outbreaks of disease or a cluster of signs and symptoms.

Therefore, this study of occupational poisoning with CH<sub>3</sub>Br underscores the need for efficient medical surveillance of exposed workers as an integral part of good fumigation practice. It also shows the difficulties that may be encountered in the diagnosis and clinical management of the neurological manifestations of chronic systemic CH<sub>3</sub>Br intoxication.

It is worth noting that in Italy the fumigation market is an oligopoly. The headquarters of most companies are in a small town in Sicily. Fumigant mixtures are produced in countries outside the EU, and the liability of manufacturers falls outside European regulations. Control over the enforcement of preventive measures and the medical surveillance of workers are not always effective in rural areas, especially in Southern Italy. The planned phase-out of methyl bromide is having a drastic effect on the use pattern of fumigants. To replace methyl bromide as a soil fumigant, increased levels of metam-sodium (sodium methylthiocarbamate) are being used, and concentrations of chloropicrin are being increased to replace a portion of methyl bromide used during soil fumigations. In agricultural areas where greenhouses and dwellings intermingle, the general population as well as the fumigators may be exposed to fumigation products.

There could be even greater problems for public health if the process of substituting methyl bromide (involving agents of unknown human toxicity) does not occur under strict hygienic surveillance.

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