INVESTIGATIONS ON ADVERSE PROPERTIES OF THE FUMIGANTS METHYL BROMIDE AND PHOSPHINE. 1. RESIDUES OF METHYL BROMIDE IN FUMIGATED COMMODITIES

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Abstract

The fumigants have been subjected to intense scrutiny in recent years because of potential adverse effects and several materials have been de-registered or banned for real or suspected effects. Some materials have been banned because of previously unknown problems and others have caused concern because of possible problems they might produce.

To avoid further restrictions on fumigants still in use appropriate investigation is required so that any potential hazard can be reduced or avoided. We have initiated investigations to provide some additional information on properties of methyl bromide and phosphine that could affect their use and we are currently examining the following aspects of concern.

1. The mode of action of phosphine and development of insect resistance.
2. The corrosion of copper by phosphine.
3. The mode of action of methyl bromide and its relationship to insect resistance.
4. Residues of methyl bromide and the reaction products formed in treated foods.

In this communication are given some results from one of these studies, i.e. residues of methyl bromide occurring in fumigated foods. Information is given on the quantities and distribution of ^{14}C labelled methyl bromide residues in yellow dent corn (maize) and Granny Smith apples after treatment with the fumigant. In the corn it was found that the germ part of the kernel, which made up less than 12% of the mass of the kernel, acquired nearly half of the residue from the fumigant and in the treated apples much of the residue was found in the seeds with less in the skin and the least in the fleshy pulp. Methods for extracting and purifying components of the residue for identification are being developed.

Introduction

In recent years pesticides have been subjected to intense scrutiny because of the potential adverse effects they might have on human health or the environment. The fumigants in particular have been viewed with grave concern, and even fear, because of potential harmful effects; some fumigants have been banned because of previously unknown or poorly understood problems and others have caused concern

Proc 4th Int Work Conf Stored-Product Protection, Tel Aviv, Israel, Sept 1986 [Eds. E. Donahaye and S Navarro], pp. 327-334

because of possible problems they might produce. This concern has been carried to the point where all of the fumigants might be de-registered and thus eliminated from use on food commodities.

Two fumigants (and essentially the last two remaining fumigants i.e. methyl bromide and phosphine) are very widely used at the present time and the reason they are so widely used is that they will give control of many stored product infestations with greater efficacy and economy than any other procedure yet devised. If they were eliminated the control of many of these infestations could be seriously jeopardized and extensive losses of stored food on a world-wide scale could result.

To avoid possible restrictions or complete bans on these chemicals the properties that might cause concern need to be investigated. All adverse properties that could affect use should be studied so that any hazard that might be found could be reduced or avoided. If the fumigants are to be retained for future use it is essential that some very clear answers to real or supposed problems be provided. It is essential to have this information if these materials are to be used with the degree of safety and efficacy which modern society demands. If, perchance, any unacceptable hazard is found and a chemical is to be banned then the decision to ban should be made for the right reasons. Any ban or restriction should be based on sound scientific information and not on public hysteria and political reaction.

To help avoid further unwarranted restrictions on the use of fumigants some investigations have been initiated at the Agricultural Research Centre, London, Canada, to provide additional information on methyl bromide and phosphine to further clarify their positions as to safety and efficacy. In this communication information is given on the quantities and distribution of residues from ^{14}C labelled methyl bromide in yellow dent corn (maize) and Granny Smith apples after treatment with the fumigant.

Residues of methyl bromide in fumigated commodities

The significance of methyl bromide residues to the consumer is not completely known. Many investigations have shown that residues may range from very low, sometimes undetectable levels, to moderate levels of a few parts per million but sometimes to high levels of over 100 ppm. For example Roehm et al (1942) found 149 ppm in rolled oats at one week after the treatment and Halliday and Prevett (1963) found up to 152 ppm in decorticated ground nuts six months after treatment. In most investigations methyl bromide residues have been reported in terms of the total bromide, i.e. the methods of analysis have been based largely on detection of bromide ion. Even for organic bromide the bromine has been converted to the inorganic form for measurement by titration, photometry, x ray fluorescence etc. (Getzendaner 1975).

Only a very few investigations have considered the nature of the residue remaining in the treated products. In 1955 Winteringham and Bridges used $^{14}CH_3$ Br to study the fate of the fumigant in wheat. They found that the gluten fraction was responsible for some 80% of the decomposition of the absorbed fumigant and 50% of this was in the form of N-methyl derivatives. The principal reaction was with the histidine residue of the protein and their investigations lead to the conclusion that loss of the semi-essential amino acid was negligible i.e. the fumigation had little effect on the nutritive value of the product.

New information on the possible hazards of both unchanged methyl bromide and of its reaction products suggest that further investigation would be valuable. A study on methyl bromide administered to the stomach of rats has suggested that the fumigant is carcinogenic (Danse et al 1984). Although this report requires further confirmation to assess its significance, the possibility of carcinogenic effects is very important. Health authorities are very concerned about residues of any chemical that has carcinogenic properties. Also the reaction products formed when methyl bromide reacts with components of food could be of significance. The decomposition of methyl bromide takes place largely through methylation of a substrate and some of the methylated compounds so formed, particularly secondary amines, can lead to the formation of nitrosamines.

The possible formation of N-nitroso precursors when alkylating agents like methyl bromide are used to fumigate food stuffs has been investigated by Dunkelberg (1980). He found that methyl bromide reacts with a number of amino acids to form n-methyl compounds. Glycine, alanine, valine, leucine and glutamic acid all react with methyl bromide, depending on pH and length of exposure time. Other nitrogen containing components of food could also be methylated. The extent of such reactions, particularly in food materials that have a high retention of methyl bromide residue, is unknown. Whether or not such reactions could pose a hazard to human health can only be determined when the nature and the quantity of the reaction products are identified. Considering the current hysteria over pesticide residues in food and the apparent phobia against anything that may be carcinogenic, the need for intensive investigation into this matter is urgent. The future approval of methyl bromide may depend on more information on its residues.

Residue levels in fumigated corn

The first part of the investigation was designed to establish the quantities of residue remaining in food commodities that may be subjected to treatments with methyl bromide. Yellow dent corn (maize) was selected as the test material and the fumigation procedure involved 5 consecutive weekly treatments applied over a period of 5 weeks. Using a dosage of 48 mg l^{-1}, ^{14}C methyl bromide with a total activity of 2 x 10^6 DPM and an exposure period of 18 h the amounts of residue found in corn at two moisture levels were determined using methods previously developed (Starratt and Bond 1981). Five successive treatments were made at weekly intervals and the radioactivity levels were determined both immediately after treatment and after an aeration period of 7 days (Table 1).

Table 1. Residues from methyl bromide in yellow dent corn after 5 consecutive treatments at weekly intervals with 48 mg/l^{-1} of the fumigant and an 18 h exposure period.

Treatment No.	Residue levels (ug/g corn)[1] immediately after treatment		7 days after treatment	
1	153.5	150.9	122.6	108.0
2	174.8	171.8	154.3	135.6
3	264.9	226.7	245.5	208.8
4	311.2	319.0	286.2	227.8
5	327.0	325.2		

[1] Data in columns 1 and 3 are for corn of 11% and in columns 2 and 4 of 13% moisture content. Quantities of residue were calculated from No. of DPM found in the sample.

Examination of the data from Table 1 shows that some residual fumigant (ranging from 7 - 28% in different treatments) was lost in the 7 day aeration period after treatment. When the corn was subjected to the repeated treatments, the level of residue increased so that more than twice the amount remained after treatment number 5 as occurred after the first treatment.

Investigation of the distribution of residue in the corn kernel showed that a large proportion of the labelled material accumulated in the germ (embryo) region of the seed. The quantities found in the endosperm and germ after five consecutive treatments with methyl bromide are shown in Table 2.

Table 2. Residue from ^{14}C methyl bromide in corn kernels after fumigation at 48 mg/l for 18 h with 5 consecutive treatments at weekly intervals (averages from 12 kernels, based on No. of DPM in the samples).

	Whole kernel	Endosperm	Germ
Av. Wt. (mg)	280.9	248.7	32.2
Residues per kernel (ug)	77.7	40.2	37.5
Residue per g tissue (ug)	276.6	160.8	1164.6

The data from this test show that although the germ part of the kernel makes up less than 12% of the mass it acquires nearly half of the quantity of residue from the fumigant. On a weight per weight basis the germ tissue contains more than 7 times the quantity of labelled material found in the endosperm. The presence of the high residue in the germ part of the seed could be of significance both from the standpoint of seed germination and in the use of the germ for human or animal food. The significance of this residue will be dependent on both the type and the quantities of compounds formed as methyl bromide combines with components of the food.

Residue levels in fumigated apples

In similarly designed experiments on Granny Smith apples the quantities of ^{14}C methyl bromide absorbed, desorbed and remaining as fixed residue were also determined. A considerable amount of labelled carbon was found to remain in the seeds of treated apples with less in the skin and the least in the fleshy pulp (Table 3).

Table 3. Amounts of ^{14}C methyl bromide absorbed, desorbed and remaining as residue in Granny Smith apples fumigated with 32 mg/l CH_3Br for 2.5 h at 25°C.

Apple No.	Apple Wt(g)	CH_3Br absorbed (mg)	CH_3Br Desorbed (mg) 0-1 day	1-7 days	^{14}C Remaining after 14 days(ug/g) Skin	Pulp	Seeds
1	172	7.4	6.0	0.2	32.0	8.1	150
2	167	7.2	7.1	0.2	30.1	5.7	153
3	149	9.3	6.9	0.1	34.4	8.3	225
4	168	9.5	4.7	0.2	37.8	7.7	167
5	177	9.9	5.3	0.2	57.5	8.7	229
6	157	9.5	5.1	0.2	43.3	7.2	188

When apples were treated at 10°C (as they may be in many commercial treatments) the quantity of residue was found to be considerably lower than in apples treated at 25°C, even though the concentration to which they were exposed was 1.5 times greater (Table 4).

Table 4. Distribution of ^{14}C methyl bromide in Granny Smith apples treated with 32 mg/l at 25°C and 48 mg/l at 10°C.

Time After Treatment (days)	Content of ^{14}C residue ug/g Pulp	Skin	Seed
	25°C		
0	14.6*	33.7*	22.4*
1	3.0	22	79
7	2.7*	18*	109*
14	2.3*	8.1*	83.5*
	10°C		
0	3.8	7.3	0
1	1.8	10.7	54
7	2.9	11.9	58
14	1.9	9.7	59

Data are averages of 2 samples from each of 4 or 6* apples.

From these data it is evident that appreciable residue does remain in treated commodity and the significance of these residues can only be determined when the nature of the components of the residue are known.

Extraction of residues from fumigated commodity

The next phase of this investigation was concerned with the extraction of labelled compounds in the residue so that they could be isolated and purified for eventual identification. For this work fumigated corn was ground and extracted with appropriate solvents as the first step towards isolation of the components labelled with ^{14}C. After grinding to a fine powder the corn was first extracted with ether to remove any fat soluble reaction products and then extracted with solvents to fractionate the proteins. A summary of the activity found in one extraction run (used as an example) is given in Table 5.

Table 5. Radioactivity found in extracts from ^{14}C methyl bromide treated corn after extraction with various solvents.

		Activity (DPM)[1]	
Solvent	Extract	Supernatant[2]	Precipitate[2]
Water	67,498	67,736	19,878
0.5 M NaCl	43,821	8,417	31,965
55% 2-Propanol	24,643	2,731	24,756
55% 2-Propanol-0.6% mercaptoethanol	32,085	108	38,571
0.2% NaOH	53,830	13,452	44,201

1 The total activity in the treated corn after grinding was 236,680 DPM/g and one g was used for the extraction.
2 The protein in each fraction was precipitated by addition of 25% trichloroacetic acid to a final concentration of 5%. Discrepancies in values between total extract and the supernatant plus precipitate are attributable to differences in procedures for preparation and counting of samples.

From the data in Table 5 it can be seen that radioactive residue is distributed throughout the various fractions (corresponding to albumins, globulins, Zein-1, Zein-2 and glutelin) with a large part in the aqueous extract (amino acids). Negligible activity was found in the ether extract. The proteins separated in these extracts were hydrolysed and the hydrolysates further purified by column chromatography and thin-layer chromatography.

This work is continuing so that the reaction products formed in food materials can be identified and their possible significance to human health can be assessed. The techniques that are being developed for isolating and identifying reaction products on corn will be used for investigations on other fumigated commodities, particularly on commodities that accumulate appreciable quantities of residue. Analysis will also be made for the presence of unchanged methyl bromide in the treated materials.

Acknowledgements. The part of this investigation concerned with isolation and identification of reaction products is being carried out with Dr. A. N. Starratt of this laboratory. Valuable technical assistance has been given by Mr.

S. Hobbs and Miss S. Maunders. We are grateful to the Ontario Pesticides Advisory Committee for funds to initiate this investigation and to the Great Lakes Chemical Corporation and the Pesticides Division of Agriculture Canada for continuing support

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