Bromine in blood, EEG and transaminases in methyl bromide workers

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ABSTRACT In 33 methyl bromide users, slight electroencephalographic changes (in 10 subjects) and a small increase in serum transaminases were found which could be related to bromine concentration in blood. No relationship was found with subjective symptoms, general neurological examination or the results of serum protein electrophoresis.

Methyl bromide is used to kill harmful organisms in food stores and in soil used for horticulture. Application may be hazardous to the health of the workers, especially inside greenhouses. Severe and sometimes fatal intoxication has been reported in the United States (Hine, 1969), Japan (Araki *et al.*, 1971), France (Merellio *et al.*, 1974), Spain (Garcia Rico *et al.*, 1974) and the Netherlands (Hartzuiker, 1968). A few years ago, the Dutch Labour Inspectorate made a study of the use of the bromine level in blood as an indicator of the early effects of methyl bromide exposure.

In acute serious intoxication methyl bromide causes pulmonary oedema, convulsions and hyperthermia. In such cases EEG disturbances have been reported. Chronic exposure may cause neuropathy, ataxia, disturbance of renal and liver function and behavioural changes. There is no generally accepted guideline for normal bromine levels in blood (Br-B).

Methods

We studied 33 men (age range 21–63 yr, median 32 yr) engaged in soil disinfection inside greenhouses. They used mainly methyl bromide and no other bromine-containing pesticides. Application was by means of fumigation tubes (23 subjects) or cans (10 subjects). Duration of employment in this work ranged from a few months (8 subjects) to 11 yr with a median of 2 yr; the estimated amounts of pesticide applied per person during the last season were

1500–2500 kg (12 subjects) and 5000–6000 kg (21 subjects). Five subjects mentioned that they had symptoms of methyl bromide intoxication 2–4 yr previously. Each subject was examined once in the University Hospital on a Thursday afternoon in groups of four during the application season.

Venepuncture was carried out to determine Br-B, liver function (s-AsT (serum aspartate aminotransferase), s-ALT (serum alanine aminotransferase) alkaline phosphatase and serum protein electrophoresis.

The presence of subjective symptoms of methyl bromide intoxication was determined by a specially designed questionnaire.

A general neurological examination was performed.

An EEG was recorded using the 10–20 International Electrode System with bipolar and unipolar leads. The duration of the EEG recording was at least 30 minutes. Blocking of the alpha-rhythm was checked by opening and closing the eyes. As evocative techniques, 4-minute hyperventilation, photostimulation and hyperventilation together with photic stimulation and acoustic stimulation were used. For photic stimulation, repetitive flashes of light were presented at rates in the range of 1–30/s. For acoustic stimulation, sound of various frequencies in the range of 500–5000 Hz was used; pulse frequency was varied between 1–30 pulses/s. The patient was lying on a stretcher in a room separated from the recording room.

Br-B was measured in whole blood by non-destructive neutron activation analysis (De Goeij *et al.*, 1976). 1 ppm = 1 mg/l = 12.5 µmol/l.

The relationship between different factors was studied by means of a product moment correlation

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coefficient (r) or Student's t test. Probabilities were calculated by a one-tailed t test.

Results

BROMINE IN BLOOD

Br-B varied between 4 and 23 ppm. The precision of the determination (coefficient of variation based on seven duplicate determinations) was 7%. Because there were many low values the logarithm was used in the calculations.

SERUM ENZYMES

Alkaline phosphatase exceeded the upper limit (40 U) in two subjects (44 and 48 U); no relationship with Br-B was found ($P > 0.1$).

S-AsT and s-AIT exceeded the upper limit (12 U) in one other subject (13 and 15 U respectively); according to the manufacturer only values of over 19 U are pathological. Mean ($\pm$ SD) s-AsT activity was 9.3 ± 2.1 U and s-AIT 7.6 ± 2.2 U. A relationship with Br-B was found for s-AsT ($r = 0.32$, $P < 0.05$) and to a lesser extent for s-AIT ($r = 0.26$, $0.1 > P > 0.05$). Re-

gression analysis shows that an increase of Br-B from 4 to 14 ppm was related to an increase in s-AsT of 1.9 ± 0.8 U ($m \pm SE$) and s-AIT of 1.6 ± 1.1 U.

SERUM PROTEIN ELECTROPHORESIS

Except for small deviations in two subjects all values were in the normal range. No relationship was found with Br-B.

QUESTIONNAIRE

For each subject, affirmative answers (maximum 36) to questions relating to symptoms were summed. Scores varied between 0 (8 subjects) and 15 (1 subject) with a median of 3. Subjects with a score of 4 or more had no higher Br-B than those with a score less than 4 ($P > 0.1$). When a symptom was mentioned by six or more subjects, their Br-B was compared with that of the others, but no difference was found. Thirty-one subjects replied 'Yes' to the question 'Do you feel healthy?'

GENERAL NEUROLOGICAL EXAMINATION

Slight signs, which theoretically could have been caused by methyl bromide, were found in five subjects: these were hyporeflexia (two cases),

Fig. 1 EEG of a man (41 yr) exposed to methyl bromide. Bilateral spike and wave discharge (see arrow), increase of beta- and theta-activity together with sharp waves. Concentration of bromine in blood, 17 ppm.

Fig. 2 Bromine in blood in methyl bromide workers, with normal ($n = 23$) and slightly disturbed ($n = 10$) electroencephalogram ($P < 0.05$).

— = geometrical averages
 - - - = cut-off value (see text)

myoclonia, ataxia and nystagmus (one case each). No correlation was found between the presence of an abnormality and Br-B ($P > 0.1$).

EEG

In 10 subjects an abnormal EEG was found. Three subjects showed a diffuse increase of β activity in all cerebral regions with an amplitude of $30\text{--}50\ \mu\text{V}$. Diffuse θ activity with an amplitude of at least $50\ \mu\text{V}$ in the whole trace was found in four cases. In one subject there was a diffuse increase of β and θ activity. Two subjects showed an increase of β and θ activity together with sharp waves. One of these two subjects showed one bifrontal synchronous spike and wave activity (Figure 1). Subjects with an abnormal EEG had a higher Br-B: the geometric mean was $10.9\ \text{ppm}$ ($m \pm \text{SD}$: $6.2\text{--}19.2$) compared with $8.2\ \text{ppm}$ ($5.6\text{--}12.0$) in the others ($P < 0.05$). The results are shown in Figure 2. No relationship was found between Br-B and the specific nature of the disturbances.

EXTERNAL EXPOSURE

No relationship was found between duration of employment (less vs more than one season) and

EEG abnormalities, transaminases or score on the questionnaire.

Subjects who had applied more methyl bromide ($\geq 5000\ \text{kg}$ vs $\leq 2500\ \text{kg}$) during the last season, tended to have higher Br-B ($0.1 > P > 0.05$), but it was not related to an abnormal EEG, transaminase level or symptom score.

OTHER RELATIONSHIPS

The five subjects with neurological signs had no higher score on the questionnaire than the others. The same applied to the 10 subjects with an abnormal EEG; of these, only one subject also had a neurological sign (ataxia). There was no relationship between the presence of an abnormal EEG and high transaminase activity. There was no relationship of age to Br-B or to abnormal EEG. The five subjects with past histories of methyl bromide intoxication had no neurological signs or EEG abnormality at the time of this study.

Discussion

The results of this study need confirmation because the effects found were marginal and were not reported earlier in apparently healthy workers.

Data on bromine, determined by neutron activation analysis, in the blood of non-exposed subjects are few. In pooled human whole blood De Goeij *et al.* (1976) found $3.7\ \text{ppm}$; the range in non-exposed subjects is only a few ppm (Bowen, 1974). Therefore, bromine in the blood of most workers in this study must have been increased by occupational exposure.

Although none of the values were pathological, the correlation between serum transaminases and Br-B indicates an effect of methyl bromide, probably hepatocellular damage. The absence of pathological transaminase values indicates that the EEG abnormalities are not attributable to hepatic encephalopathy. Because the increase, only a few units over the whole range of bromine values, is very small compared with the increase of more than 100 units in manifest clinical disturbances, the present effect *per se* seems to be not unacceptable so far. However, future studies should give more information on the reversibility and other aspects of liver function.

Contrary to the findings of Shapovalov (1974) no decrease of the albumin/globulin ratio was found; this may be attributable to higher exposure or the presence of other bromine compounds in that study.

No relationship was found between Br-B and neurological signs, but demonstration of possible effects is hampered by the difficulty in practice of quantifying each symptom in every subject.

The occurrence of EEG abnormalities confirms observations in acute intoxication (Araki *et al.*, 1971; Merellio *et al.*, 1974 and others), but it was not reported earlier in apparently healthy workers. The abnormalities possibly reflect an early stage in the development of encephalopathy. In this respect it will be important to study the extent of improvement or deterioration in such subjects.

Our data do not allow us to postulate a no-effect level for Br-B with regard to EEG abnormalities because the normal and abnormal EEG group, although differing significantly, showed a large overlap of Br-B values. This may be attributable to the possibility that subjects with abnormal EEG but with low Br-B during examination may have acquired the former because of more intensive previous exposure, for example some weeks earlier. The half-life of Br-B after methyl bromide exposure is not known, but we may surmise that it is of the order of some weeks. Hine (1969) noted a five-fold decrease of bromide in blood during several months after acute intoxication and Gay (1962) measured a half-life of two weeks for bromide in blood after ingestion of inorganic bromide. However, methyl bromide first has to be converted to the bromide ion. Also, in our study subjects with recently acquired high Br-B may not have had time to develop EEG disturbances.

For practical purposes, such as screening exposed workers, it is desirable to estimate a cut-off value for Br-B which is valid for prediction of the existence of an EEG abnormality. For that purpose both sensitivity (the fraction of the group with abnormal EEG above the cut-off value) and specificity (the fraction of the group with normal EEG below this value) should be high (Zielhuis and Verberk, 1974). From our data (Fig. 2), the optimum value appears to be 12 ppm, sensitivity being $6/10 = 0.60$ and specificity $19/23 = 0.83$. These figures mean that if we had performed an EEG only in workers with Br-B above 12 ppm, 60% of the abnormal EEGs and 17% of the normal EEGs would have been found.

The proportion (60%) of EEG abnormalities in workers with Br-B > 12 ppm is impressive compared with the 10% found by the same observer and the same method in a healthy non-exposed population. Of the workers with Br-B ≤ 12 ppm, 17% had an abnormal EEG in this study. From these figures, a risk-ratio of $0.60/0.17 = 3.5$ can be calculated at a cut-off value of 12 ppm; this means that, with

Br-B > 12 ppm, the probability of having a slightly disturbed EEG is 3.5 times that of a worker with Br-B ≤ 12 ppm.

The absence of excess subjective symptoms in subjects with early and exposure-related EEG abnormalities was also described by Härkönen (1977) in a group of 98 styrene workers. We also conclude that the general symptom survey is hardly suited to the prediction of slight EEG abnormalities.

As long as little is known of the course of these EEG abnormalities and their long-term effect on health, more extensive and preferably longitudinal studies as well as periodical individual screening are recommended. Our study suggests that, for this purpose, bromine in blood appears to be useful as a guideline in screening workers for early effects of methyl bromide.

References

- Araki, S., Ushio, K., Abe, A., Uchara, K., and Suwa, K. (1971). Methyl bromide poisoning: a proposal for a new classification and the results of its application to fifty-seven cases so far reported in Japan. *Japanese Journal of Industrial Health*, **13**, 419-424.
- Bowen, H. J. M. (1974). Problems in the elementary analysis of standard biological materials. *Journal of Radioanalytical Chemistry*, **19**, 215-226.
- De Goeij, J. J. M., Tjioe, P. S., Pries, C., and Zwiers, J. H. L. (1976). Vergelijkend onderzoek van sporemetalen in menselijk bloed. *Report Interuniversity Reactor Institute No.* 133/76/10.
- Garcia Rico, A. M., Garcés Brusés, J., Mas Marfani, J., and Nolla Panades, R. (1974). Intoxicación aguda colectiva por bromuro de metilo. *Medicina Clinica*, **63**, 291-296.
- Gay, H. H. (1962). Blood and urinary levels following exposure to bromides. *Industrial Medicine and Surgery*, **31**, 438-439.
- Härkönen, H. (1977). Relationship to occupational styrene exposure and to the findings of electroencephalographic and psychological examinations. *International Archives of Occupational and Environmental Health*, **40**, 231-239.
- Hartzuiker, J. A. (1968). Methylbromide intoxicatie. *Nederlands Tijdschrift voor Geneeskunde*, **112**, 1934-1936.
- Hine, C. H. (1969). Methylbromide poisoning. A review of the cases. *Journal of Occupational Medicine*, **11**, 1-10.
- Merellio, T., Gaultier, M., and Bismut, C. (1974). Electroencephalogram during acute methylbromide poisoning. *European Journal of Toxicology*, **7**, 119-132.
- Shapovalov, Yu. D. (1974). Metabolic changes in workers producing elementary bromine and its compounds (bromine salts and bromomethane). *Vrachebnoe Delo*, **130**-134.
- Zielhuis, R. L., and Verberk, M. M. (1974). Validity of biological tests in epidemiological toxicology. *International Archives of Occupational and Environmental Health*, **32**, 167-190.