trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture (USDA) and does not imply its approval to the exclusion of other products or vendors that may be suitable. This work was supported in part by USDA Tobacco Cooperative Agreement No. 58-43YK-5-0036 and represents a cooperative investigation by the USDA—ARS and the Kentucky Agricultural Experiment Station (KAES), Lexington. Paper No. 87-3-87 of the journal series of the KAES, Lexington, published with the approval of the Director.

Methylation of DNA of Maize and Wheat Grains during Fumigation with Methyl Bromide

Alvin N. Starratt* and Edwin J. Bond

The possibility that methylation of DNA occurs during fumigation of foodstuffs with methyl bromide was investigated in two grains, maize and wheat, using ^{14}C-labeled fumigant. 7-Methylguanine and 1-methyladenine were identified as major products along with lesser amounts of 3-methylcytosine and 3-methyladenine. 3-Methylguanine was probably also formed in minor amounts. Although less than 1% of the bound radioactivity was associated with the DNA isolated, the results indicated that 0.5–1% of the guanine residues in the DNA of these grains was methylated during treatment with 48 mg/L methyl bromide for 72 h.

Methyl bromide, long used as a fumigant to control insects, rapidly dissipates from foodstuffs after treatment and appears to offer little risk to the consumer (Thompson, 1966; Alexeeff and Kilgore, 1983). Some reaction, however, occurs during fumigation, resulting in methylation of food constituents and the concomitant formation of bromide ion. Although appreciable toxicological effects have not been observed in animals fed food treated with methyl bromide [see Winteringham (1955) and Alexeeff and Kilgore, 1983)], this remains a serious area for concern since so few studies have been conducted.

Proteins are the major site of methylation when commodities are treated with this fumigant; the amount of methylation as indicated by the level of inorganic bromide or of unrecoverable methyl bromide appears to be directly related to protein content (Lewis and Eccleston, 1946; Winteringham and Harrison, 1946; Cova et al., 1986). Studies on wool (Blackburn et al., 1941); wool, silk, collagen, and gelatin (Blackburn and Phillips, 1944); wheat flour (Winteringham et al., 1955; Bridges, 1955); and cocoa beans (Asante-Poku et al., 1974) have indicated that N-, O-, and S-methylation of proteins occurs.

Methyl bromide has been demonstrated to be mutagenic in several systems (Djalali-Behzad et al., 1981; Ehrenberg et al., 1974; Kramers et al., 1985). It also was reported to be carcinogenic in rats (Danse et al., 1984), but this conclusion was not substantiated when the evidence was reexamined (Anonymous, 1984), and it has not been confirmed by other work (Boorman et al., 1986; Reuzel et al., 1987). Identification of labeled 7-methylguanine as a component of DNA from mice exposed to [^{14}C]methyl bromide (Djalali-Behzad et al., 1981) indicates that this fumigant can react with nucleophilic sites of DNA in a manner similar to that observed for other alkylating agents (Singer and Kuśmierek, 1982; Jeffrey, 1985). The methylation of the DNA of foodstuffs during fumigation with methyl bromide, however, does not appear to have been investigated despite the possibility that methylated purines and/or pyrimidines, nucleotides, or other methylated fragments of the nucleic acids may be reincorporated [see Saffhill et al. (1985)] into the DNA of the food consumer, potentially leading to mispairing of the bases and mutation. The present study is concerned with the methylation of maize and wheat DNA during fumigation with [^{14}C]-methyl bromide.

Research Centre, Agriculture Canada, 1400 Western Road, London, Ontario N6G 2V4, Canada.

MATERIALS AND METHODS

Chemicals. [^{14}C]Methyl bromide (8.95 μCi/μmol) from Pathfinder Laboratories (St. Louis, MO) and unlabeled methyl bromide (99.5% pure) from Matheson Gas Products (Whitby, Ontario) were used for the treatments. Concentrations of methyl bromide were determined by gas chromatography using a 2 m × 3 mm (i.d.) nickel column packed with 120/140-mesh Chromosorb 102, in a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector.

3-Methylcytosine, 1-methyladenine, 3-methyladenine, and 7-methylguanine were obtained from Sigma Chemical Co. (St. Louis, MO), and 3-methylguanine and 7-methyladenine were purchased from Chemical Dynamics Corp. (South Plainfield, NJ). O^6-Methylguanine (6-methoxy-2-aminopurine) was prepared from 6-chloroguanine (Sigma) as described by Balsiger and Montgomery (1960).

Radioanalysis. A Beckman LS-9000 liquid scintillation spectrometer was used for quantitating radioactivity. Aliquots of methyl bromide, methylated DNA hydrolysis products, and chromatographic fractions were added to scintillation cocktail (Omnifluor; Dupont Canada, NEN Products, Lachine, PQ). For estimation of the radioactivity in treated maize and wheat, samples were combusted in an R. J. Harvey Instrument Corp. (Hillsdale, NJ) biological oxidizer, Model OX300, and the $^{14}CO_2$ was collected in 15 mL of carbon-14 cocktail (R. J. Harvey).

Methyl Bromide Treatments. Maize grain (30 g) was placed in a 290-mL flask fitted with adapters and septa. A mixture of labeled and unlabeled methyl bromide to give a final concentration of approximately 48 mg/L (calculated on total volume of empty flask) was introduced by means

0021-8561/88/1436-1035$01.50/0 Published 1988 by the American Chemical Society

of a gas syringe for 1- and 3-day exposures at room temperature. Initial concentrations were established by gas chromatography. Wheat grain was treated similarly for 3 days.

Defatting of Treated Grains. After aeration overnight, methyl bromide treated maize and wheat were ground with use of a Phillips coffee bean grinder and defatted by extracting with diethyl ether in a Soxhlet apparatus. The defatted meals were stored at 4 °C until use.

Isolation of DNA. Defatted and ground grain (approximately 4-g batches) in a 50-mL polyallomer centrifuge tube was extracted at 70 °C with 20 mL of 1% cetyltrimethylammonium bromide buffer for 30 min, essentially as described by Rogers and Bendich (1985). After the incubation period, the sample was centrifuged (9000 rpm, 2 min) and the supernatant transferred to another tube for extraction with an equal volume of chloroform–isoamyl alcohol (24:1). Other steps were as described (Rogers and Bendich, 1985) with the exception that longer centrifugation periods were used.

Hydrolysis of DNA. Samples of methylated DNA from 8 to 16 g of grain were hydrolyzed by heating in Reacti-vials (Pierce Chemical Co., Rockford, IL) with 98% formic acid (0.5 mL) at 175 °C for 30 min or with 0.1 N HCl (0.5 mL) at 70 °C for 30 min. After cooling, the formic acid was removed in vacuo and the bases were redissolved in 0.1 N HCl (500–800 μL). Marker methylated bases were added to the 0.1 N HCl solutions, and the preparations were stored at 4 °C until HPLC separation.

Chromatographic Separation of Purines and Pyrimidines. Chromatographic separations were performed with a Waters Scientific (Mississauga, Ontario) HPLC system consisting of two Model 510 pumps, a system interface module, a U6K injector, and a Model 481 variable-wavelength detector operated at 254 nm. This system was controlled by a Waters 840 data and chromatography control station. High-purity water was obtained from a Milli-Q system (Millipore Ltd., Mississauga, Ontario). All chromatography was performed at room temperature. The following columns and systems were used: (1) Partisil 10 SCX, 4.6 mm × 25 cm (Whatman, Clifton, NJ). Solvents: A, 20 mM ammonium formate, pH 4.0, containing 6% methanol; B, 200 mM ammonium formate, pH 4.0, containing 8% methanol. Elution: linear gradient from 0 to 25% B over 25 min at 1.5 mL/min. (2) Vydac 201HS104, 4.6 mm × 25 cm (The Separations Group, Hesperia, CA). Solvents: A, 10 mM potassium acetate adjusted to pH 5.0 with trifluoroacetic acid; B, acetonitrile–water (1:3). Elution: linear gradient from 5 to 50% B over 15 min at 1.5 mL/min. (3) LC-7 ODS, 10 μm, 3.9 mm × 30 cm (CSC Inc., Ville Mont-Royal, PQ). (a) Solvents: A, 10 mM heptafluorobutyric acid in water; B, acetonitrile. Elution: linear gradient from 0 to 5% B over 15 min and then 5% B for 10 min at 2.0 mL/min. (b) Isocratic elution at 1.0 mL/min with 50 mM ammonium formate, pH 3.5, containing 10% methanol.

Several runs (100–200 μL/injection) were used to separate the DNA hydrolysis products. Fractions were collected with a Gilson FC-80 fraction collector, and eluants were removed in a Savant Speed Vac concentrator.

RESULTS AND DISCUSSION

Preliminary results showed that DNA from [^{14}C]methyl bromide fumigated maize, isolated essentially as described by Rogers and Bendich (1985), contained a low level of radioactivity. After hydrolysis, the major portion of this was indicated chromatographically to be associated with the methylated purine bases 7-methylguanine and 1-methyladenine. Larger lots of DNA were prepared to confirm these identifications and to determine whether other methylated bases were also present. Table I presents results showing that 0.3–0.4% of the bound radioactivity in the treated maize was associated with the fraction containing DNA.

Table I. Content of Radioactivity in DNA of Maize Fumigated with [^{14}C]Methyl Bromide

run	wt of maize,[b] g	treatment time, h	total bound radioact, dpm	DNA[a] radioactivity, dpm	DNA[a] % of total
A	15.9	72	3558616	13488	0.38
B	11.8	72	2792768	8758	0.31

[a] Determined after formic acid hydrolysis of the isolated DNA. [b] Defatted.

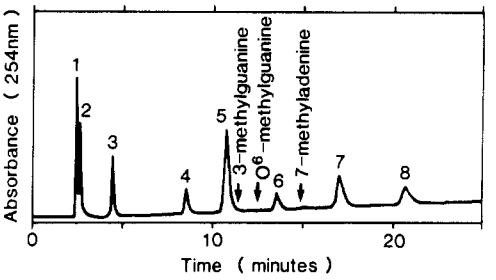

Figure 1. Elution pattern of DNA bases and several methylated derivatives on Partisil 10 SCX, a strong cation-exchange column (see Materials and Methods). Peaks: 1 = thymine; 2 = uracil; 3 = guanine; 4 = 7-methylguanine; 5 = cytosine and adenine; 6 = 3-methylcytosine; 7 = 1-methyladenine; 8 = 3-methyladenine. Arrows mark the retention times of other methylated purines.

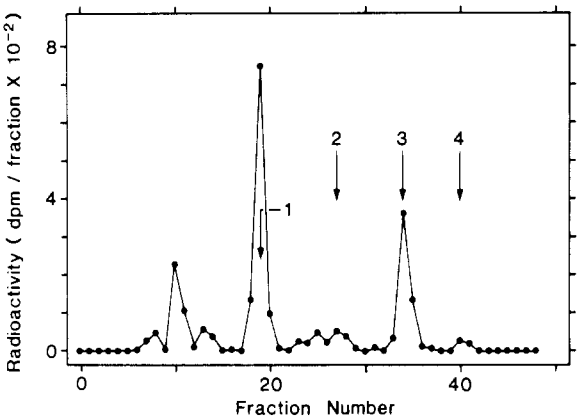

Figure 2. Radioactivity of 0.5-min (0.75-mL) fractions from HPLC separation of an aliquot of the formic acid hydrolysate of DNA from [^{14}C]methyl bromide treated maize on a Partisil 10 SCX column (see Materials and Methods). Arrows show the positions of UV absorption peaks for added markers: 1 = 7-methylguanine; 2 = 3-methylcytosine; 3 = 1-methyladenine; 4 = 3-methyladenine.

The strong cation-exchange column Partisil 10 SCX readily separated the usual purines and pyrimidines of DNA from the methylated bases 7-methylguanine, 3-methylcytosine, 1-methyladenine, and 3-methyladenine (Figure 1). These substances could also be separated on a reversed-phase column. Since the order of elution was quite different, these two chromatographic systems are complementary; use of both also permits the separation and unambiguous identification of 3-methylguanine, 7-methyladenine, and O^6-methylguanine. Chromatography of 25% of the formic acid hydrolysate of DNA from run A (see Table I) on the Partisil 10 SCX column and radioanalysis of 0.5-min fractions showed the presence of two major peaks attributable to 7-methylguanine and 1-methyladenine (Figure 2). Small amounts of radioactivity

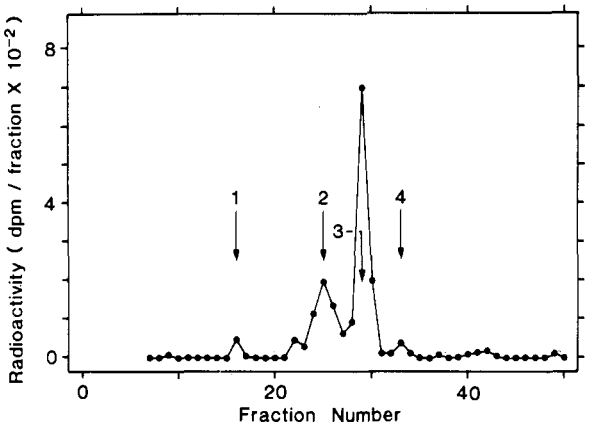

Figure 3. Radioactivity of 0.5-min (1.0-mL) fractions from HPLC separation of an aliquot of the formic acid hydrolysate of DNA from [^{14}C]methyl bromide treated maize on an LC-7 ODS reversed-phase column with acetonitrile–10 mM heptafluorobutyric acid as eluent (see Materials and Methods). Arrows show the position of UV absorption peaks for added markers: 1 = 3-methylcytosine; 2 = 1-methyladenine; 3 = 7-methylguanine; 4 = 3-methyladenine.

were eluted throughout the area in which the methylated bases are found. This included zones with elution times corresponding to 3-methylcytosine and 3-methyladenine, marker substances added before chromatography. A major peak, which eluted earlier than the standards and remains unidentified, was probably due to incompletely hydrolyzed DNA components or to non-DNA material that was methylated since levels and elution times of the radioactivity in this area varied considerably with the preparation and chromatographic system used for analysis. Only two major peaks, corresponding to 1-methyladenine and 7-methylguanine, were obtained with another aliquot of the same hydrolysate chromatographed on LC-7 ODS, a reversed-phase column, with a linear gradient of 0–5% acetonitrile in 10 mM heptafluorobutyric acid (Figure 3). Further basis for this assignment was obtained when material in these peak fractions was rechromatographed under isocratic conditions on the same column with 50 mM ammonium formate (pH 3.5) containing 10% methanol. Again, peaks of radioactivity were obtained with elution times corresponding to those of the respective standards.

For additional confirmation regarding the identity of the methylated bases in DNA isolated from maize treated with labeled methyl bromide, a second sample of DNA (run B, Table I) was hydrolyzed with formic acid and separated on the Partisil 10 SCX column. Results similar to those shown in Figure 2 were obtained when aliquots of fractions were analyzed by scintillation counting. Fractions corresponding to the elution times of 7-methylguanine, 1-methyladenine, 3-methyladenine, and 3-methylcytosine were separately combined. Rechromatography of material in the first three peaks on the Vydac reversed-phase column, using the solvent system described in Materials and Methods, and the fourth on the LC-7 ODS column, using the 10 mM HFBA–acetonitrile system, gave peaks of radioactivity coincident with the elution times of the respective standards. Additional support for the identity of the two major methylated bases, 7-methylguanine and 1-methyladenine, was obtained when remaining material from the Vydac column separation was divided and rechromatographed on the LC-7 ODS column with the two systems described in Materials and Methods. Again, in each case, the peaks of radioactivity corresponded to the elution times of the respective standards. On the basis of radioactivity measurements after the second chromatographic step, the ratio of 7-methylguanine to 1-methyladenine to 3-methylcytosine to 3-methyladenine was 9.1:7.4.:2.6:1.

Table II. Relationship of Time of Treatment to Level of Methylation of Maize Constituents during Fumigation with [^{14}C]Methyl Bromide

wt of maize, g	treatment time, h	[^{14}C]CH_3Br level, mg/L	[^{14}C]CH_3Br act., dpm/μg	[^{14}C]CH_3Br appl dpm	total bound radioact, dpm
30	24	52.3	2368	36 261 600	3 082 200
30	72	52.8	2096	30 392 000	5 200 290
30	72	51.1	2318	34 556 400	5 383 710

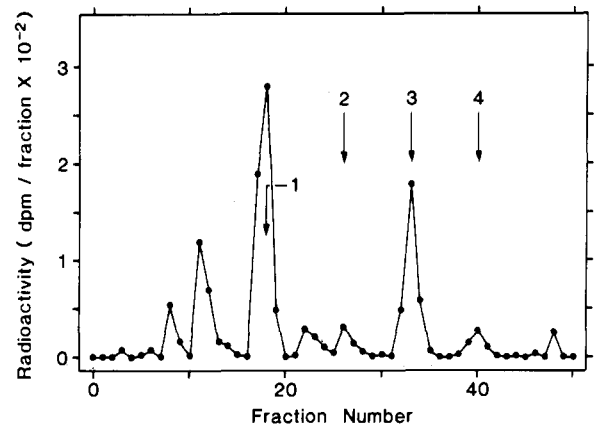

Figure 4. Radioactivity of 0.5-min (0.75-mL) fractions from HPLC separation (Partisil 10 SCX column) of aliquots of a formic acid hydrolysate of DNA from maize treated with [^{14}C]methyl bromide for 24 h. Arrows show the positions of UV absorption peaks for added markers: 1 = 7-methylguanine; 2 = 3-methylcytosine; 3 = 1-methyladenine; 4 = 3-methyladenine.

The recommended level of methyl bromide and treatment time for the control of insect pests in maize is approximately 40 mg/L for 24 h. Although the level is near that used for the maize, the time of treatment is significantly shorter than the 72 h generally used for the present study. For comparison, batches of maize were treated with [^{14}C]methyl bromide at approximately the same level for periods of 24 h (one batch) and 72 h (two batches). As shown in Table II, almost 10% of the methyl bromide used in the treatment reacted with the maize over 24 h while a larger proportion reacted when the treatment time was extended to 72 h. After thorough aeration and grinding, maize treated for 24 h had 102 740 dpm/g while the two batches fumigated for 72 h had 173 340 and 179 460 dpm/g, respectively. The increase in radioactivity with time of exposure is not surprising; in a much earlier study with wheat flour, Lewis and Eccleston (1946) found that the amount of reaction of methyl bromide with the flour increased with treatment period. DNA from 15 g of 24-h treated maize had 2610 dpm, which represents only 0.17% of the bound radioactivity contained in the maize or about half the percentage observed after the 72-h treatment (0.29% of bound). Analysis of the formic acid hydrolysate on the cation-exchange column (Figure 4) again showed that 7-methylguanine and 1-methyladenine were the major methylated bases. Smaller levels of radioactivity were observed at the elution volumes of 3-methylcytosine and 3-methyladenine. Radioactivity measurements for fractions corresponding to the UV absorption peaks of marker substances indicated that 7-methylguanine, 1-methyladenine, 3-methylcytosine, and 3-methyladenine were in a ratio of 9.6:5.3:1.4:1. A similar determination with DNA from one of the batches of maize treated under the same conditions for 72 h indicated a ratio of 8.1:4.5:1.1:1 for these

methylated bases. On the basis of correspondence of retention times with a standard chromatographed subsequent to these experiments, a small peak of radioactivity eluting between 7-methylguanine and 3-methylcytosine (see Figure 4) may by due to 3-methylguanine.

An estimate of the amount of DNA methylation occurring may be obtained from the specific activity of the [^{14}C]methyl bromide. For example, the 2610 dpm found for the DNA preparation from the maize treated for 24 h with fumigant having a specific activity of 2368 dpm/μg (see Table II) represents residue from approximately 1 μg of methyl bromide. A direct measurement of DNA was not obtained, but HPLC analysis of guanine in the hydrolysate leads to an estimate of 218 μg in the sample. This level indicates that the yield of DNA obtained was significantly higher than that obtained by Rogers and Bendich (1985) when a smaller amount of maize was extracted. On the basis of content of guanine, the specific activity of the [^{14}C]methyl bromide, and the radioactivity of the 7-methylguanine (683 dpm), it may be estimated that 0.21% of the guanine residues in this DNA preparation were methylated at the N-7 position.

Three times as much radioactivity was found in a DNA preparation from maize treated with [^{14}C]methyl bromide (2318 dpm/μg) for 72 h as for 24 h. Analysis of the formic acid hydrolysate of DNA from 14.8 g of maize showed 7690 dpm radioactivity of which 2069 dpm was contained in HPLC peak fractions for 7-methylguanine. HPLC analysis indicated that the hydrolysate contained 233 μg of guanine. On the basis of these data, it may be estimated that 0.61% of the guanine residues in this 72-h preparation was methylated at the N-7 position.

In order to test the generality of these observations, methylation of DNA in wheat treated with [^{14}C]methyl bromide was also investigated. Two samples of wheat grain treated with labeled fumigant (1133 dpm/μg) at levels of 48.9 and 46.7 mg/L for 72 h had almost identical levels of radioactivity of (106 470, 106 580 dpm/g) when analyzed after aeration and grinding. As indicated by radioactivity determinations, approximately 20% of the methyl bromide applied reacted with the wheat. This value is similar to that observed for the maize when treated for 72 h (see Table II). DNA from 8-g portions of the treated wheat had 5015 and 5150 dpm, respectively, which represents 0.6% of the bound radioactivity contained in the wheat, an amount substantially above that observed for the maize.

The profile of methylated bases obtained when a formic acid hydrolysate of DNA from wheat treated with [^{14}C]-methyl bromide was chromatographed on the cation-exchange column and fractions were analyzed for radioactivity (Figure 5) was similar to that obtained for the maize. Peaks corresponding to the elution times of 7-methylguanine, 1-methyladenine, 3-methylcytosine, 3-methyladenine, and 3-methylguanine in a ratio of 6.7:4.2:1.2:1:1 were observed. A ratio of 5.9:4:1.5:1:1.1 was found for the hydrolysate of a second wheat DNA preparation.

HPLC analyses indicated guanine levels of 259 and 271 μg in the hydrolysates of the DNA from the two 8-g samples of treated wheat. These quantities are similar to those obtained for DNA isolated from considerably larger batches of maize, indicating that either the wheat used in this study must contain higher levels of DNA than the maize or the isolation was more efficient in the case of the wheat DNA. Rogers and Bendich (1985) obtained a slightly higher yield of DNA from wheat grain than from maize. By the same considerations as described above for the maize, it is estimated that 0.92 and 0.76% of the guanine residues in the two wheat DNA preparations were

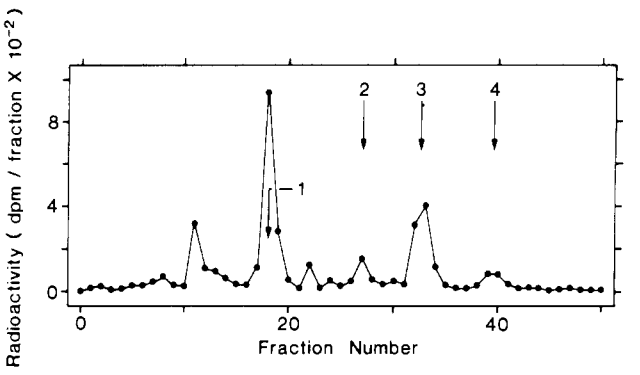

Figure 5. Radioactivity of 0.5-min (0.75-mL) fractions from HPLC separation (Partisil 10 SCX column) of aliquots of a formic acid hydrolysate of DNA from wheat grain treated with [^{14}C]-methyl bromide for 72 h. Arrows show the position of UV absorption peaks for added markers: 1 = 7-methylguanine; 2 = 3-methylcytosine; 3 = 1-methyladenine; 4 = 3-methyladenine.

methylated at the N-7 position.

Even allowing for considerable error, it seems obvious from these results that methylation of DNA occurs to a significant extent when grains are fumigated with methyl bromide. In discussing the decline in germination of rice and maize seeds with exposure to methyl bromide, Sittisuang and Nakakita (1985) suggested that it is likely that changes in certain proteins and enzymes are a main cause of the loss of seed viability. It seems probable that methylation of seed nucleic acids is an additional factor.

Many studies have described the in vivo and in vitro methylation of DNA by a variety of agents [for example, see Jeffrey (1985), Singer (1985), and Blackburn and Kellard (1986)]. With those reacting by the S_N2 mechanism, methylation of the DNA bases occurs almost entirely on nitrogen while those exhibiting S_N1 character show an enhanced, although still small, preference for alkylation at oxygen (Blackburn and Kellard, 1986). It is believed that the relative mutagenicity and/or carcinogenicity of various simple alkylation agents may be correlated with the ability to react with oxygen (Singer, 1985). Since methyl bromide belongs to the former class, most of the methylation would be expected on nitrogen. An attempt to detect O^6-methylguanine in a HCl hydrolysate of DNA from 3.2 g of treated maize was inconclusive; due to a low yield of methylated bases only 7-methylguanine could be identified in the hydrolysate. However, in view of the low proportion (1 part in 300) of O^6-methylguanine observed in the methylation products of DNA treated with methyl methanesulfonate (Lawley and Shah, 1972), another agent acting predominantly by an S_N2 mechanism, it is obvious that larger samples of DNA would be required to determine whether O^6-methylguanine is formed upon methyl bromide treatment of maize and wheat.

In this work, 7-methylguanine was the major methylated base identified as expected since the N-7 position of guanine is generally the primary site of attack when DNA is exposed to alkylating agents including the organophosphorus pesticides which have a long history of use for control of insects associated with food commodities. For example, 7-methylguanine was isolated in a yield of about 1% of the available guanine after in vitro treatment of calf thymus DNA with dichlorvos (Löfroth, 1970). A number of organophosphorus insecticides including dichlorvos (Wennerberg and Löfroth, 1974), trichlorphone and butonate (Dedek et al., 1976), tetrachlorvinphos (Zayed et al., 1983), chlorpyrifos (Mostafa et al., 1983), and methamidophos (Zayed and Mahdi, 1987) have also been reported to methylate guanine in vivo. A major difference

between these results and those for work with the two grains treated with methyl bromide is the relatively large quantity of 1-methyladenine formed in the latter. In most studies with other alkylating agents only small amounts of alkylation at the N-1 position of adenine have been found. Lawley and Brookes (1963) observed the formation of significantly more 1-methyladenine in RNA and heat-denatured DNA than in undenatured DNA when treated with methyl methanesulfonate. Recently, it was reported (Wiaderkiewicz et al., 1986) that thermally denatured DNA yielded more 1-methyladenine than undenatured DNA when treated with the insectide methylbromphenvinphos. In contrast to the maize and wheat results, however, 7-methyladenine and 3-methylcytosine were at approximately the same levels as 1-methyladenine in the denatured DNA after treatment. Perhaps of significance also is the fact that the grains were treated directly with methyl bromide whereas in the other studies the DNA was dissolved in buffer. The results appear to indicate that the N-1 position of adenine in maize and wheat DNA is more accessible relative to the N-7 site of guanine than it is in buffer solutions of undenatured DNA.

ACKNOWLEDGMENT

We thank L. Ross for excellent technical assistance, S. Hobbs for assistance with the gas chromatography, and G. Lambert for the illustrations. Funding for this project was provided by the Methyl Bromide Industry Panel.

Registry No. Methyl bromide, 74-83-9; 7-methylguanine, 578-76-7; 1-methyladenine, 5142-22-3; 3-methylcytosine, 4776-08-3; 3-methyladenine, 5142-23-4.

LITERATURE CITED

Alexeeff, G. V.; Kilgore, W. W. "Methyl Bromide". *Residue Rev.* **1983**, *88*, 101–153.

Anonymous "No Evidence of Methyl Bromide Carcinogenicity Found by NTP Panel". *Pestic. Toxic Chem. News* **1984**, *13(3)*, 9–10.

Asante-Poku, S.; Aston, W. P.; Schmidt, D. E. "Site of Decomposition of Methyl Bromide in Cocoa Beans". *J. Sci. Food Agric.* **1974**, *25*, 285–291.

Balsiger, R. W.; Montgomery, J. A. "Synthesis of Potential Anticancer Agents. XXV. Preparation of 6-Alkoxy-2-aminopurines". *J. Org. Chem.* **1960**, *25*, 1573–1575.

Blackburn, S.; Phillips, H. "Experiments on the Methylation and Acetylation of Wool, Silk Fibroin, Collagen and Gelatin". *Biochem. J.* **1944**, *38*, 171–178.

Blackburn, G. M.; Kellard, B. "Chemical Carcinogens". *Chem. Ind. (London)* **1986**, 607–613.

Blackburn, S.; Carter, E. G. H.; Phillips, H. "The Methylation of Wool with Methyl Sulphate and Methyl Halides". *Biochem. J.* **1941**, *35*, 627–639.

Boorman, G. A.; Hong, H. L.; Jameson, C. W.; Yoshitomi, K.; Maronpot, R. R. "Regression of Methyl-Bromide-Induced Forestomach Lesions in the Rat". *Toxicol. Appl. Pharmacol.* **1986**, *86*, 131–139.

Bridges, R. G. "The Fate of Labelled Insecticide Residues in Food Products. III. N-Methylation as a Result of Fumigating Wheat with Methyl Bromide". *J. Sci. Food Agric.* **1955**, *6*, 261–268.

Cova, D.; Molinari, G. P.; Rossini, L. "Residues after Fumigation with Methyl Bromide: Bromide Ion and Methyl Bromide in Middlings and Final Cereal Foodstuffs". *Food Addit. Contam.* **1986**, *3*, 235–240.

Danse, L. H. J. C.; van Velsen, F. L.; van der Heijden, C. A. "Methylbromide:Carcinogenic Effects in the Rat Forestomach". *Toxicol. Appl. Pharmacol.* **1984**, *72*, 262–271.

Dedek, W.; Lohs, K.; Fischer, G. W.; Schmidt, R. "Alkylation of Guanine in Mice *In Vivo* by Organophosphorus Insecticides I. Trichlorphone and Butonate". *Pestic. Biochem. Physiol.* **1976**, *6*, 101–110.

Djalali-Behzad, G.; Hussain, S.; Osterman-Golkar, S.; Segerbäck, D. "Estimation of Genetic Risks of Alkylating Agents. VI. Exposure of Mice and Bacteria to Methyl Bromide". *Mutat. Res.* **1981**, *84*, 1–9.

Ehrenberg, L.; Osterman-Golkar, S.; Singh, D.; Lundqvist, U. "On the Reaction Kinetics and Mutagenic Activity of Methylating and β-Halogenoethylating Gasoline Additives". *Radiat. Bot.* **1974**, *14*, 185–194.

Jeffrey, A. M. "DNA Modification by Chemical Carcinogens". *Pharmacol. Ther.* **1985**, *28*, 237–272.

Kramers, P. G. N.; Voogd, C. E.; Knaap, A. G. A. C.; van der Heijden, C. A. "Mutagencity of Methyl Bromide in a Series of Short-Term Tests". *Mutat. Res.* **1985**, *155*, 41–47.

Lawley, P. D.; Brookes, P. "Further studies on the Alkylation of Nucleic Acids and Their Constituent Nucleotides". *Biochem. J.* **1963**, *89*, 127–138.

Lawley, P. D.; Shah, S. A. "Reaction of Alkylating Mutagens and Carcinogens with Nucleic Acids: Detection and Estimation of a Small Extent of Methylation at O-6 of Guanine in DNA by Methyl Methanesulphonate *In Vitro*". *Chem. Biol. Interact.* **1972**, *5*, 286–288.

Lewis, S. E.; Eccleston, K. "Residues of Wheat Flour Fumigated with Methyl Bromide". *J. Soc. Chem. Ind., London* **1946**, *65*, 149–152.

Löfroth, G. "Alkylation of DNA by Dichlorvos". *Naturwissenschaften* **1970**, *57*, 393–394.

Mostafa, I. Y.; Adam, Y. M.; Zayed, S. M. A. D. "Bioalkylation of Nucleic Acids in Mice by Insecticides. I. Alkylation of Liver RNA and DNA by Chlorpyrifos". *Z. Naturforsch., C: Biosci.* **1983**, *38C*, 461–464.

Reuzel, P. G. J.; Kuper, C. F.; Dreef-van der Meulen, H. C.; Hollanders, V. M. H. "Chronic (29-Month) Inhalation Toxicity and Carcinogenicity Study of Methyl Bromide in Rats"; Report No. V86.469/221044; Netherlands Organization for Applied Scientific Research, Division for Nutrition and Food Research TNO: Zeist, 1987.

Rogers, S. O.; Bendich, A. J. "Extraction of DNA from Milligram Amounts of Fresh, Herbarium and Mummified Plant Tissues". *Plant Mol. Biol.* **1985**, *5*, 69–76.

Saffhill, R.; Margison, G. P.; O'Connor, P. J. "Mechanisms of Carcinogenesis Induced by Alkylating Agents". *Biochim. Biophys. Acta* **1985**, *823*, 111–145.

Singer, B. "In Vivo Formation and Persistence of Modified Nucleosides Resulting from Alkylating Agents". *Environ. Health Perspect.* **1985**, *62*, 41–48.

Singer, B.; Kušmierek, J. T. "Chemical Mutagenesis". *Annu. Rev. Biochem.* **1982**, *51*, 655–693.

Sittisuang, P.; Nakakita, H. "The Effect of Phosphine and Methyl Bromide on Germination of Rice and Corn Seeds". *J. Pestic. Sci.* **1985**, *10*, 461–468.

Thompson, R. H. "A Review of the Properties and Usage of Methyl Bromide as a Fumigant". *J. Stored Prod. Res.* **1966**, *1*, 353–376.

Wennerberg, R.; Löfroth, G. "Formation of 7-Methylguanine by Dichlorvos in Bacteria and Mice". *Chem.-Biol. Interact.* **1974**, *8*, 339–348.

Wiaderkiewicz, R.; Walter, Z.; Reimschussel, W. "Sites of Methylation of DNA Bases by the Action of Organophosphorus Insecticides *In Vitro*". *Acta Biochim. Pol.* **1986**, *33*, 73–85.

Winteringham, F. P. W. "The Fate of Labelled Insecticide Residues in Food Products. IV. The Possible Toxicological and Nutritional Significance of Fumigating Wheat with Methyl Bromide". *J. Sci. Food Agric.* **1955**, *6*, 269–274.

Winteringham, F. P. W.; Harrison, A. "The Sorption of Methyl Bromide by Wheat". *J. Soc. Chem. Ind., London* **1946**, *65*, 140–149.

Winteringham, F. P. W.; Harrison, A.; Bridges, R. G.; Bridges, P. M. "The Fate of Labelled Insecticide Residues in Food Products. II. The Nature of Methyl Bromide Residues in Fumigated Wheat". *J. Soc. Food Agric.* **1955**, *6*, 251–261.

Zayed, S. M. A. D.; Mahdi, F. M.; "Methylation of Guanine *In Vivo* by the Organophosphorus Insecticide Methamidophos". *Z. Naturforsch., C: Biosci.* **1987**, *42C*, 17–20.

Zayed, S. M. A. D.; Mostafa, I. Y.; Adam, Y.; Hegazi, B. "In Vivo Methylation of Guanine by the Organophosphorus Insecticide Tetrachlorvinphos". *J. Environ. Sci. Health, Part B* **1983**, *B18*, 767–779.

Received for review August 3, 1987. Accepted March 7, 1988.