ENVIRONMENTAL FATE AND ANALYSIS OF INSECTICIDAL FUMIGANT RESIDUES IN STORED GRAIN AND GRAIN-BASED PRODUCTS

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CONTENTS

SUMMARY

The work described in this thesis is principally concerned with the environmental fate and analysis of halogenated hydrocarbons commonly used as insecticidal fumigants on stored grains and their by-products. The fumigation of stored products with these volatile compounds, which has been a common practice for centuries, is designed to destroy insects infesting the food and the storage area. The fumigated grains are normally used as food for human and animal consumption. The chemicals applied on them are highly toxic and are environmental hazards. There has been consistent evidence that some of these chemicals remain associated with foodstuffs as residues for a long period after treatment and therefore become a potential risk to human beings and the environment as a whole.

Environmental pollution, an increasing threat to the world, is often not directly perceptible and therefore its protection has become a major goal all over the world. In attempting to predict the fate of fumigants in cereal grains, a full understanding of how the many parameters may influence the physical or chemical interaction of fumigants with grains or other food commodities and the ability to detect and determine the residues remaining in such food is essential to understanding their impact upon the environment. The effective use of these pesticides and the control of their contamination of the food supply

and the environment, depends heavily on the ability to detect, identify and quantify them, often in very low concentrations.

Analytical methods have been developed for the detection of fumigants as residues on fumigated food products or in the environment. Such methods served a variety of purposes; 1) studies designed to determine optimum fumigation parameters required analytical methods for investigating the effects of different conditions on the effectiveness of fumigation, 2) residues methodology was used to assure that toxic compounds are within internationally established safe limits before consumption of the fumigated products by the general public, 3) tests for fumigant vapours in the atmosphere were needed for the protection of those persons occupationally exposed to a fumigated area and its vicinity, 4) studies of the penetrating power of different compounds aided in decisions on promising new fumigants, and 5) investigations into the nature of terminal residues utilized residue methodology in elucidating the site of reactions that occur between fumigant and commodity.

The work carried out here is basically a description of an attempt to meet the main objectives of the research project discussed in Chapter One, namely; 1) critical literature review on modern fumigants and methods of fumigation, 2) environmental fate and reaction of these fumigants with the constituents of foodstuffs, and 3) development of a method or methods for fumigant residues

or multiresidue analysis.

Many pesticides have been used on stored grains and their products for many centuries. However, like any other sections of technology undergoing revolution, stored grain pesticides have changed drastically from the most toxic and persistent organochlorine pesticides to the more friendly ones like insecticidal fumigants now commercially used on grains. Although these fumigants are volatile and easily dissipate away after fumigation, their presence has been effectively controlled and monitored in order to reduce their toxicity towards living organisms.

Chapter Two describes a comprehensive review of the existing literature pertaining to important fumigants and their method fo application with special reference to maize and wheat grains and their by-products. The study which was carried out during the first part of the research, revealed that only about six insecticidal fumigants are currently in use largely in tropical countries. These are the volatile halogenated hydrocarbons which are applied as vapour to penetrate through the piles of grains in bulk. These fumigants are methyl bromide (CH_3Br), carbon disulphide (CS_2), ethylene dibromide ($C_2H_4Cl_2$) and ethylene dibromide ($C_2H_4Br_2$). In addition, chloroform ($CHCl_3$) and carbon tetrachloride (CCl_4) are usually added as fire and/or explosive retardants or to aid in distribution and penetration and therefore are included as fumigants. Another important fumigant dichlorvos, a solid on resin strips, has been

excluded in this study. Further studies reported that fumigation techniques depend on the chemical used, prevailing conditions and vary from country to country.

Environmental fate discussed in Chapter Three illustrated that the fumigants used for fumigation in storage are not only a public health hazard to those directly involved in fumigation or other related occupations but also those beyond the fumigation and storage premises. These chemicals have been detected in the air, water and soil especially around the fumigation area and therefore a great need for their monitoring and detection in the environment. Much of the literature on this chapter is based on the food commodity as a representative of the diverse environment. Methyl bromide was found to react chemically with wheat constituents with the formation of inorganic bromide and methylated products. There was no evidence of any significant reaction between other fumigants and the constituents of food.

The bulk of the work was however taken up with the development of the analytical method or methods suitable for the detection and quantification of fumigant residues or multiresidue in grains. Many available methods have been tested and validated wherever possible. Chapter Four deals exclusively with developments of methods that are specifically suitable for single fumigant residues. Three analytical methods with high precision and good overall recoveries were developed which allowed the determination

of such a volatile fumigant as methyl bromide in very low concentrations. The three methods are the headspace method, the purge and trap method and the Association of Analytical Chemist Official first method (AOAC). These methods were also found to be suitable for simultaneous multiresidue analysis. With the help of other improved parameters such as the electron capture detector, the capillary column and the tenax adsorbent trap, efficient and sensitive detection with good reproducibility and high recoveries was observed (Chapter Five).

Chapter Six describes the advantages and disadvantages of fumigant residues methodology and evaluation assessment and compares its effectiveness with other available methods. The underlying fact revealed that a more broad and integrated method of assessment which utilises all effective methods is advisable.

Finally, Chapter Seven concludes the findings, with recommendations for further vigilance and monitoring of the fumigant residues in the food supply and the environment. This suggests that, for any meaningful reduction in the risk from these chemicals and to check that internationally recommended maximum residue levels are fully adhered to, determination of fumigant residues is essential before any treated commodity is consumed by humans or animals.

CHAPTER ONE

INTRODUCTION AND THESIS OBJECTIVES

1.1 INTRODUCTION

The history of man is the record of a hungry creature in search of food. Today, around a half billion of the world's population are hungry and suffering from malnutrition, and with current high birth rates and the prolongation of life by the human application of medical science, the problem of raising sufficient food to meet all human needs appears to be increasing. This problem is not new; history is filled with evidence of man's precarious position in the face of famine and disease. Much of this trouble has been caused by insects. For as long as man has been able to gather or produce more food than he could consume immediately, he has been troubled by these insect pests and other organisms such as rodents, birds etc. which eat, contaminate, or otherwise depreciate it. As man realized the advantages of storing surpluses, however small, the insects and other living organisms found veritable mountains of nutritious food. The types of materials stored, types of storage facilities, length of storage, and climatic conditions vary, but the basic problems associated with the preservation of food still remain from those earliest days.

Storage of grain and other cereal products occur in many forms and places, e.g., grain bins, mills, bakeries,

homes, grocer's stores, warehouses and mobile storage in transportation vehicles. Man will always need effective storage to hold food products safely from one harvest to the next, to maintain surpluses, usually for several years, and to transport such goods from places of abundant harvest to those of demand where warehousing is available and where retailing and consumption occur. During storage and in transit, insects infest these commodities and cause a great deal of losses and damage.

Storage losses from insect attack are often as great or greater than those sustained by the growing crops. Moreover, losses in growing crops are frequently obvious, whereas losses in stored grain are likely to be insidious. Experienced grain workers cannot detect internally infested kernels without employing special techniques. Insect damage to growing crops may be counterbalanced to some degree by partial recovery of the damaged plants or increased yield from the survivors, but insect damage to stored grain is final and without compensatory adjustments. When the grain is to be used as human food, losses extend beyond the amount actually consumed and must include the effects of contamination from dead insects, odours, webbing, cast skins, and fragments in the manufacture or processed product. Also, the losses must include damage resulting from insect-caused heating and the translocation of moisture, with subsequent molding and caking.

Estimates of losses to the world's supply of stored grain from insect damage range from 5 to 10 percent of the world's production (Grisamore et al., 1991). In certain tropical and subtropical countries, estimates are higher. Destruction of food by stored grain insects is a major factor responsible for the low levels of subsistence in many tropical countries especially in Africa. In Kenya, for example, approximately 3.03 million tonnes of maize are produced annually. In December 1991, national maize consumption had outstripped the annual production by more than 2.5 million bags which necessitated some importation of grain. A substantial amount of this reduction has been blamed on the increasing high birth rate of about 4 percent annually. There is also concrete evidence to suggest that insect infestation which increased to about 30 percent contributed highly to this overall reduction (Ng'weno et al., 1992). If these losses could be prevented, it would alleviate much of the food shortages in the famine areas of the world.

In many developing countries today, the rate of population growth is such that these countries face difficulties in producing sufficient food for their people. Agricultural practice is still subsistence in nature and the little that is produced is inadequately stored with the result that a significant proportion of the harvest may be lost in storage. This necessitated therefore, a need to protect these essential commodities in order to be more self-reliant with regard to food.

Grain protection has been in practice for centuries in the tropics. Earlier protection included use of materials such as ash and dry neem leaves, which probably acted as an abrasive and repellent respectively (Singh and Benazet, 1974). In the late 1940's, the concept of synthetic chemical control was established and spread during the 1950's. It gradually evolved from mixing of BHC and DDT dust to approved products such as the application of malathion and phosphine. During this period research on relative efficacy of various chemicals and fumigants under tropical storage conditions was also conducted. Hence, the development of the insecticidal fumigants nowadays used as pesticides.

The term pesticide is used generally to include all toxic chemicals used in the control of undesirable illness-producing or economically inacceptable insects, plants, fungi, rodents, nematodes and other pests. Practically, they are considered to be substances or mixtures of substances intended for preventing, destroying, repelling or discouraging anything considered to be a pest, and any substance or mixture of substances intended for use as a plant regulator, defoliant or dessicator. Generally, pesticides form into three main groups, namely; insecticides, herbicides and fungicides. Insecticides are much used and sometimes misused in many tropical regions and therefore represent the topic of discussion in this project. Insecticides are further classified into different subgroups, namely;

organochlorines (OC's), organophosphates (OP's), carbamates and fumigants. As people become aware of the dangers caused by most of the much used highly toxic insecticides, greater emphasis has shifted to lesser toxic or less persistent chemicals such as the fumigants which leave very little or no residue in foodstuffs.

Fumigants (from latin "fumus" or smoke) are gaseous pesticides. Hence, a fumigant can be defined as a chemical that can exist in the gaseous state in sufficient concentration at a required temperature and pressure to be lethal to a given pest organism. The ability to protect stored foodstuffs from attack by insects and mites using toxic volatile gases has been recognised since the end of the last century, when hydrogen cyanide (HCN) and carbon disulphide (CS_2) were two of the earliest compounds used. Other chemicals such as ethylene oxide and methyl bromide are lethal to a wide range of pest organisms and were introduced as both fumigants and sterilants. Most fumigants are readily available volatile industrial solvents, chemical intermediates, or gases that have, until recently, escaped the stringent evaluation now required before modern pesticides are accepted for general use. However, concern regarding the effect of a wide range of contaminants in the environment has resulted in fumigants increasingly coming under scrutiny, and concern about the undesirable toxicological properties of many halogenated hydrocarbons has increased pressure to phase out and replace many of the existing fumigants. Those

used today vary from country to country and with climatic region but some such as gaseous methyl bromide, liquids - carbon disulphide, carbon tetrachloride, ethylene dichloride, and ethylene dibromide and solids - Dichlorvos (DDVP) are generally common.

1.2 ENVIRONMENTAL FATE

The central theme of the continuing public controversy over pesticides has been the detectable presence of residues in the environment particularly in food. Consumers demand that these residues be kept, at least, to a minimum. The residues remaining in food and animal feeds have received most attention in the pesticide residue field, particularly from the public health viewpoint. This is probably as it should be because food represents the most common route of exposure to pesticides that most people experience.

During the course of fumigation, vapour is absorbed by food, by the fabric and structure of buildings, or by soil. As fumigants may be employed at concentrations approaching their saturated vapour pressure, sorption of vapour under such circumstances can be considerable. With volatile compounds such as methyl bromide, sorption is much lower and will depend on many factors, including the properties of the compounds used, ambient temperature, and the commodity being treated and its moisture or lipid content as well as its physical condition. However, after treatment with any fumigant, varying amounts of the

parent fumigant will be held strongly by the commodity for a considerable period after removal of vapour from the surrounding airspace. The rate at which this vapour is lost will depend on the volatility and reactivity of the fumigant, the temperature, and the properties and composition of the fumigated products (Scudamore, 1988).

The wide use of organic fumigants for control of insect infestation during storage of grains and their by-products has caused some concern that residues of the parent fumigants may remain in the food long after application. The volatility of these compounds suggests that they should rapidly dissipate. However, because of their toxicity more positive proof of their absence is desirable.

A fumigant residue is the amount of original or unchanged gas or vapour (parent molecule), or degradation product, metabolite, adduct or conjugate thereof (offspring molecule) that remains on or in a substrate (animal, plant or mineral product) after application of a fumigant. It should be noted that toxicity as such is not mentioned, although it is known that the parent molecule or its offspring derivative must be toxic to the metabolism of insects, mites, nematodes, etc. to be effective. Nevertheless, depending on the nature and the amount of the fumigant derivative, fumigant residues may not be toxic to mammalian or plant systems. Thus, by chemical transformation (photodecomposition, enzymatic hydrolysis, biodegradation), fumigant residues may be

converted to inorganic compounds with minimal mammalian or plant toxicity, although there might be other factors in the toxicity syndrome.

Sorption phenomena influences fumigant behaviour and fumigant residues. Sorption may be: (a) physical in nature, and is mainly due to adsorption, whereby the gas molecules are held by surface (Van de Waals) forces to a solid or liquid surface, and may subsequently be desorbed, the rate of desorption depending on the intensity of the surface forces; or (b) chemical in nature, involving covalent interlocks or bonding, which are permanent. Thus, in addition to miscellaneous fumigant fission (decomposition) products resulting from hydrolysis, oxidation, reduction and other biodegradation mechanisms, there might be physically bound residues, which are reversible, temporary, non-specific with respect to target or substrate which generally reach a maximum rapidly, are sorbed more rapidly at lower temperatures, and can be desorbed by solvents, reduced pressure, increased temperature or prolonged aeration. Chemically bound residues, which are irreversible, permanent, specific with respect to substrate, and increase with temperature and reaction time (contact or exposure period). The nature of the substrate is important. At low concentrations, the amount of gas chemisorbed is a direct function of applied concentration. Depending on the environmental conditions, all fumigants show chemisorption capabilities to some extent. It is conceivable that chemisorption has a role

in detoxification and in resistance of some test species to fumigants. It is not possible presently to estimate the extent to which chemisorption occurs in fumigation, because the criteria to fit all cases are impractical to assess due to the wide diversity in environmental conditions.

Residues remaining after treatment may therefore be classified as (1) unchanged fumigant, physically bound to the commodity; (2) simple reaction products; (3) products due to modification of constituents of the fumigated products, such as those resulting from methylation or alkylation of protein, amino acids and vitamins; or (4) other compounds present in the original formulation either as impurities or added intentionally, such as warning agents. The relative importance of each type of residue will depend on the fumigant, but chemical reactivity can result in undesirable effects of economic importance such as the occurrence of off-odours, taints, and loss in the viability of seeds.

Apart from food, these pesticides have also been detected in water, air and soil, which are collectively termed as environment in this project. In carrying out a general fumigation of a building, sufficient gas to kill the insects must be liberated into the free space and then maintained at the toxic level for a defined period of time. After the treatment, the residual gas remaining in the building is dispersed into the environment outside the mill or fumigation chamber. The normal procedure for

aeration involves either opening doors and windows to allow the gas to diffuse or the operation of exhaust fans to blow it outside the mill. Thus, the residual fumigant can be quickly diluted to low concentrations by mixing with the outside atmosphere. The rate of the dispersal and diluting process is thought to be rapid because of the great amount of space into which the gas is liberated. However, the question of dispersing the toxic gas into the environment, where it might pose a hazard to human beings and other natural living organisms has been a subject of great concern for a long time. One of the main questions is whether the gas does disperse as rapidly as has been assumed or whether it flows out of the buildings in plume-like currents without appreciable dilution to create a potential hazard. This concern has pointed out the need for information on the levels of fumigants that occur in the atmosphere in and around buildings during a fumigation treatment and to ascertain their general fate beyond the walls of the premises.

To check for the recommended level of these toxic chemicals in the environment, effective methods of analysis must be developed and/or validated in order to be able to detect these very volatile compounds in very minute quantities. It has proved difficult to develop a successful method or methods for more than a few fumigants because of the wide range in their boiling points and the differences in their physical and chemical properties. However, a number of methods have been reported for the

simultaneous determination of usually about four or five fumigants.

1.3 THESIS OBJECTIVES

Analysis of pesticides falls generally into three areas; (1) pesticide residues in the environment (Environmental); (2) on-stream analysis in the manufacturing process (Quality control); and (3) analysis of developmental and/or experimental compounds (Formulation analysis or Research and Development). The most important and difficult of these probably are the analysis of residues because of the nature of the samples and the need for detection in very low concentrations i.e. parts per billion (ppb) or less. Research in the pesticide residue field currently falls into three main categories; (a) methodology, (b) metabolism studies, and (c) dissipation relationships. In the past few years, there has been a tremendous upsurge in research on all phases of pesticide methodology including extraction, clean-up detection, identification, and quantification. The emphasis on improved methodology will undoubtedly continue in the future since pesticide metabolism and dissipation studies are wholly dependent on sound methodology.

In the protection of stored food, particularly grain, against loss or deterioration due to insect damage, some millions of tonnes of product are treated with chemical agents throughout the world each year. The

application of insecticidal fumigants as grain protectants, is a well-established practice that, properly carried out, leaves only residues which conform with internationally agreed tolerances. The exact treatment of such consignments is often known and, in addition, government and public authorities carry out analytical surveys to ensure that these limits are not exceeded. Fumigation of foodstuffs is, however, frequently carried out on an ad-hoc basis, as a means of controlling an infestation which has already built up. It may be necessary to treat bulk stores more than once, particularly in tropical climates, and alternative fumigants may be used. Many of these compounds, although exerting their main effect in the vapour phase, are applied as liquids by spraying.

In contrast to the controlled application of solid insecticides, fumigants, particularly liquid formulations, are applied in quantities which, if they remained in indefinite association with the foodstuffs would be deleterious to health since they are in almost all cases mammalian poisons. However, precise dosages are recommended and particular treatments should be carried out on the basis that adequate time and aeration or processing before consumption will allow the removal of, ideally, the whole of the remaining fumigant by volatilization and diffusion, or by breakdown to harmless compounds. Nevertheless, the uncertain fumigation treatment of many consignments moving in international

trade and indeed, the lack of knowledge concerning post-treatment handling after farm or warehouse fumigation of stored produce in the country of consumption presents a situation in which it is desirable that means should be available for monitoring amounts of residual fumigant present in all kinds of foodstuffs at the time of consumption by humans or animals or at some convenient earlier stage. A number of working parties in residues analysis reported that further work was required on the development of general schemes of analysis for unknown residues of insecticides and on the need for simpler means for independent and unambiguous identification of, among others, unchanged halogenated fumigant residues in these foodstuffs.

The range of boiling points of the fumigant normally used, is wide and, in general, the higher the boiling point, the more likely is the fumigant to remain associated with the commodity for a long period after treatment, though other factors such as physical adsorption, lipid solubility and chemical stability play their part. As an example, ethylene dibromide, (B.P. 131°C) a component of liquid formulations, has been known to remain for at least three or more months after application, this initiated its banning by the United Nations in 1984 (Anon, 1984).

To make sure that limits recommended by national and international governments and agencies are routinely and correctly adhered to, methods to analyse these

residues must be developed and validated in order to protect the consumer and the environment as a whole. The main objectives of this thesis are therefore; (1) to critically review the existing literature on fumigant residues in maize and wheat, which are both major basic foods in Kenya, (2) to develop and/or validate a method or methods suitable for general residue or multiresidue analysis and appraise, evaluate and modify wherever necessary the currently accepted methods of analysis under conditions available in Kenya, (3) to determine whether fumigant residues accumulate in food (grains) and to determine the extent of the residues in the grain and its by-products, and (4) to carefully study the metabolism, environmental fate and interactions of these chemicals in the food chain.

CHAPTER TWO

FUMIGANTS AND FUMIGATION

2.1 INTRODUCTION

In modern terminology, as discussed in Chapter One, a fumigant is a chemical which, at a required temperature and pressure, can exist in the gaseous state in sufficient concentration to be lethal to a given pest organism. This definition implies that a fumigant acts as a gas in the strictest sense of the word.

This definition excludes aerosols, which are particulate suspensions of liquids or solids despersed in air, and which are popularly referred to as smokes, fogs or mists. It is important to make this distinction at the outset because it emphasizes one of the most important and useful properties of fumigants: as gases they diffuse as separate molecules. This enables them to penetrate into the material being fumigated and to diffuse away afterwards.

Fumigants are widely used for the control of insects and other animal pests that infest on stored grains and their by-products. This may be attributed largely to the great adaptability of the fumigation technique.

In many cases, fumigation is carried out on infested grains without disturbing it in any way such as in storage as in silos or warehouses, in railway cars,

motor trucks, ships or barges. The development of lightweight plastic sheets, such as those made from polyethylene, has extended the use of gastight tarpaulins or tents for fumigating large piles of grains. Entire buildings can be completely covered and treated simultaneously inside and out.

Fumigation does not require highly skilled personnel unless complicated machinery is used, such as vacuum fumigation chambers or equipment for large-scale recirculation of gases through grain in permanent installations. For most operations, the qualities required are reasonable physical fitness, mental alertness, and the ability both to understand verbal or written instructions and to follow these out carefully and intelligently. In this field, physical fitness includes absence of any repiratory trouble which might make the operator unduly susceptible to the effect of gases. In many operations involving field applications, much of the work may be done by relatively unskilled people working under competent and well-trained foremen.

This chapter deals principally with the use of fumigants as insecticides used in grain protection. The control of birds and mammalian pests is also briefly mentioned in connection with certain individual fumigants. Soil fumigation and control of nematodes which is mainly an aspect of soil fumigation is totally excluded since it is usually taken as a complete separate subject. The currently marketed fumigants are introduced and discussed

and the act of fumigation is summarized.

2.2 PRINCIPLES OF FUMIGATION

2.2.1 Choice of fumigant

There are many chemical compounds which are volatile at ordinary temperatures and sufficiently toxic to fall within the definition of fumigants. In actual practice, however, most gases have been eliminated on account of unfavourable properties, the most important being chemical instability and destructive effects on materials. Damage to materials may take place in several ways, as follows:

1 Excessively corrosive compounds attack shipping containers or spoil the structure and fittings of fumigation chambers or other spaces undergoing treatment.

2 Reactive chemicals form irreversible compounds which remain as undesirable residues in products. In foodstuffs, as it will be seen later in this thesis, such reactions may lead to taint or the formation of poisonous residues. Other materials may be rendered unfit by visible staining or by the production of unpleasant odours.

3 Physiologically active compounds may adversely affect the germination of seeds.

Highly flammable compounds are not necessarily excluded if dangers or fire and explosion can be controlled by the addition of other suitable compounds, or if fumigation procedures are carefully designed to eliminate these hazards. Toxicity to human beings is not usually a cause for exclusion. All known fumigants are toxic to man to a greater or lesser degree, and ways can be devised for their safe handling under the required conditions of application. Table 2.1 below lists six common fumigants used in this project and which are part of the limited number of insecticidal fumigants in common use today. Many of the fumigants used today do, in fact, exhibit one or more of the undesirable properties discussed above. This fact shows that the ideal fumigant has not as yet been found, and it is quite probable that it never will be. Nevertheless, these fumigants are highly useful in their own particular spheres of application. (Anon, 1969).

2.2.2 Evaporation of fumigants

The Boiling Point of different chemical compounds generally rises with the increase of molecular weights. This generalization is borne out by data for the six most commonly used fumigants shown in Figure 2.1, where molecular weights are plotted against boiling points. The relationship stated above holds very well except for methyl bromide, and it demonstrates that important compounds such as carbon tetrachloride or ethylene dibromide will evaporate very slowly under practical

Table 2.1 Essential properties of fumigant commonly used for insect control in stored grains (Source: Anon, 1969)

Name and Formula	Molecular Weight	Boiling Point (at 760 mm pressure)	Solubility in Water (g/l)	Flammability by Volume in Air	Commodities Treated and Remarks
Methyl bromide (CH_3Br)	94.95	4.0	13.4 at 25°C	non-flammable	General Fumigant
Carbon disulphide (CS_2)	76.13	46.0	2.2 at 22°C	1.25-44	Grain - as ingredient of nonflammable mixtures
Chloroform ($CHCl_3$)	119.38	61.0	1.49 at 15°C	non-flammable	Used mainly with other fumigants to aid in penetration and distribution
Carbon Tetrachloride (CCl_4)	153.84	77.0	0.8 at 20°C	non-flammable	Used chiefly in mixture with flammable compounds in grain fumigation to reduce fire hazard and aid distribution
Ethylene dichloride ($CH_2Cl.CH_2Cl$)	98.17	83.0	8.7 at 20°C	6-16	Seeds and grains. Usually mixed with CCl_4
Ethylene dibromide ($CH_2Br.CH_2Br$)	187.88	131.0	4.3 at 30°C	non-flammable	General fumigant

fumigating conditions. If the highest possible concentrations are required at the beginning of the fumigation with such compounds, more rapid volatilization will have to be effected in some way.

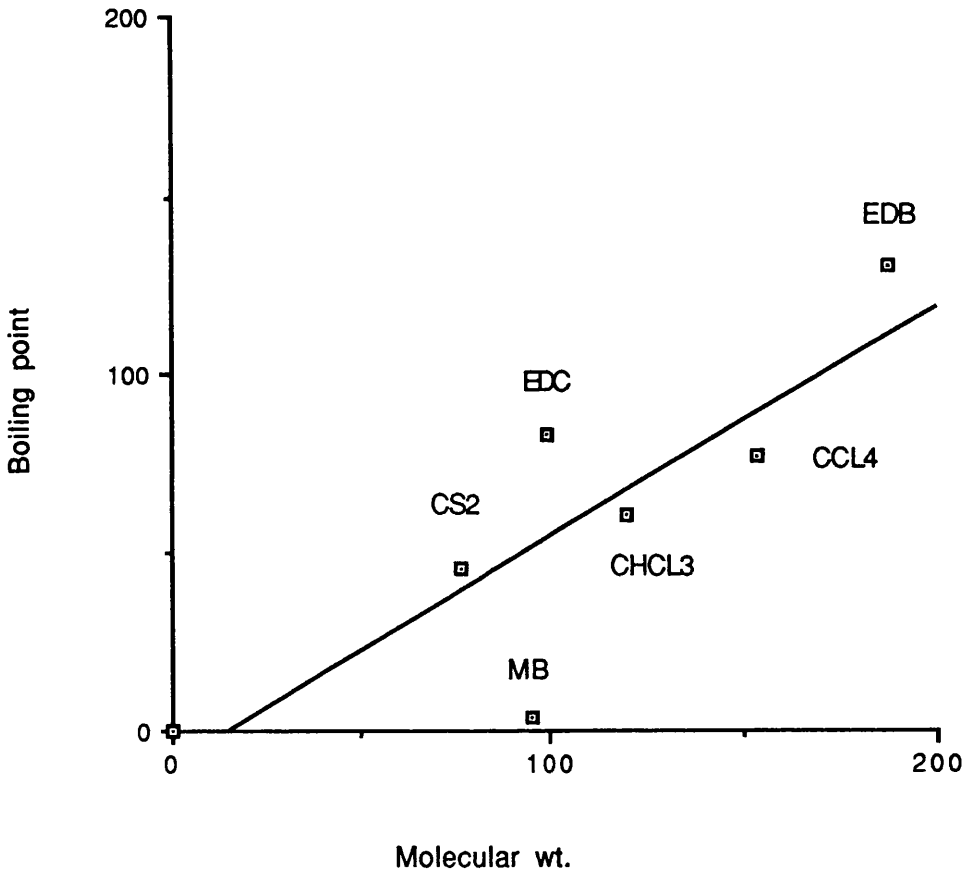
Figure 2.1 shows that, from the physical standpoint, fumigants may be divided into two main groups according to whether they boil above or below room or moderate outdoor temperatures (20°C to 25°C). The low Boiling Point fumigants, such as methyl bromide, may be referred to as gaseous-type fumigants. These are normally kept in cylinders or cans designed to withstand the pressure exerted by the gas at the highest indoor or outdoor temperatures likely to be encountered.

The second main group of fumigants contains those with high boiling points; these are usually described as liquid-type or solid-type according to the form in which they are shipped and handled. In some kinds of work, such as grain fumigation, the slow evaporation of certain liquids is an advantage because the initial flow leads to a better distribution of the gas subsequently volatilized. In other applications where personnel have to distribute the fumigants, slow evaporation of the liquids or solids makes them safer to handle. (Anon, 1969).

2.2.2.1 Maximum concentrations

The maximum weight of a chemical that can exist as a gas in a given space is dependent on the molecular weight of that chemical. This fact, implicit in the well-known hypothesis of Avogadro, has an important

Figure 2.1 Relationship between molecular weight and boiling point of important fumigants.



practical application. It is useless attempting to volatilize in an empty chamber more fumigant than can exist in the vapour form. Table 2.2 shows the maximum amounts of the more important fumigants that can be vaporized in a given space. It will be noted that the fumigants with low boiling points, such as methyl bromide, may be released in large amounts compared with high boiling point compounds, such as ethylene dibromide. The data in Table 2.2, while useful for comparative purposes, apply only to empty spaces. Sorption of the fumigant by the material treated in a given space will permit greater amounts to be volatilized. Nevertheless, the figures given will still apply to the amount which can exist as vapour in the free air space surrounding the fumigated material. (Anon, 1969).

2.2.2.2 Latent heat of vaporization

Unless it is sustained by warming from an outside source, the temperature of an evaporating liquid constantly drops owing to the fall in energy caused by the escape of molecules with greater than average energy. Thus, evaporation takes place at the expense of the total heat energy of the liquid. The number of calories lost in the formation of one gramme of vapour is called the latent heat of vaporization of the liquid. Some fumigants have higher latent heats than others.

The factor of latent heat is of important practical significance. The high pressure fumigants, such as methyl bromide, are usually kept under pressure in suitable

Table 2.2 Maximum weights of some common fumigants which can exist in vapour form in an empty fumigation space at different temperatures (Source: Anon, 1969)

Fumigant	Maximum weight in mg l^{-1} at indicated temperature							
	0°C (32°F)	5°C (41°F)	10°C (50°F)	15°C (59°F)	20°C (68°F)	25°C (77°F)	30°C (86°F)	35°C (95°F)
Methyl bromide	3839.3	4152.8	4079.4	4008.6	3940.2	3874.1	3810.1	3748.3
Carbon disulphide	568.1	701.1	843.7	1010.9	1297.2	1430.8	1740.9	2096.3
Carbon Tetrachloride	288.5	363.0	460.9	572.6	730.9	916.8	1145.4	1398.5
Ethylene dichloride	133.4	173.7	223.7	282.0	350.1	430.3	537.1	668.2
Ethylene dibromide	38.5	54.1	63.7	83.5	112.8	141.2	173.6	214.7

cylinders or cans. On release into the atmosphere, volatilization takes place rapidly and unless the lost heat is restored, the temperature of the fumigant may fall below the boiling point, and gas may cease to be evolved. Also, as the liquid changing to gas is led through metal pipes and tubes, or rubber tubing, the fall in temperature may freeze the fumigant in the lines and prevent its further passage. In many applications it is advisable to apply heat to the fumigant as it passes from the container into the fumigation space.

Fumigants that are liquids at normal temperatures and are volatilized from evaporating pans or vaporizing nozzles, may require a source of heat, such as a hot plate, in order that full concentrations be achieved rapidly.

2.2.3 Diffusion and penetration

As stated earlier, fumigants are used because they can form insecticidal concentrations: (a) within open structures, or (b) inside commodities and in cracks and crevices into which other insecticides penetrate with difficulty or not at all. Hence, it is necessary to study the factors that influence the diffusion of gases in every part of a fumigation system. This study includes the behaviour of fumigants both in empty spaces and also in structures loaded with materials into which the gas is required to penetrate. (Anon, 1969).

2.2.3.1 Law of diffusion

Graham's Law of diffusion of gases states that the velocity of diffusion of a gas is inversely proportional to the square root of its density. Also the densities of gases are proportional to their molecular weights. Therefore, a heavier gas, such as ethylene dibromide, will diffuse more slowly throughout an open space than a lighter one, like methyl bromide. While this basic law is of importance, especially for empty space fumigations, the movement of gases in contact with any internal surface of the structure or within any contained materials is greatly modified by the factor of sorption discussed later.

The rate of diffusion is also directly related to temperature, so that a given gas will diffuse more quickly in hot air than in cold air. (Anon, 1969).

2.2.3.2 Specific gravity and distribution

Many of the commonly used fumigants are heavier than air. If a gas heavier than air is introduced into a chamber filled with air, and it is not agitated by fans or other means, it will sink to the bottom and form a layer below the air. The rate of mixing between the two layers may be very slow.

In good fumigation practice, settling or stratification is not encountered. This is because a proper fumigation makes provision for such distribution of the gas from the very beginning of the treatment. This is ensured by employing singly or in suitable combination: (a) multiple gas inlets, (b) fans or blowers, and (c)

circulation by means of ducts and pipes.

Contrary to popular belief, once a gas or number of gases heavier than air have been thoroughly mixed with the air in a space, settling out or stratification of the heavier components takes place very slowly; so slowly, in fact, that once a proper mixture with air has been secured, the problem of stratification of a heavier-than-air fumigant is of no practical importance for the exposure periods commonly used in fumigation work.

2.2.3.3 Sorption

A most important factor affecting the action of fumigants is the phenomenon known as sorption. It is not possible in this chapter to give a complete explanation of sorption because the interaction of all forces involved is complex. Fortunately, for the purpose of understanding fumigation practice, it is possible to give a general account of the important factors concerned.

In the relationship of gases to solids, sorption is the term used to describe the total uptake of gas resulting from the attraction and retention of the molecules by any solid material present in the system. Such action removes some of the molecules of the gas from the free space so that they are no longer able to diffuse freely throughout the system or to penetrate further into the interstices of the material. In fumigation practices, collision with air molecules tends to slow down gaseous diffusion through the grain material, and sorption takes place gradually. Thus, there is a progressive rather

than immediate lowering of the concentrations of the gas in the free space. This gradual fall in concentration is illustrated in the graphs in Figure 2.2a and 2.2b. The curves of each of the two compounds show clearly the differences in degree of sorption of the fumigants by the same load in the chamber. Throughout the exposure period of six hours, the fall in concentration of methyl bromide (Figure 2.2a) was proportionately less compared with that of ethylene dibromide (Figure 2.2b), both in the empty chamber and with the two loads of grains. This was due to the fact that the internal surface of the chambers and the loads of grains both sorbed less of the methyl bromide than of ethylene dibromide in proportion to the applied dosage. Sorption under a given set of conditions determines the dosage to be applied, because the amount of fumigants used must be sufficient both to satisfy the total sorption during treatment and also to leave enough free gas to kill the pest organisms (Methodology: Ch. 4).

The general term sorption covers the phenomena of adsorption and absorption. These two are reversible because the forces involved, often referred to as van der Waal's forces, are weak. On the other hand, a stronger bonding called chemisorption usually results in chemical reaction between the gas and the material (grain) and is irreversible under ordinary circumstances (Berck, 1975b).

2.2.3.4 Physical sorption

From the point of view of practical fumigation, adsorption and absorption, being both physical in nature

Figure 2.2a Relationship between load (Kgs of grains) and concentration of fumigant in gas phase at atmospheric pressure and 21 º C.

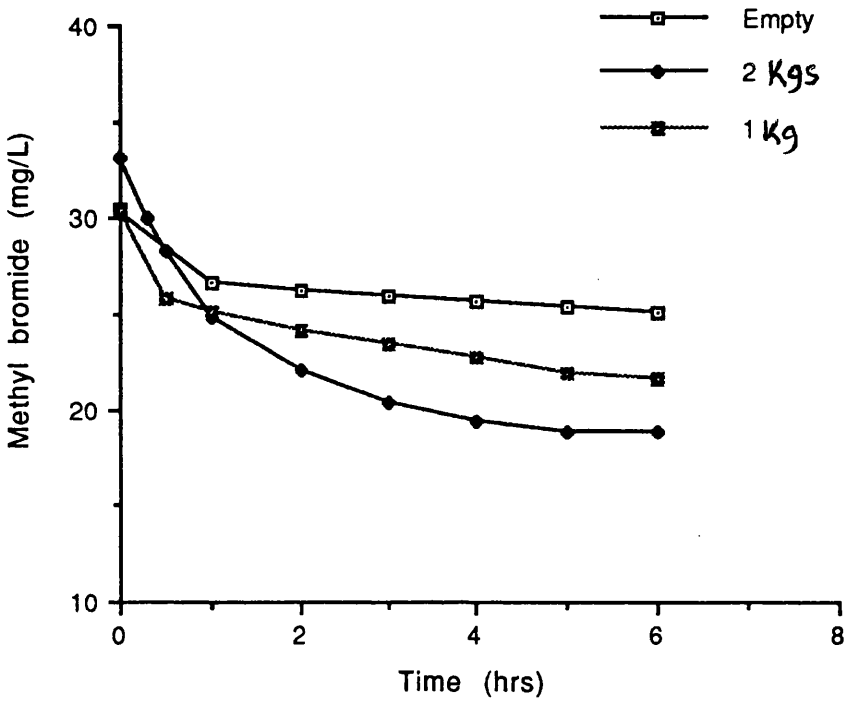
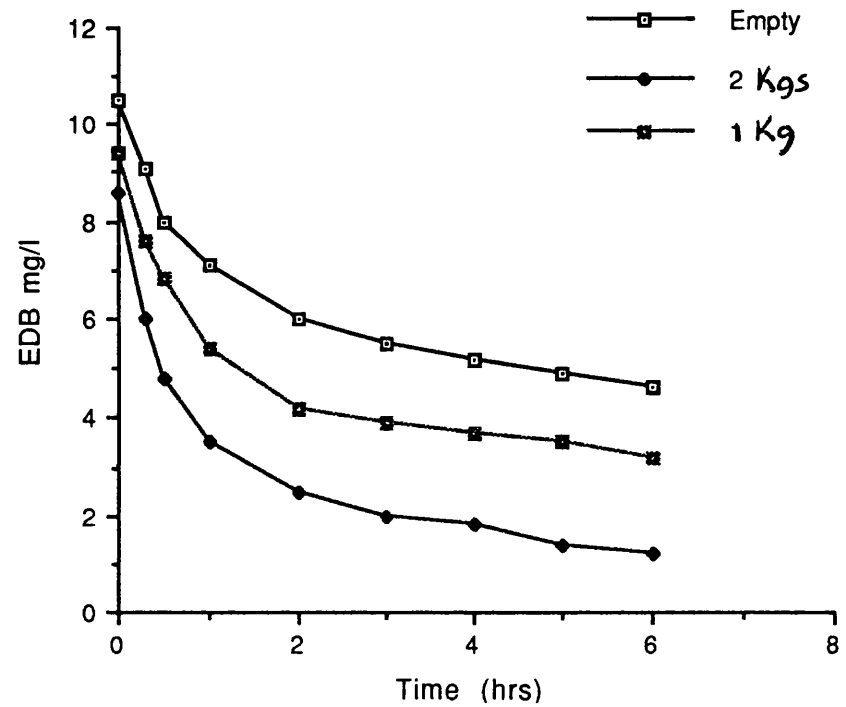


Figure 2.2b Relationship between load (Kgs of grains) and concentration of fumigant in gas phase at atmospheric pressure and 21 º C.

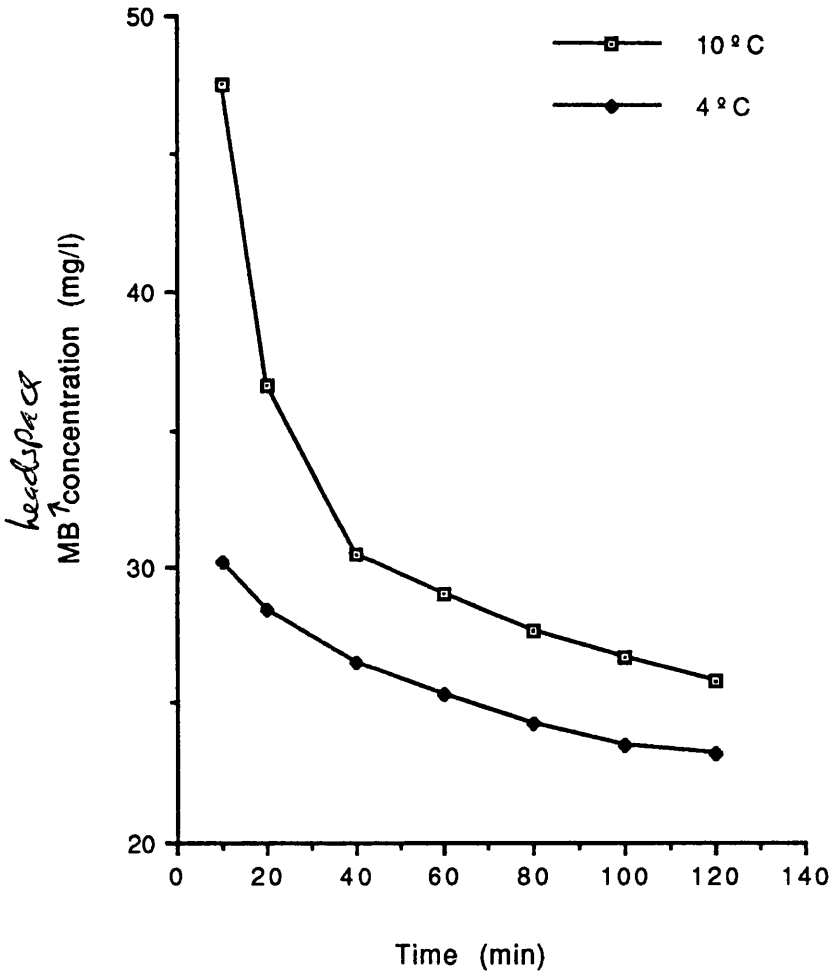


and reversible, may be discussed in this chapter under the§heading of physical sorption. However, it is necessary to make some distinction between them at the outset because the forces involved may be less with adsorption than with absorption.

Stated briefly, adsorption is said to occur when molecules of a gas remain attached to the surface of a grain. Absorption occurs when the gas enters the solid or liquid phase, such as the aqueous phase of grain, and is held by capillary forces that govern the properties of solutions. Physical sorption, considered generally, is an extremely important factor affecting the successful outcome of fumigations. Apart from specific reactions between certain gases and commodities, it may be stated as a general rule that those fumigants with higher boiling points tend to be more highly sorbed that the more volatile compounds. This is illustrated, as was seen, in the graphs in Figure 2.2a and 2.2b; with this particular load there is greater sorption of ethylene dibromide (boiling point 131°C) than of methyl bromide (boiling point 4.0°C) (Methodology derived from Chapter 4).

Physical sorption varies inversely as the temperature, and is thus greater at lower temperatures. This fact has important practical applications. It is one of the reasons why dosages have to be progressively increased as the temperature of fumigation is lowered (Figure 2.3). (Methodology derived from Chapter 4).

Figure 2.3 Effects of temperature on sorption of fumigant by identical loads (weight) of grains.



Sorption may also be influenced by the moisture content of the commodity being fumigated. This was demonstrated by Lindgren et al. (1968) in the fumigation of a number of foodstuffs with methyl bromide; at higher moisture contents more fumigant was sorbed. This effect may be important with fumigants which are soluble in water to any significant degree.

The specific physical reaction between a given gas and a given commodity cannot be accurately predicted from known laws and generalizations. Usually, a certain fumigant must be tested with each material concerned before a recommendation for treatment can be drawn up.

2.2.3.5 Desorption

When treatment of grains is completed and the system is ventilated to remove the fumigant from the space and the grain, the fumigant slowly diffuses from grain. This process is called desorption and is the reverse of physical sorption with the common fumigants and the commodities usually treated, residual vapours are completely dissipated within reasonable periods, although the length of time varies considerably according to the gas used and the material treated. Because of the inverse effect of temperature, dissipation takes place more slowly when the commodity or material is cold, and loss of residual fumigant may be hastened by warming the space and its contents. Also, removal of the gas may be speeded up artificially by employing fans and blowers to force fresh air through the commodity. Natural ventilation may be

quickened by taking the goods out of doors where advantage may be taken of wind, thermal air currents, and in tropical countries like Kenya, the warming effect of sunlight.

An amount of the fumigant, usually very small but perhaps harmful to human beings and animals will not be desorbed because it will react chemically with the grain constituents. It is this amount, left behind in foodstuffs that this project will be dealing with quantitatively.

2.2.3.6 Chemical reaction

If chemical reaction takes place between the gas and the grain constituents new compounds are formed. This reaction is usually characterized by specificity and irreversibility. If the reaction is irreversible, permanent residues are formed. Example is the appearance of inorganic bromide compounds after treatment of some foodstuffs with methyl bromide (Page et al., 1989).

Because this type of reaction is essentially chemical it may be expected that its intensity varies directly with the temperature. Lindgren et al. (1968) found an increase in the bromide content of wheat as the temperature during fumigation rose from 10° to 32°C. This will be demonstrated further in the coming chapters.

2.2.4 Significance of residues in foods

2.2.4.1 Maximum residue levels

In recent years attention has been focused on the nature and possible effects on human beings of insecticidal residues appearing in foodstuffs. World-wide interest in this problem is reflected in the fact that international organizations such as the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) have set up special committees to investigate and report on the nature and significance of residues formed in foodstuffs as the result of the application of pesticides at different stages as in storage and transporation prior to human consumption. These special committees review a number of pertinent factors involved in the use of each pesticide. Important factors, among others, are the toxicological significance of any residues formed and the average fraction of the total diet likely to be constituted by a food containing this residue. Through the Codex Alimentarius Committee these organizations undertake "to recommend international maximum residue levels for pesticide residues in specific food products".

Such recommendations are not binding on member nations of these organizations but are intended to be used as guides when particular countries are formulating their own regulations for pesticide maximum residue levels.

The following definitions of the terms used in work on pesticide residues are given by the joint FAO/WHO

Committee on Pesticide Residues: (Anon, 1983)

1) Residue: A pesticide chemical, its derivatives and adjuvants in or on plant or animal. Residues are expressed as parts per million (ppm) based on fresh weight of the sample.
2) Food factor: The average fraction of the total diet made up by the food or class of foods under discussion.
3) Aceptable daily intake: The daily dosage of a chemical which, during an entire lifetime, appears to be without appreciable risk on the basis of all the facts known at the time. "Without appreciable risk" is taken to mean the practical certainty that injury will not result even after a lifetime of exposure. The acceptable daily intake is expressed in milligrammes of the chemical, as it appears in the food, per kilogramme of body weight (mg/kg/bw).
4) Permissible level: The permissible concentration of a residue in or on a food when first offered for consumption, calculated from the acceptable daily intake, the food factor, and the average weight of the consumer. The permissible level is expressed in ppm of the fresh weight of the food.
5) Maximum residue level (tolerance): The permitted concentration of a residue in or on a food, derived by taking into account the range of residue actually remaining when the food is first offered for consumption (following good agricultural practice) and

the permissible level. The MRL is also expressed in ppm. It is never greater than the permissible level for the food in question and is usually smaller.

Many of the fumigants used today form hazardous residues when used on foodstuffs for insect control. The nature and significance of any residues formed during the treatment of grains will be mentioned briefly under the heading of each particular fumigant (Section 2.4) and discussed further in Chapter Three.

2.2.5 Dosages and concentrations

There should be a clear understanding of the difference between dosage and concentration. The dosage is the amount of fumigant applied, and is usually expressed as weight of the chemical per volume of space treated. In grain treatments, liquid-type fumigants are often used, and the dosage may be expressed as volume of liquid (litres or gallons) to a given volume (amount of grain given as litres or bushels) or sometimes to a given weight (metric tons or tons). (Anon, 1969).

From the moment that a given dosage enters the structure being fumigated, molecules of gas are progressively lost from the free space either by the process of sorption and solution described earlier, or by actual leakage from the system, if this occurs. The concentration is the actual amount of fumigant present in the air space in any selected part of the fumigation system at any given time. The concentration is usually determined by taking samples from required points and

analysing them. It may thus be said that the dosage is always known, because it is a predetermined quantity. Concentration has to be determined because it varies in time and position according to the many modifying factors encountered in fumigation work.

There are three methods of expressing gas concentrations in air: weight per volume, parts by volume, and percent by volume.

2.2.5.1 Weight per volume

For practical designation of dosages, this is the most convenient method because both factors - the weight of the fumigant and the volume of the space - can be easily determined. In countries using the metric system, this is usually expressed in grammes per cubic metre (g/m^3), whereas in countries using the British system of weights and measures, expression is usually in terms of pounds per 1000 cubic feet ($lb/1000ft^3$).

In reports of laboratory experiments, dosages and concentrations are usually given in milligrammes per litre (mg/l), equivalent to grammes per cubic metre. Measurements in this project will be in metric systems unless otherwise stated. (Anon, 1969).

2.2.5.2 Parts or percent by volume

Parts by volume and percent by volume will be discussed here together because both modes of expression give the relative numbers of molecules of gas present in a given volume of air. The values for both modes have the

same digits, but the decimal points are in different places (3475 parts per million by volume of a gas is the same as 0.3475 percent by volume). (Anon, 1969).

Parts per million of gases in air are used in human and mammalian toxicology and in applied industrial hygiene. Percent by volume is used in expressing the flammability and explosive limits of gases in air.

2.2.5.3 Concentration x time products

In the past, most fumigation treatments have been recommended on the basis of a dosage given as the weight of chemical required for a certain space - expressed as grammes per cubic metre or pounds per 1,000 cubic feet, or as volume of liquid applied to a certain weight of material - expressed as litres per kilogramme or gallons per 1000 bushels. Usually this designation of dosage is followed by a statement of the length of the treatment in hours and the temperature at which the schedule will apply. While such recommendations may be based on treatments that have proved successful under certain conditions, they do not take into account the fact that certain factors may modify the concentrations left free to act against the insects. One important factor already mentioned is the effect of loads of different sizes (Figure 2.2a and 2.2b). Another is the leakage from the structure undergoing treatment (Anon, 1969).

What is really important is the amount of gas acting on the insects over a certain period of time. For instance, it is known (Bond and Monro, 1961) that in

order to kill 99 percent of larvae of Tenebroides Mauritanicus (L.) at 20°C, a concentration of 33.2 milligrammes per litre must be maintained for 5 hours. The product 33.2 milligrammes per litre x 5 hours = 166 milligrammes per litre x hours is known as the concentration x time product needed to obtain 99 percent control of this insect (Figure 2.4). It can be abbreviated and referred to as§the ct or (c.t.) product. In the literature it is often expressed numerically with the notation mg hr/l (milligramme hours per litre).

In order to apply this method of treatment designation to practical fumigations, it is necessary to make reasonably correct determinations of the fumigant concentrations required to kill the insects under certain specific conditions; important modifying conditions are temperature and humidity. One such determination is illustrated graphically in Figure 2.4. It has to be noted that in this figure the concentration curve tends to flatten out for short exposures at high concentrations and long exposures at low concentrations and at these extremes, which are not likely to be employed in practice, the constant value for the ct product does not hold. To illustrate specifically the use of the data in Figure 2.4, Table 2.3 sets out the required concentration x time products to bring about 99 percent mortality of T. Mauritanicus using methyl bromide at 20°C and 70 percent relative humidity for various exposures (Anon, 1969):

Table 2.3 - Required concentration x time (ct) products to obtain 99 percent mortality of Tenebroides Mauritanicus. (Source: Anon, 1969)

Concentration methyl bromide in mg/l	Exposure (hours)	ct product mg hr/l
83.0	2	166
55.3	3	166
41.5	4	166
33.2	5	166
23.7	7	166
16.6	10	166

It must be emphasized again that before they are applied in practical use these ct products must be worked out for each specific set of conditions. Each product must be calculated for a given stage of an insect species at a certain temperature and humidity. Under practical conditions variations in temperature are particularly important. In practice, several insect species or stages of a given insect may be treated and therefore the ct product which is applied is that which is effective against the most resistant species or stage present in the system.

The value and possible application of the ct product for the fumigation of insects has been

investigated by a number of workers, (Whitney, 1962; Harein, 1974; etc.). These and other recent studies have confirmed that ct products may be utilized effectively against insects as long as the treatments are conducted under conditions which have been established by experiment and confirmed in practice. The important modifying effects of temperature, humidity and the moisture content of the commodity are emphasized. Kenaga (1961) describes the use of graphs to estimate the effective use of ct products of 8 different fumigants against Tribolium Confusum Duv. under varying conditions of time and temperature. Heseltine and Royce (1960) showed how integrated ct products of methyl bromide may be applied in practice with the aid of specifically designed concentration indicators in the form of sachets.

The use of integrated ct products is particularly useful in routine fumigations when the reaction of a particular species or groups of species has been carefully worked out under the range of conditions likely to be encountered.

Figure 2.5 and Table 2.4 show how an integrated ct product of methyl bromide may be applied in dealing with specific problems. In this instance a hypothetical situation is illustated in simplified form to show how the method could be applied under more complex conditions with multiple gas sampling points. The target of the fumigation is an insect which requires for complete control, under prevailing conditions of temperature and

Figure 2.4 Insect mortality and concentration x time products.

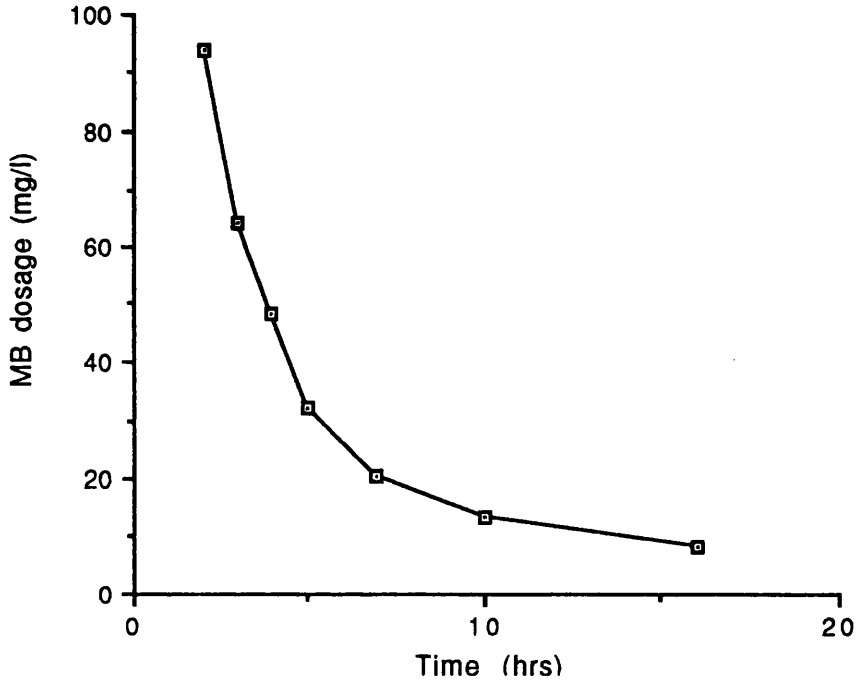
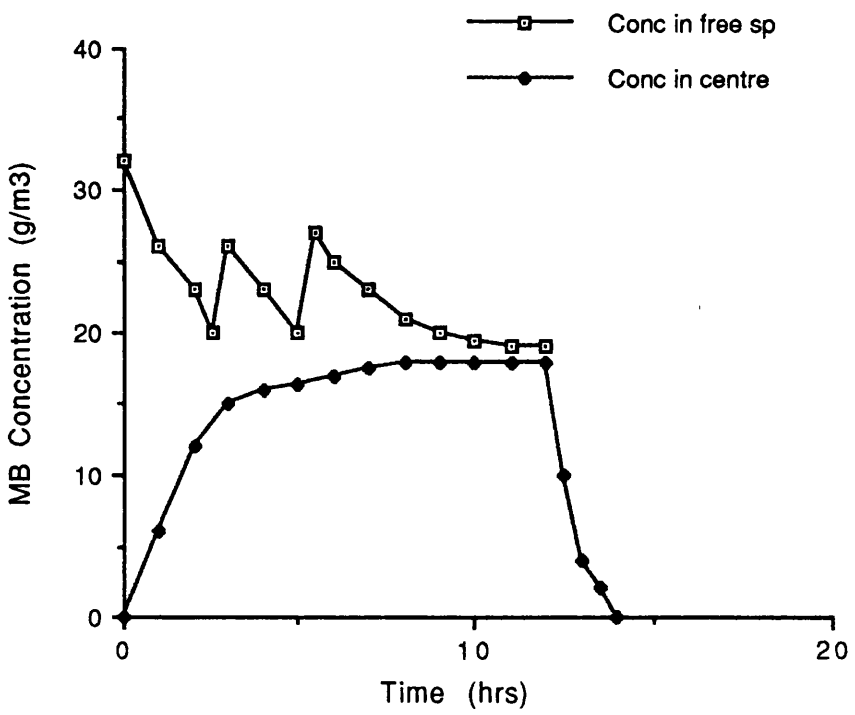


Figure 2.5 Chart of progress of fumigation with Methyl bromide designed to achieve a cumulative CT of 190 g h /m3



humidity, a ct product at 190 gramme hours per cubic metre (g hr/m^3) which is equivalent to 190 milligramme hours per litre (mg hr/l). Leakage from the 100 cubic metre structure and sorption by the commodity are two factors that in this instance influence the concentration of fumigant in the free space and thus within the commodity. It is known that on initial dosage of 32 grammes per cubic metre (g/m^3) may bring about the desired conditions for this load of commodity in a 12-hour exposure period if the concentration in the free space is maintained above 20 milligrammes per litre (mg/l) during the entire exposure. This nominal dose is introduced and concentration readings are made at regular intervals and then suitably plotted from sampling points in the free space and at the centre of the commodity. At the beginning, particular attention is paid to the free space readings. After 2.5 hours it is clear that the free space concentration will fall below the stipulated 20 milligrammes per litre, and 5 kilogrammes of fumigant are added to the system. Again, after a further 2.5 hours (total elapsed time 5 hours) another 5 kilogrammes are added to sustain the concentration. After 11.7 hours the desired ct product of 190 gramme hours per cubic metre has been attained and the treatment is terminated by initiating aeration. The integrated ct product obtained within the commodity, calculated from the concentration plot, is arrived at as shown in Table 2.4 (Heseltine and Royce, 1960).

Table 2.4 - Integrated concentration x time products within the infested commodity.

Hours	Rectangle	Triangle	Total area	Cumulative mg hr/l
1	0	3	3	3
2	6	2.9	8.9	11.9
3	11.7	1.65	13.35	25.25
4	15	1	16	41.25
5	17	0.5	17.5	58.75
6	18	0.5	18.5	77.25
7	19	0.25	19.25	96.5
8	20	-	20	116.5
9	20	-	20	136.5
10	20	-	20	156.5
11	20	-	20	176.5
11.7	13.5	-	13.5	190.0

The use of certified ct products is now, more than before, a practical possibility because accurate and sensitive techniques of fumigant determination, such as the method gas chromatography analysis by electron-capture detector, have been developed and tested. (Refer Section 2.5.1 for types of storage).

2.3 COMMONLY USED FUMIGANTS

2.3.1 Carbon disulphide

2.3.1.1 Introduction

S — C — S

Carbon disulphide (Empirical Formula CS_2) has a boiling point of 46°C, M.P. of -108.6°C and a molecular weight of 76.13, solubility in water 2.2 g/litre at 32°C and miscible with organic solvents. It was one of the first fumigants employed on a large scale. It was first used in France in 1869 against the pest of grapes. For many years afterwards, CS_2 was widely used as both a soil and foodstuff fumigant. Carbon disulphide penetrates well, and is still commonly used in certain parts of the world. It is of practical value in tropical countries, like Kenya, where the high temperatures favour volatilization. Because of its low flash point and explosive nature it is usually formulated in mixtures with nonflammable compounds such as carbon tetrachloride when used for fumgation of grains. (Anon, 1987).

2.3.1.2 Toxicity

Carbon disulphide ranks rather low among the insect fumigants because relatively large dosages by weight are required. It is however, toxic to man. Because it is used in certain manufacturing processes, it is used an important industrial poison. High concentrations of the vapour produce a narcotic effect, and if exposure is continued, unconsciousness and death may ensue from

paralysis of the respiratory centre. Repeated exposure to low concentrations for periods of a few weeks or longer may result in a variety of nervous manifestations which may make correct diagnosis difficult. Persons exposed to low concentrations may lose their ability to detect the odour of the chemical and thus may continue to work in a toxic atmosphere without being aware of it.

Adsorption of high concentrations may take place through the skin as well as by inhalation. Prolonged contact of the skin with high concentrations of vapour or with the liquid may result in severe burns, blistering or neuritis. (Anon, 1987).

2.3.1.3 Chemical properties

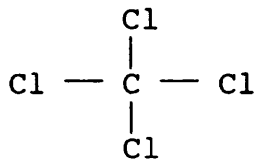
Dense, extremely flammable vapour. It decomposes very slowly on standing. Unpleasant odour due to presence of impurities.

2.3.1.4 Residues in foodstuffs

Reduction in viability of moist seeds has been reported (Bailey et al., 1982). However, the germination of cereal grains (wheat, barley, corn and millet) was not affected at normal fumigation dosages (Heikes, 1987), but when this fumigant was used in a mixture with carbon tetrachloride, some effect on germination was reported (Heikes, 1987). Reaction with proteins and amino groups in food constituents has been suspected, but no studies on this have been reported yet. The available information refers to the possibility of CS_2 itself appearing as

residue in fumigated grains. This will be experimentally determined and reported in Chapters Four and Five.

2.3.2 Carbon tetrachloride



2.3.2.1 Introduction

The principal use of carbon tetrachloride as a fumigant has been for the treatment of bulk grain or for local spot treatment in mills. Because it is relatively nontoxic to insects, nonflammable in any concentration in air, it has usually been applied in mixtures with other compounds such as ethylene dichloride, ethylene dibromide, carbon disulphide to serve in reducing fire hazards or as a carrier liquid which aid in distribution. Its use as a fumigant is declining but it is still permitted for treatment of grain in some developing countries. The widespread use of carbon tetrachloride as an industrial solvent and its global occurrence in the environment means that it may, and it does occur in grain and other foodstuffs as a result of contamination from fumigation and other resources.

2.3.2.2 Physical and chemical properties

Carbon tetrachloride (tetrachloromethane, perchloromethane, CCl_4) has a boiling point of 77°C, M.P. of -23°C, and molecular weight of 153.84. Solubility in

water is at 0.8 g/litre at 20°C and readily soluble in many organic solvents. It is a heavy, stable, nonflammable and nonexplosive liquid.

2.3.2.3 Toxicity

Although, compared with other commonly used fumigants, carbon tetrachloride is not very toxic to insects, it is however, now known to be extremely poisonous to human beings. Toxicity to man is attributed mainly to extensive liver damage, irritation of mucous membranes, headache and nausea.

Human poisoning may be acute as the result of exposure to high concentrations but in practice it more often occurs as a chronic condition from comparatively low concentrations inhaled over extended periods of time. Suspected carcinogen. (Anon, 1987).

2.3.2.4 Residues in foodstuffs

No reaction of carbon tetrachloride with food constituents has been reported. However, breakdown during steam distillation of carbon tetrachloride residues from grain has been reported with the production of chloroform (Scudamore and Heuser, 1973).

Carbon tetrachloride persists in cereals unchanged, gradually disappearing by volatilization. This loss is accelerated by airing and grinding, but low levels of it can be detected in maize meals and bread baked from flour milled from fumigated wheat.

2.3.3 Ethylene dibromide

$$Br - CH_2 - CH_2 - Br$$

2.3.3.1 Introduction

The insectidical properties of ethylene dibromide were reported by Neifert et al. in 1925. One of its main uses as a fumigant has been as a toxic component of a number of liquid fumigant mixtures used on stored grain in the tropics. It has also been used as a soil fumigant, a subject outside the scope of this thesis, and as an industrial solvent, intermediate, or additive.

Although ethylene dibromide is a fumigant of considerable utility, it has a high boiling point, nonvolatile and is greatly sorbed by grains. It has been proved to produce cancers in laboratory animals (Olson et al., 1973), decrease the size and number of eggs from laying hens fed with fumigated animal feed (Bondi et al., 1955, Caylor and Laurent, 1960), and caused malformations of sperm cells in bulls (Fanini et al., 1984). It has, therefore, been banned by EPA in USA (1984) as a food fumigant but, although restricted in many other countries (EEC countries were required to monitor for EDB at 0.01 ppm from last year (1991)), it still finds its use in many developing and tropical countries such as Kenya and many others in Africa and Asia.

2.3.3.2 Properties

Ethylene dibromide (1,2-dibromoethane, EDB or $C_2H_4Br_2$) has a boiling point of 131°C, M.P. of 9.3°C, and

a molecular weight of 187.88. Solubility in water 4.3 g/litre at 30°C and very soluble in many organic solvents. It is a heavy liquid and stable at room temperature.

2.3.3.3 Toxicity

Among the fumigants commonly employed ethylene dibromide is one of the most toxic to insects. However, it is more toxic to human beings than methyl bromide. It causes severe skin irritation and high vapour concentrations may affect the lungs and result in injury to the liver and kidneys. It is suspected carcinogen and known to cause reproductive problems in some animals and birds. (Anon, 1987).

2.3.3.4 Residues in fumigated foodstuffs

Ethylene dibromide (or EDB), in contrast to methyl bromide, does not normally react to any significant degree with the constituents of foodstuffs, but there has been evidence of the formation of small amounts of inorganic bromide in addition to the naturally occurring bromides. At normal temperatures ethylene dibromide breaks down slowly to produce ethylene glycol, which may react with the methionine in cereal proteins (Bridges, 1956; Olomucki and Bondi, 1955).

The main problem with EDB is that, on account of low volatility, it is physically sorbed by fumigated grains; considerable aeration and a long interval of time are required before the vapours are completely dissipated by volatilization or chemical breakdown. Both mechanisms

are accelerated by heat. Residues of great concern therefore, are the inorganic bromide formed and unchanged ethylene dibromide.

Berck (1975b) has shown that the uptake of EDB by wheat increases significantly with increase of moisture content from 9 to 18.5 percent. Both grinding and milling greatly increased sorption.

As Kenaga (1961) has pointed out, the problem of residual EDB in food is likely to be a more serious problem with animal feedstuffs which are often fed without any processing other than blending. On the other hand, food for human consumption other than fruits is subjected to a considerable amount of manipulation and aeration before it is cooked, and any residual fumigant must disappear or be reduced to insignificant amounts. It is clear, however, that great care must be exercised to ensure that residual vapours of ethylene dibromide are fully dissipated from fumigated foodstuffs before they are consumed. The fate of ethylene dibromide in food will be discussed in detail in Chapter Three and its residue analysis will feature predominantly in both Chapter Four and Five.

2.3.4 Ethylene dichloride

$$Cl — CH_2 — CH_2 — Cl$$

2.3.4.1 Introduction

Ethylene dichloride (EDC) is not as toxic to insects as other commonly used fumigants, but it is

useful in the fumigation of grains and other foodstuffs. Because both the vapours and the liquid are flammable, EDC is mixed with some nonflammable compound, usually carbon tetrachloride in the proportion of 3 parts EDC to 1 part CT by volume. The mixture applied according to recommendations has no adverse effect on the milling qualities of grain. Because EDC is soluble in fat and oils, it is usually not recommended for use on cereals or foods with a high oil content. Its use as a food fumigant, therefore, has declined in recent years and is now only restricted to a few tropical countries.

2.3.4.2 Properties

The other synonym name for EDC is 1,2-dichlo-methane with empirical formula $C_2H_4Cl_2$, a boiling point of 83°C, M.P. -36°C, molecular weight of 98.17 and solubility in water of 8.7 g/litre at 20°C. It is readily soluble in many other organic solvents such as chloroform, ethanol, acetone and benzene. It is a heavy liquid, stable and noncorrosive. (Anon, 1987).

2.3.4.3 Toxicity

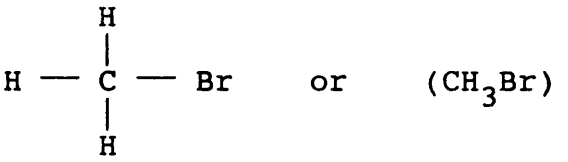
Ethylene dichloride has the property of causing injury to the human liver and kidney from either excessive single or repeated exposures. Acutely, it is somewhat more toxic than carbon tetrachloride and under these conditions it is also a central nervous system depressant and lung irritant. Chronically, it is safer than carbon tetrachloride. In practice, most people will not tolerate

it or will be nauseated by sublethal concentrations.

2.3.4.4 Residues in foodstuffs

No reaction of EDC with food constituents has been reported. During fumigation of cereal grains with ethylene dichloride or its mixture with other fumigants, relatively heavy and continuous sorption of the fumigant takes place (Scudamore et al., 1982). The amount sorbed is higher at lower temperatures. The adsorbed fumigant airs off slowly from whole grains over a period of months. During handling, cleaning or milling processes the amount of adsorbed fumigant is progressively reduced. After milling, a greater proportion of ethylene dichloride is found in the bran than in the whole grain before milling (Berck, 1974). Residues in bread baked from fumigated flour were generally below 0.05 mg/kg (Anon, 1984).

2.3.5 Methyl bromide



or (CH_3Br)

2.3.5.1 Introduction

Since the first reports of its toxicity to insects (Le Goupil, 1932), methyl bromide has been extensively used as a volatile insecticide. Fumigant schedules (Bond, 1984) list dosage rates for a wide range of products including grain stored in bulk or in silos, nuts, dried fruit, cocoa beans, cigarette tobacco, and many other

important stored commodities. Methyl bromide can also be used for treatment of actively growing or dormant plant material, fresh fruit and vegetables, flower bulbs, corms and seeds, and for disinfestation of mills, empty warehouses, railway cars, and aircraft. Rodent infestation can also be controlled with this fumigant.

In most cases methyl bromide disappears rapidly after the end of treatment by volatilization or chemical reaction, but particularly with oily commodities or under cold storage conditions, residues of the unchanged compound may persist for a considerable period. As methyl bromide is a highly toxic compound, recommended maximum guideline levels have been proposed for some commodities (Anon, 1984).

2.3.5.2 Physical and chemical properties

Methyl bromide (bromomethane) has a boiling point of 4.0°C, M.P. -93°c, molecular weight of 94.95 and soluble in most organic solvents. Solubility in water at 25°C, 13.4 g/kg and forms a crystalline hydrate with ice water.

Chemically it is stable, nonflammable liquid, dissolves many organic materials, especially natural rubber, corrosive to aluminium, magnesium and their alloys. Methylating agent reacting with —SH, —OH or —NH groups. (Anon, 1987).

2.3.5.3 Toxicity

The effect of methyl bromide on man and other mammals appears to vary according to the intensity of exposure. At concentrations not immediately fatal, this chemical produces neurological symptoms. High concentrations may bring about death through pulmonary injury and associated circulatory failure. The onset of toxic symptoms is delayed, and the latent period may vary between 0.5 to 48 hours according to the intensity of the exposure and the personal reaction of the patient (Von Oettingen, 1955). Contact of the human skin with the liquid or with strong concentrations of the gas may cause severe local blistering.

Against insects, methyl bromide appears to exert its principal toxic effect on the nervous system. As in man, the onset of poisoning symptoms may be delayed, and with many species of insects definite conclusions as to the success of a treatment should be delayed for at least 24 hours.

2.3.5.4 Reaction and residue analysis

The main reaction of methyl bromide is methylation, although under certain circumstances methyl bromide may be hydrolyzed to methanol. When methyl bromide is used as recommended, problems are seldom encountered, but overdosing or misuse can lead to excessive reaction, which may have important economic consequences. These include production of off-odours or taints, such as in bread baked from fumigated wheat and flour. Studies (Dumas et al.,

1977) have shown that reaction is mainly with NH, OH, or SH groups. Hence, the off-odours that may be produced in bread are due to methylation of sulfur-containing protein amino acids such as methionine, which on hydrolysis may release volatile sulfur compounds. The effect on the B-vitamins is due to the reaction with NH groups.

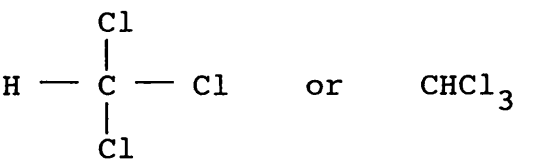
Methyl bromide is widely used today especially in most African countries, for the fumigation of almost every type of cereal product. Because it penetrates densely packed materials, it is especially useful for the treatment of stored grains such as maize, wheat, and their by-products such as flour and meals.

Fumigation with methyl bromide almost invariably leads to an increase in bromide content of the commodity. Concern regarding bromide intake in the total diet of the ever growing world population has led to recommended maximum residue levels of a range of commodities (Anon, 1984). This necessitates reliable analytical methods for the detection of inorganic bromide in addition to the parent compound.

Following fumigation of foodstuffs such as grains, the greater part of methyl bromide is desorbed and diffuses away quickly. Under normal circumstances, gaseous methyl bromide does not present a residue problem. However, there is usually a small, variable amount of permanent residue resulting from the chemical reaction between this fumigant and some constituents of the grain. The reaction product, which is usually easily detectable,

is inorganic bromide. In considering this subject it must be borne in mind that many foodstuffs contain naturally occurring bromides. It is therefore, very important to have a clear understanding of the nature of these residues. As already mentioned, these may consist of (1) residues of the unchanged fumigant, methyl bromide absorbed in the food commodity; (2) total bromide, which is the sum of all compounds such as methyl bromide, ethylene dibromide or other organobromine compounds present; (3) inorganic bromide - the ionic bromide present as a natural constituent by the food plus that resulting from degradation of organobromine compounds applied to the food; and (4) total bromide - the sum of bromide present from all sources. (Anon, 1969).

2.3.6 Chloroform



2.3.6.1 Introduction and its properties

Chloroform is not an insecticide in its true form but normally used in mixture with other insecticidal fumigants used on stored grains. It is utilized to synergize the penetration and distribution of other fumigants and as a nonflammable compound helps as a fire retardant. Chloroform is a colourless and heavy liquid with an ethereal odour. It boils at 61°C, molecular weight 119.37 and a density of 1.49845 at 15°C. It has

a melting point of -63.5°C.

2.3.6.2 Toxicity and residues in foodstuffs

Chloroform has been widely used as an anaesthetic, however due to its toxic effects, this use is being abandoned. It has been found therefore to be toxic to both humans and animals. It damages the liver, heart and kidneys. In the initial stages there is a feeling of warmth of the face and body, then an irritation of the mucous membrane and skin followed by nervous aberration. Prolonged inhalation will bring on paralysis accompanied by cardiac respiratory failure and finally death. It is a suspected human carcinogen.

Chloroform, like other volatile compounds dissipates away from foods. However small quantities of it have been detected in grain and grain-based products like flour and meals. There is not enough information to suggest any reaction of this compound with constituents of foodstuffs (Anon, 1987).

2.4 FUMIGANT MIXTURES

Fumigants are sometimes marketed in mixtures with other compounds. This is for a number of reasons, the more important for practical purposes being:

1) The flammability risk of the toxic ingredient is reduced or prevented altogether by the addition of another chemical. Examples are the addition of carbon tetrachloride to carbon disulphide.

2) With liquid-type fumigants in grain fumigation, mixtures may be made to provide ingredients whose vapours have differing rates and patterns of diffusion. After the liquid has been applied to the surface the ingredients evaporate. The distance the ingredients diffuse downward depends largely on the extent to which they are sorbed by the grain. (This is, in effect, the same as the separation of gases brought about by a chromatographic column). It may therefore be necessary to have various types of ingredients in the mixture to kill insects at various depths and locations in the grain (Kenaga, 1961). An example is a mixture of ethylene dibromide, ethylene dichloride, carbon disulphide and carbon tetrachloride which is used for grain fumigation in farm storage units and country elevators.

3) A highly volatile fumigant such as methyl bromide may diffuse downward too rapidly, so that the upper part of a load of infested goods may not receive an adequate insecticidal treatment. Another less volatile fumigant, such as ethylene dibromide, is added to ensure that the material at the top is properly fumigated. A mixture of this type may be useful under tropical conditions when commodity temperatures may range as high as 38°C (Majunder and Muthu, 1964).

4) The principal toxic ingredient may be diluted so that its distribution becomes more uniform. Carbon

tetrachloride, though only moderately insecticidal in itself, aids in the distribution of other fumigants, such as ethylene dibromide, which does not by itself diffuse well through masses of grain (Berck, 1975b).

2.4.1 Some important mixtures

The mixtures of fumigants in common use today are discussed below. The proportions of fumigants which are in a liquid state at normal temperatures are usually expressed in terms of volume. Mixtures containing volatile ingredients such as methyl bromide are expressed by weight of the components. There are many mixtures of fumigants but only a few very common ones have been mentioned in summary here. (Anon, 1969).

2.4.1.1 Carbon tetrachloride:carbon disulphide (80:20) mixture

A mixture of carbon disulphide (CS_2) and carbon tetrachloride (CCl_4) formulated with 4 parts of CCl_4 to 1 part of CS_2 and commonly known as 80:20 mixture, has been used as a grain fumigant for more than 5 decades. Due to the flammable nature of carbon disulphide, investigations were made in the 1920's by Neifert to find a nonflammable compound such as carbon tetrachloride to act as a fire suppressant. 80% carbon tetrachloride and a 20% carbon disulphide mixture was therefore found to be one of the most commonly and effectively used liquid fumigant formulations. Because of the explosive nature of CS_2, a substitute mixture containing 75% ethylene dichloride and

25% carbon tetrachloride was introduced in its place. There are however many other forms of formulations depending on the country of origin and the method of treatment.

2.4.1.2 Dowfume (EB-5) mixture

This is a general fumigant mixture containing 7.2% ethylene dibromide, 29.2% ethylene dichloride and 63.6% carbon tetrachloride. It is commonly known as Dowfume EB-5. As indicated earlier, ethylene dibromide is highly insecticidal and under normal conditions the fixed residues formed by chemical reaction with the constituents of foodstuffs are of a low order. These advantages are offset by the fact that EDB is highly sorbed by grains undergoing fumigation. As a result there may be poor penetration during actual treatment and prolonged persistence of the vapours in the fumigated commodity during the aeration process. Attempts to utilize the advantages and overcome the disadvantages have resulted in the formulation of a wide variety of ethylene dibromide mixtures. The inclusion of carbon tetrachloride (CT) as a high proportion of the EB-5 formulation appears to add greatly to the effectiveness of EDB principally because carbon tetrachloride acts as an eluant for EDB in a column of grain and assists effectively in its downward migration (Berck, 1975b). The following are some commonly used liquid formulations containing EDB in admixture with other compounds for grain fumigation (percent by volume).

EDB 5:CT 95

EDB 7:EDC 29:CT 64

EDB 7:CS_2 12:CT 81

EDB 3.5:EDC 10:CS_2 10:CT 76.5

EDB 5:CS_2:24:$CHCl_3$ 71

EDB 5:EDC:35:CCl_4 60

2.4.1.3 Dawson (D-73) mixture

Mixtures of ethylene dibromide (EDB) 70% with 30% of methyl bromide (MB) have been found useful for three main purposes.

1) In the tropics to treat bagged grains under gasproof sheets.

2) As local fumigants for treating food handling equipment in mills and food processing plants generally.

3) As a fumigant for grain stored in bulk.

In India, for instance, mixtures of the two fumigants in various proportions are recommended for application under sheets or in chambers to treat different types of grains in bags under warehouse conditions (Majumder, 1962). Treatment with the fumigant mixtures accompanied by simultaneous prophylactic applications of liquid insecticides to prevent reinfestation is known as the "Durofume" process (Majumder and Muthu, 1964). In local fumigation, the mixture is best introduced by special applicators into holes bored at strategic points in the food handling equipment. The proportions of the

mixture for bulk grain fumigation may vary according to the grain being treated and the method of application. For cereal grain treatments the ratio 70:30 EDB to methyl bromide (best known as D-37) is recommended for gravity distribution in smaller bins or farm storages. For forced distribution system, 30:70 EDB to methyl bromide (D-73) is normally preferred. The combination of methyl bromide and ethylene dibromide in D-73 and D-37 increases the downward movement of ethylene dibromide in grain column only slightly. The ethylene dibromide assisting effect of methyl bromide is evidently minimal.

2.5 FUMIGATION OF GRAIN IN BULK

Fumigation of sacked grain is more or less the same as that of bulk grain. However, bulk grain fumigation represents a wider part in most cases and this section reviews briefly the general view of grain fumigation. As already mentioned, fumigants are still used for disinfesting grain in most countries of the world. The chemicals used and the methods of application vary greatly. Differences in techniques may be influenced by the nature of the grains and by the wide range of climatic conditions encountered. One of the most important variables lies in the diversity of structures used for storage. The shape, size, and type of construction of each particular structure creates special problems in achieving and maintaining the concentrations required for the control of the insects and mites present in the

grain. (Anon, 1969).

The more important grain-infesting insects are cosmopolitan; they have been transported through international commerce to many parts of the world. A treatment effective in one country may therefore be successfully adapted in another if due allowance is made for the variables which are necessary to carry out the treatment. The relative amount of destruction caused by certain species varies somewhat from continent to continent, or from country to country. Therefore, the most serious pests of grain may differ in their order of importance from one area of the world to another.

2.5.1 Types of storage

As already stated, the shape and size of a given storage unit are important considerations. Grain storage units are usually broadly classified for fumigation purposes as follows:

1) Upright (vertical) storage. In this type, the height is greater than the length or width. It is mainly found in the form of silo bins in storage units which have elevators. In cross-sction, the bins may be almost any shape, but are usually circular or rectangular.

2) Flat (horizontal) storage. One dimension, either length or width is greater than the height. This type includes a wide variety of structures, including many temporary (sometimes called "distress") storage units.

Railway freight cars (wagons) or motor vans (trucks or lorries) may be included under this heading.

3) Farm-type bins and storage units. These are usually small and often loosely constructed, and their treatment requires special consideration. (Anon, 1969).

In the following description of methods of grain fumigation, brief mention will be made of special applications suited to these three types of storage.

2.5.2 Methods of grain fumigation

The differences between methods of grain fumigation are related primarily to the manner in which the fumigant is initially applied to the mass (Tables 2.5 and 2.6).

1) Direct mixing (vertical storage). By this method, the fumigant is applied to the grain so that it is distributed as evenly as possible from the beginning of the treatment. This method is often used when infestation is general throughout the mass and there is access to the grain stream as the infested grain is being run into storage for the first time, or is being transferred from one bin to another. Only liquid or solid-type fumigants are used in this way. As the grain enters vertical storage units, currents of air containing these toxic fumigant vapours may be forced up into the working place and to the environment in general. This, apart from the residue left behind by these fumigants in the food chain, is toxic to the

Table 2.5 Bulk fumigation of grain in vertical storage
(Source: Anon, 1969)

Fumigant(s)	Dosage per m^3	minim-exposure (days)	Remarks
Direct mixing;			
EDC 75:CT 25	15 litres	4	Good for maize
CS_2 16:CT 84	9 litres	4	Mixture may contain 1 to 2% SO_2
EDB 7: EDC 30:CT 63	8 litres	4	Good for maize
Surface application;			
EDC 75:CT 25	15 litres	8	Good for maize
CS_2 16:CT 84	11 litres	8	Good for maize
EDB 7: EDC 30:CT 63	9 litres	8	Good for maize
CT	19 litres	15	More often used in combination
Recirculation;			
CH_3Br	908 g	1	Should be removed by aeration after 24 hours
CH_3Br 70:EDB 30	454 g	2	Should be removed by aeration

NB: CT - Carbon tetrachloride

EDC - Ethylene dichloride

CS_2 - Carbon disuphide

EDB - Ethylene dibromide

CH_3Br - Methyl bromide

Table 2.6 Bulk fumigation of grain in horizontal storage
(Source: Anon, 1969)

Fumigant(s)	Dosage per m^3	minim-exposure (days)	Remarks
Surface application;			
EDC 75:CT 25	17 litres	8	Good for maize
CS_2 16:CT 84	19 litres	8	Good for maize
EDB 7: EDC 30:CT 63	17 litres	4	Good for maize
CH_3Br	1100 g	1	Must be aerated after 24 hours
Recirculation;			
CH_3Br	1400 g	1	Should be aerated
CH_3Br 70:EDB 30	700 g	1	Should be aerated after 24 hours

fumigation worker inside and harmful to the environment outside. This therefore should be a matter of great concern to everyone.

2) Surface application (flat storage). This method has so far been used mainly with liquid-type fumigants. The liquids are sprayed evenly over the top surface of the grain. The vapours slowly evolve and diffuse downward through the pile of grain. Under modern conditions, this method is usually employed only when the grain cannot conveniently be turned, or as an emergency

measure. Diffusion may be slow, and distribution with some fumigants is often not uniform. In this type of treatment, the grain is usually not aerated and the vapours remain in the grain until dissipated by leakage. This gives rise to desorption of the fumigant from the grain to form harmful residues which must be verified before consumption.

3) Farm storage fumigation. The gastightness of the structure is an important factor in small scale storage because the bulk grain is not large enough in itself to retain vapours once leakage begins at any point. Steel, concrete or tight wooden structures are usually satisfactory without alterations. Most wooden storage structures are leaky, thus polluting the environment and it is advisable to line the floors and walls on the inside with stout roofing paper and to nail boards over any visible openings in the walls. If possible, fumigation during windy weather should be avoided because strong air currents hasten leakage.

The application of fumigants to large masses of grain in various types of structures and storage units involves the dispensing of considerable amounts of relatively toxic fumigants. Under these conditions safety measures are of prime importance not only to protect the operators but also those working in the vicinity and the environment in general. It is always necessary to ensure that all fumigated grain is thoroughly aerated before it

is released to customers and/or consumers.

2.6 FUMIGATION AS PART OF AN INTEGRATED PEST MANAGEMENT PROGRAM

The prevention and control of pest organisms in grain is achieved by a number of policies and procedures that are employed in food storage systems. Careful planning in the design of storage and handling facilities, so that infestation is impeded or prevented, is of great importance. Any peculiarity of the system that might allow the introduction and increase of pest organisms not only endangers the quality of the product but it can also allow cross contamination and undermine the integrity of an entire pest control program. Facilities that are amenable to sanitation and disinfestation procedures are likely to be the most beneficial to food storage and handling systems. Best results may be attained by careful integration of all the procedures that will prevent contamination, suppress development or eliminate pest organisms. (Berck, 1975b).

Fumigation is a widely used procedure that has served an important role in pest control programs for many years. As fumigants can penetrate into materials to eradicate pest organisms, mainly insects, from sites where no other form of pest control is feasible, they are invaluable agents for use in food preservation programs. The continued utility of fumigants is dependent, not only on their penetrating properties and effectiveness as pest

control agents, but also on the introduction of new and adaptable procedures that will meet the demands of an ever-changing food storage technology and on safety procedures that will allow them to be used without harm to human beings and the environment as a whole. Safety measures, both for workers handling fumigants and for consumers eating treated products, must progress to meet the requirements of present day health standards. Although a great amount of research has been done on fumigants and much information is available on their effectiveness, considerably more information is required to fully exploit the potential applications of these materials for pest control, fully understand the impact of the toxic residues left behind in foods and to ensure that the fumigants are used with minimal hazard to both man and his environment. Continued acceptance of the fumigants by health authorities and the general public is generally dependent on such vital information from fully integrated programmes.

2.6.1 Hazards, safety and health standards for fumigants

Fumigants are toxic to man as well as to insects and any exposure before, during or after a fumigation treatment can be harmful to human beings. Safety from occupational hazards and from contamination of food has become a matter of increasing concern in the utilization of fumigants for treatment of food commodities. The need for improved safety measures has become more evident as

new technologies for detection and analysis have become available and as new information on the toxic hazards of fumigants has been revealed. Both the applicator that uses fumigants, the consumer that eats treated goods and the natural environment must be protected from potentially harmful exposures. Regulatory agencies must move to provide guidelines that will adequately cope with these occupational and residue problems. (Berck, 1975b).

Occupational hazards for personnel that handle the toxic gases fall into two categories - acute and chronic hazards. The acute effects of fumigants have long been known and appropriate precautions are usually taken to avoid them. If proper care is taken, work with fumigants is no more hazardous than any other industrial or domestic technique that uses potentially harmful chemicals (COSSH). Chronic or long term effects, which may result from overdose to a single exposure of a toxic gas or from repeated exposure to low levels over a period of time, are less evident. The effects may not appear until long after exposure to the fumigant has taken place and, in some cases, they may not be easily associated with it. As already mentioned elsewhere in this chapter, chronic effects may take the form of injury to liver, kidneys or other organs or tissues or there may be other delayed effects. Some of the fumigants have the ability to produce cancer in animals under experimental conditions and it is believed that they may be potential carcinogens for humans. Carbon tetrachloride, ethylene dibromide and

ethylene dichloride are all "suspected" carcinogens (ACGIH, 1981, National Cancer Inst., 1978). Consequently, threshold limit values have been reduced to very low levels and instructions for their use have been revised accordingly, to reduce these hazards.

Residues of fumigants in food commodities have also taken on increasing importance as awareness of their presence in food has come about. The major part of this project will deal with the determination and analysis of these residues and will be covered subsequently in detail in Chapters Four and Five. New instruments and new methods of analysis that can detect and measure residues down to low ranges - parts per billion or less have indicated the occurrence of residues that were previously unsuspected. Consequently the setting of meaningful tolerance levels (maximum residue levels) has become extremely difficult. Acceptable daily intake studies for degradation products and, in some cases, for unchanged fumigant are incomplete and often lacking. Also the toxicological significance of exposure to low levels of fumigants or their residues for extended period is little known. Authorities in many cases are reluctant to set maximum residue levels for residues, particularly carcinogens where the long term effects have not been determined.

With the ultra-sensitive methods of detection that are now available, the possibility of fumigating a commodity without leaving some residue that can remain

undetected is remote. The question of health hazards may not be resolved until the toxic effects of these residues have been determined. For fumigants that are suspected carcinogens the question of health hazards is further complicated by the dearth of knowledge of the cancer-causing process. The possible cumulative effects of repeated exposure to ultra-levels of carcinogens are not fully understood. Consequently the hazards brought about by exposure to very low levels of suspected fumigant carcinogens must not be predicted. Further developments in the field of cancer research will be needed, along with additional toxicological data and new developments in safety procedures to adequately cope with this problem.

To protect human beings from the toxic effects of airborne substances, such as fumigants, threshold limit values must be established. These values are based on the best available information from industrial experience, from experimental human and animal exposures and, when possible, from a combination of all three. In recent years threshold limit values for many of the fumigants have been reduced to make allowance for known or suspected toxicological effects (Table 2.7). (Anon, 1990).

Table 2.7 Threshold limit values for fumigants for 1964, 1981, 1989 (ACGIH)

Fumigant	1964	TLV 1981	1989
Carbon disulphide	20	2	2
Carbon tetrachloride	10	5	5
Ethylene dibromide	25	5	5
Ethylene dichloride	50	15	10
Methyl bromide	20	5	5

While threshold limit values are believed to allow adequate protection for customary working situations, i.e. an 8 hour day and 40 hour week, they do not apply to other situations. For fumigation treatment where personnel may be exposed for longer periods of time acceptable thresholds values have not been established and the criteria for arriving at such values have not yet been developed.

In one extensively practised fumigation procedure, i.e. "in transit" ship fumigation in close proximity to the fumigated areas for continuous and extended periods of time. If current standards of health safety are to be followed it is essential that appropriate threshold limit values should be established to cover such situations. Health authorities state that established threshold limit values based on intermittent exposures cannot be

transposed nor related directly to continuous prolonged exposures. The toxicological effects resulting from prolonged exposure could be very different from short intermittent exposures. New information on long term dose-effect relationships is needed to establish meaningful maximum residue level values for these treatments. Also adequate methods for monitoring the low concentrations of fumigant occurring in such circumstances will be required to meet current health criteria.

Even the data on the toxicity and toxicology of fumigants that are used at present is inadequate as measured by present day health standards. Since fumigants were first used as pesticides in times when knowledge of the hazards of chemicals to human health was far less advanced than it is today, the requirements for health standards were less demanding. Fumigants were registered in those bygone times and are still being used, especially in the developing tropical world, like Africa, without the information that is presently required for the approval of new pesticides. Many regulatory agencies are faced with the dilemna of either by-passing legislative requirements to give continued approval for the fumigants or of de-registering invaluable materials that cannot be replaced.

A review and re-registration process has and is being undertaken in many countries to bring the fumigants in line with the health safety standards set for other pesticides. For this re-registration a considerable

amount of toxicological data, that is presently unavoidable, will be needed to establish appropriate safety regulations. The question of how to obtain the necessary data and who should underwrite the cost is a difficult one to resolve. Because of the high cost of gathering toxicological data that will meet present day standards, because of the limited markets for fumigants and because most of the fumigants can no longer be covered by patents, chemical manufacturers are reluctant to invest large sums of money in these materials. The benefits derived from the information would, however, be shared by users of fumigants through the world as well as by the manufacturers and suppliers.

This seems to be an area where an international co-operative effort would be invaluable. If several national governments could combine their efforts together with the appropriate industries, to finance such an operation and carry out the research, the information could be obtained without excessive burden on any group or even country.

The lack of essential data on fumigant toxicology could jeopardize the development of realistic health standards and might imperil the future approval of fumigants for use on food commodities. Regulatory authorities could be forced into the position of banning useful materials because of the fear of harmful effects. It would be unfortunate if some of these useful fumigants were lost simply because the required toxicological data

were unavailable.

2.6.2 Fumigation and pest management program

Fumigation is just one of a number of methods that can be used for controlling pests in stored products. Pest control is likely to be obtained when all appropriate measures are taken to eliminate pest organisms. In an effective pest management program, methods of prevention and control are integrated to give maximum protection of goods at the lowest possible cost. A number of other procedures that have been found effective in preventing and controlling infestations are as follows:

1) Sanitation
2) Exclusion of pests
3) Low temperature - "freeze-outs", refrigeration, aeration
4) High temperatures - heating of mills
5) Moisture control - grain drying
6) Aeration - cooling, drying, elimination of temperature gradients
7) Protectants - chemicals, inert dusts, natural compounds
8) Residual insecticide sprays
9) Atmospheric gases - carbon dioxide, nitrogen
10) Gamma radiation, radio and sonic waves, microwaves, infrared radiation
11) Pheromones
12) Insect growth regulators

13) Insect pathogens

14) Predators

15) Insect resistant packaging

16) Resistant varieties.

An effective integrated pest management system should begin with comprehensive planning to include all aspects of the problem, followed by the application of appropriate preventative and control methods. For example, the planning of pest management for a commodity like farm-stored grain may be divided into five major categories:

1) Exclusion of the pest organism

2) Inspection procedures

3) Good housekeeping and sanitation

4) Physical and mechanical control

5) Chemical control.

Infestation problems can often be reduced by careful planning so that the possibilities of pest organisms reaching the commodity will be minimized. Location of the storage relative to sources of infestation is important as well as quality of the structure. Well built storage structures with a minimum of sites where debris can accumulate and insects develop are desirable. Other features of the storage that should be considered include - facilities for conditioning such as aeration systems or driers, provision for proper inspection and cleaning and appropriate facilities for pest control

procedures. An effective pest management program may include the following steps:

1) Use of sound structures for storage of commodities.
2) Maintaining clean conditions around stores.
3) Removal of residues of grain or other material from storage facility 4 to 6 weeks prior to storing newly harvested produce.
4) Spraying of storage with approved residual insecticide after removal of food residues.
5) Storage of commodity in a condition suitable for optimum storage e.g. grain is best stored at low moisture levels.
6) Treatment with appropriate insecticide protectant at time of storage may be desirable.
7) Use of aeration or other procedures to cool grain and maintain uniform temperature below those favourable for development of pest organisms.
8) Regular inspection to determine:
    a) evidence of insect activity or the development of micro-organisms.
    b) accumulation of moisture.
    c) changes in temperature.
9) If insects are detected grain should be fumigated; where field infestation occurs grain should be fumigated within 6 weeks after harvest. If micro-organisms are developing, further drying may be required.

Several fumigation techniques may be combined or incorporated with other practices such as controlled atmosphere techniques or aeration and drying procedures.

By careful planning and management, fumigation may be incorporated into food preservation systems so that fumigants can be used more effectively and safely than when used independently. They should never be used as a substitute for sound management and good sanitation procedures. The benefits derived can include: reduced cost of storage with improved food quality, reduced residues in food materials, greater occupational safety and less environmental contamination. All of these benefits are of great concern to the general public and will be factors that have to be taken into consideration in the future use of fumigants. The ultimate goal in the control of pests in stored products should be to so improve the methods of handling, storing and processing commodities, that the need for pesticides will decrease. However, the protection of grain from the ravages of pest organisms, particularly insects, will still depend on the judicious use of fumigants for many years in the future. New approaches to effective fumigant utilization can only come through intensive investigation of all the factors that relate to insect control. To date, research on fumigants has trailed far behind other developments in science and technology and users of fumigants have failed to make maximum use of research data and technology innovations. Hopefully increased effort will be made in

the future to provide and to employ the necessary information and instrumentation particularly in developing countries where these fumigants are still being used today, so that these valuable materials can be utilized with the greatest efficacy in comprehensive pest management programs. (Berck, 1975b).

CHAPTER THREE

ENVIRONMENTAL FATE OF FUMIGANT RESIDUES

3.1 INTRODUCTION

The more that is known of the fate of fumigant residues in the environment, the easier it should be to set overall acceptable daily intakes for man. Fumigants, like other commonly used pesticides dissipate to the surrounding environment in different ways and quantities of which if left undetected can result in dangerous consequences to human beings, animals and the entire natural environment. The environment in this context comprises mainly the atmosphere (air), the lithosphere (land - soil and food included), and the hydrosphere (water). This Chapter will deal comprehensively with fumigant residues in the food environment which is one of the most affected areas in the entire system.

Public concern over pesticide residues in food has been increasing particularly during the last decade. A 1988 Food and Agricultural Organization (FAO) Survey showed that approximately 75 percent of consumers are very concerned about pesticides in their food. The percentage is higher than that of consumers worried about cholesterol, fats, salt, additives or any other components. Contributing to such concerns have been the discovery of hazardous effects from certain pesticides once deemed safe such as ethylene dibromide, and the high

level of uncertainty concerning the health effects of other fumigant residues. This Chapter will discuss the fate of fumigant residues in cereal grains and their by-products, both commercially and in the shopping basket, with special reference to the insecticidal fumigants mentioned in Chapter Two and their metabolites.

It will become evident that normal food preparation practices, as an incidental effect, will physically remove substantial amounts of these residues from the stream of agricultural products as they are converted into prepared human food. On the other hand, the same residues may be reintroduced to the stream with process byproducts that are fed to food-producing animals.

The FAO/WHO Pesticide Residues Committee and other regulatory agencies, both national and international, seeking to limit the human intake of pesticide residues through consumption of food and/or exposure to air, have established systems of maximum residue levels. A legal maximum level is the concentration of the pesticide allowed in a particular crop or food product. Its value is generally no higher than that residue level likely to result from "good agricultural practice", and the pattern of established maximum levels must in all cases be judged as safe in that it controls the probable daily pesticide intake by humans below an acceptable upper limit. Conceptually maximum residue levels apply to human food, but in actual practice this is not so. Most raw agricultural commodities or food products moving in

commerce, the items on which maximum levels are established, are subjected to further processing or cleansing and preparations at home before they become human food. For very practical reasons maximum residue levels are enforceable on food products moving in commerce, but not for food on the dining table. It has been found that there is a substantial difference between insecticidal fumigant residue intake inferred by some from existing maximum levels patterns and the probable actual intake from human consumption of finished foods.

3.2 THE FATE, TOXICOLOGICAL AND NUTRITIONAL SIGNIFICANCE OF METHYL BROMIDE RESIDUES IN WHEAT

When wheat grains or flour is exposed to methyl bromide under the conditions of fumigation against insect pests the fumigant is rapidly sorbed. A high proportion of the sorbed fumigant reacts chemically with the wheat constituents with the formation of water-soluble bromide and methylated products. At the end of the fumigation period any undecomposed methyl bromide would either be rapidly desorbed or undergo ultimately complete decomposition. In fumigated wheat flour the end-products appear therefore to consist almost entirely of inorganic bromide, O-methyl, S-methyl, dimethyl sulphonium, and N-methylhistidine derivatives. There is no reason to believe that these products would differ if the wheat were exposed to methyl bromide as the whole grain or as the milled product. The total sorption and consequent rate

of decomposition would, however, be higher in milled wheat grain than in whole grain. It is the purpose therefore of this section to discuss the fate, the possible toxicological and nutritional significance of methyl bromide fumigation on the basis of these results and on the basis of other available data.

3.2.1 N-methylation in methyl bromide fumigated wheat

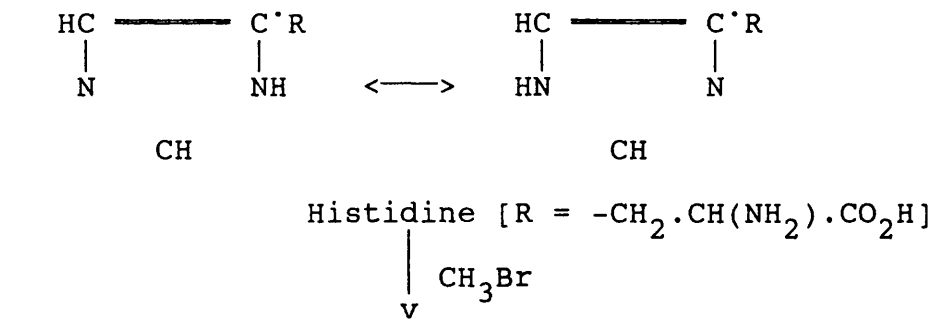
It has been shown that the larger part of the reaction occurring between methyl bromide and gluten (wheat protein) is due to methylation of the nitrogen-containing groups of the protein (Scudamore et al., 1987). The purpose of the work described in this section is to discuss the nature and the extent of the N-methylation occurring in wheat flour under the conditions of methyl bromide fumigation.

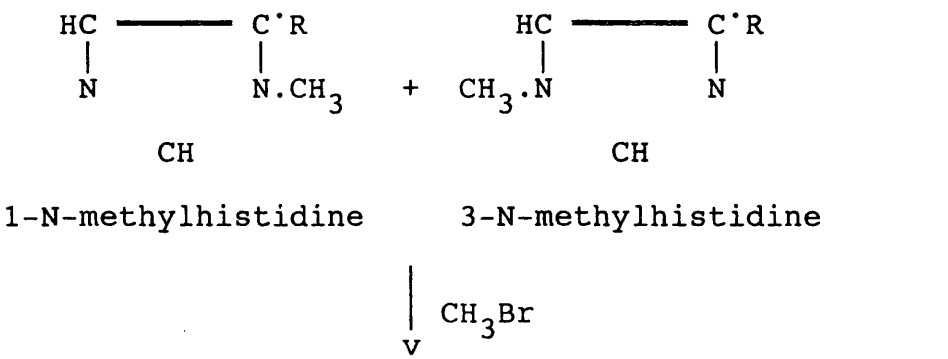
Possible sites of N-methylation in the protein are: (1) the nitrogen of the peptide links; (2) the terminal amino groups of the protein chains; (3) the nitrogen-containing groups of the three basic amino-acids, histidine, lysine and arginine, not involved in peptide links; and (4) the nitrogen of the indole group of tryptophan.

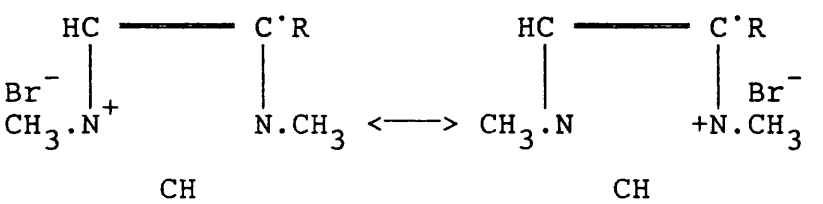
Acid hydrolysis of a protein in which groups of type (1) or (2) were methylated would yield amino-acids in which the α-amino group would be methylated. A protein in which groups of type (3) or (4) were methylated would yield a methylated amino-acid in which the α-amino group was intact. Tryptophan is decomposed on acid hydrolysis

and it is probable that N-methylated tryptophan would undergo a similar fate. In the native protein, however, many of these sites may not be readily available for methylation, as they are involved in salt links and hydrogen bonding.

Many studies have found that the particular groups undergoing N-methylation depend on the conditions of methylation. The evidence further suggests that in N-methylation of proteins the basic amino-acid residues are the most susceptible to attack.







1:3-N-dimethylhistidine bromide

3.2.2 The nature and magnitude of methyl bromide residues

In fumigation practice the mean free-space (intergranular space) concentration of methyl bromide to which infested wheat is exposed is rarely known with any precision and does, in any case, vary widely with the conditions of fumigation. It is difficult therefore to estimate the magnitude of typical residues as a result of fumigation experience. The problem may, however, be approached in a different way. Common insect pests of wheat in the United Kingdom, for example, are the grain weevil (Calandra granaria), and the flour beetle (Tribolium confusum). Since not less than a 99.9% kill at all stages of the insects' development would be an effective disinfestation measure, the minimum concentration of methyl bromide required has been estimated from toxicity data and found to be of the order of 5 mg/litre for a 48 hour exposure at 20°C. It is calculated on the basis of sorption data that in flour this would result in an ultimate residue of about 100 ppm of decomposed methyl bromide. If the average person consumed 1.75 kg of flour products per week, and all this flour has been fumigated, he would consume 175 mg of decomposed fumigant per week. Two points need emphasis. First, methyl bromide fumigation is usually applied to whole wheat rather than milled wheat so that the residues in the resulting flour would be smaller. Second, it is highly improbable that any one person would consume

exclusively and continuously fumigated flour products which had not undergone extensive dilution with unfumigated flour. On the other hand, there is inadequate legislation in force to prevent either of these possibilities, so that the case may be considered academically at least as a possible one.

Some of the methylated products of fumigation (e.g. dimethyl sulphonium salt) might decompose partly during baking or cooking before human ingestion. Since little information is available it seems safer to neglect this factor and to assume that the methylated derivatives will survive as the corresponding products of digestive hydrolysis. It will be to the toxic effects of these hydrolysis products that the body will effectively be exposed. It could be concluded that the weekly consumption of 175 mg of decomposed methyl bromide mentioned earlier, would be equivalent to the absorption of the products listed in Table 3.1.

Although little is known about the precise nature of the reaction between the starch or carbohydrate fraction of the flour and methyl bromide, it is believed that wheat starch readily undergoes O-methylation by diazomethane with the formation of methylated glucose hydrolysis products (Winteringham et al., 1955). Methylation by methyl bromide probably yields very similar products. The mammalian digestion of starch is well understood; the ultimate product of acid and enzymic hydrolysis is glucose. The starch from fumigated wheat

Table 3.1 Components absorbed as a result of eating methyl bromide-fumigated flour-products (hypothetical human case); total concentration of decomposition products equivalent to 100 ppm of methyl bromide (Source: Winteringham et al., 1955)

	Parent fraction in fumigated flour	Effective end-product of digestive hydrolysis	Percent of total decomposed fumigant represented	Weight of equivalent CH_3Br mg/wk	Weight of equivalent end-products mg/wk
1	Volatile water soluble fraction ($CH_3.OH$)	Methanol (or methanol + methylglucoses)	10.7	18.7	15.9
2	Starch, $^{-}O.CH_3$		8.0	14.0	(or 28.6 mg of methylglucuses + 11.2 of methanol)
3	Protein, $^{-}O.CH_3$		8.2	14.4	
4	Fat, $^{-}S.CH_3$	S-methyl cysteine	0.6	1.1	14.5
5	Protein, $^{-}S.CH_3$		5.2	9.1	
6	Protein, $^{-}S.(CH_3)_2$	Methyl methionine sulphonium ion, $[(CH_3)_2.S.CH_2.CH(NH_2).CO_2H]^+$	26.6	46.6	87.5
7		1-Methylhistidine	15.0	26.2	46.7
8		Dimethylhistidine ion, $[(CH_3)_2.C_3H_2N_2.CH_2.CH(CH_2).CO_2H]^+$	5.3	9.3	18.0
9	Protein, $^{-}N^{\cdot}CH_3$	3-Methyl histidine	11.4	20.2	35.6
10		E-N-Methylhistidine $CH_3.NH.(CH_2)_4.CH(NH_2).CO_2H$	4.5	7.8	13.2
11		Unidentified derivatives	4.5	7.8	-
12	Inorganic bromide	Inorganic bromide, Br^-	100.0	175.0	147.4

would thus be expected to yield methylated glucoses. Since, however, there is evidence of the presence of at least one methyl glucosidase in the mammalian intestine, methanol may be the ultimate product of intestinal hydrolysis of methylated starch. The nature of the protein O-methylation in the fumigated flour is not fully defined, but if it is due to the esterification of carboxylic acid groups, methanol would again be expected as the result of peptidase and lipase action. The effective end-product of O-methylation is thus assumed to be methanol (Table 3.1).

This neglects the possibility of the formation of stable methoxyl groups formed, for example, from the phenolic residue of protein tyrosine. A further assumption has been that the reaction between the light-petroleum-soluble fraction of wheat and methyl bromide is due to methylation of the mercapto group of cysteine-containing lipoprotein. However, this fraction respresents the smallest part of the decomposition products. This fraction, together with the S-methyl product determined directly in the protein, would therefore be expected to yield S-methylcysteine on digestive hydrolysis. The remaining end-products (Table 3.1) would be expected as the result of normal peptidase action on the derivatives determined in the fumigated-flour protein. Finally the effective end-products suggested in Table 3.1 may not in fact represent the ultimate decomposition before absorption

from the intestine. For example, dimethyl sulphonium salts may decompose spontaneously under the relatively alkaline conditions of pancreatic digestion. The end-products suggested, however, are compounds which can reasonably be assessed for their possible toxicological significance.

3.2.3 Toxicological significance of methyl bromide residues

A method widely used for detecting toxic residues as a result of fumigation or insecticidal application is that of animal-feeding tests. For this purpose the food is exposed under normal or extreme conditions and fed to test animals for a period during which the animals' behaviour, growth rate, longevity, etc. are compared with that of control animals given untreated food. Clearly, slight toxic effects, such as might induce occasional headache or nausea in man, would pass undetected by these tests and yet, if established, would be sufficient to condemn that treatment of human food. A second limitation of feeding-tests is that the toxic product may be the result of a reaction with some constituent of the food (for example, amino acid). The normal concentration of this constituent will then represent the maximum possible concentration attainable even under artificially heavy conditions of exposure, and which may yet be inadequate to demonstrate an undesirable effect. When the nature of the decomposition products is established they can be synthesized in the laboratory and fed to or injected into

animals at concentrations enormously higher than those attainable in animal-feeding tests. Moreover, identification of the decomposition products may reveal them as substances known to pharmacologists. With the limitations of animal-feeding tests in mind at least one extensive series has been carried out. Rats were maintained on control and on methyl bromide-fumigated diets for one year. No nutritional or toxicological effects were detected (Michael et al., 1980).

Methanol:- On ingestion, methanol (see Table 3.1 in Section 3.2.2) is more toxic than ethanol to man and undergoes less rapid detoxication. Ingested methanol undergoes rapid distribution in the body water and is excreted partly as methanol vapour and carbon dioxide via the lungs and partly as formate, and as methyl glucuronides via the kidney. Almost 90% of doses administered to rats has been recovered as these metabolites within 48 hours (Michael et al., 1980). It is believed that species vary in their susceptibility to methanol and ethanol poisoning and the toxicities of these alcohols on ingestion are of comparable orders. The safe maximum concentrations in air of 200 ppm and 1000 ppm (by volume) of methanol and ethanol respectively have been recommended for workers regularly exposed for 8-hour days. Both alcohols are infinitely soluble in aqueous media so that there would be corresponding regular concentrations in the blood of exposed workers. The maximum residue level of man to regular small doses of ethanol is well

known so that it seems unlikely that the ingestion of doses of methanol at least many thousands of times smaller would be harmful. It is well established that methanol is readily lost to various metabolic pathways in vivo and may even participate in biological transmethylation, which suggests strongly that there would be no accumulation of methanol or its metabolites as a result of the ingestion of trace quantities.

Methylglucose:- In Table 3.1 (Section 3.2.2) it is assumed that methylated glucose arising by the digestive hydrolysis of the methylated starch would possibly be further hydrolysed to glucose and methanol as a result of enzyme action. Since there is inadequate direct experimental evidence for this, the possibility that one or more methylglucoses are the effective end-products of digestion must be considered. Many observations have shown that 3-methylglucose (a likely product of partial starch methylation since 1- and 4- positions would not be available) is not apparently utilized by the rat in the same way as glucose, but is absorbed from the gut and excreted by the kidney unchanged. The compound had no direct or antagonistic effect on glucose metabolism in vitro.

S-methylcysteine:- When S-methyl-L-cysteine was fed to a 60 g rat at the rate of 111 mg per week in a cysteine-free diet for a period of 20 days, it was found that the methyl derivative is neither a substitute nor a precursor for

cysteine in the rat. For a 60 kg human the rate of ingestion of S-methylcysteine per unit of body weight indicated in Table 3.1 (Section 3.2.2) would be less than that in the rat experiment by a factor of 7500.

Methyl methionine sulphonium salts:- The formation of sulphonium salt which is almost certainly derived from the methionine in fumigated wheat cannot therefore be described as the introduction of a substance foreign to normal diet. A group of five weanling rats were fed on formulated casein diets which were respectively deficient in methionine and methyl methionine sulphonium bromide (control), supplemented with methionine only, and supplemented with methyl methionine sulphonium bromide only (Michael et al., 1980). The rats grew more rapidly on either of the supplemented diets compared with the control, which suggested that the growing rat was able to utilize the sulphonium salt as a substitute for methionine. The rats on the third diet received the sulphonium salt at the rate of 360 mg per week per rat for two weeks. No abnormal symptoms were detected in the rats which each received a total dose of 720 mg of the sulphonium salt and they grew at a rate comparable with that of the rats on the methionine-supplemented diet (Price, 1985). It would be unwise to generalize on the basis of such a limited test, but it may be noted that when these animals ingested the sulphonium salt for a short period at a rate about 1000 times greater than that indicated in Table 3.1 (Section 3.2.2) per unit of body

weight, no toxic effects were detected.

N-methyl derivatives:- DL-1-methylhistidine is not a substitute for histidine in the diet of albino rats (Bridges R.G., 1955). No toxic effects were noted by Bridges in a single 72 g rat which apparently received over a three-week period DL-1-methylhistidine at a rate of 168 mg per week, equivalent to about 3000 times that indicated in Table 3.1 per unit of body weight. 3-methyl-L-histidine has been identified in human urine. No dietary source of this amino-acid seems to be known but the 1-methyl isomer occurs in certain meats. When DL-1-methyl-, DL-dimethyl-, and DL-3-methylhistidine were injected intravenously into mice at doses up to 300 mg of compound per kg of body weight there were no toxic effects noted during a 14-day period after the injections (Bridges, R.G., 1955). No date relevant to the possible toxicity of the N-methyllysine identified in the fumigated wheat have been found.

Inorganic bromide:- This anion is a normal constituent of all plant and animal tissues so that the formation of bromide in fumigated wheat cannot be described as the introduction of a foreign substance into the normal diet. The increased concentration in wheat flour as a result of fumigating with methyl bromide in the example illustrated in Table 3.1 is 85 ppm. In an extensive series of tests on the possible effects of bromide therapy, 70 adult humans each received sodium bromide on a daily basis for a

period of four months at a dose rate of bromide at least 50 times greater than that implied in Table 3.1. The average bromide contents of blood and urine were significantly increased during this period, but dropped rapidly on cessation of the bromide treatments. No significant physiological or neurological effects were detected.

3.2.4 Nutritional significance of methyl bromide residues

Besides the possible formation of toxic reaction products in wheat exposed to methyl bromide, there is the possibility of deleterious nutritional effects as the result of the selective destruction of essential amino-acids, available glucose and so on. Consideration of the data of Table 3.1 (Section 3.2.2) shows that the possibility of these effects under the conditions of fumigation may be dismissed. For example, with methionine its destruction would be equivalent to approximately 27 mg of methyl bromide per kg of wheat (Table 3.1). On the basis of a simple bimolecular reaction this would represent a reduction of about 2% in the total methionine of a typical wheat. The destruction of histidine would be equivalent to approximately 31 mg of methyl bromide per kg of wheat or a reduction of 1.3% in the histidine level of a typical wheat. Not all the amino-acids are regarded as essential and with methionine there is some evidence that the reaction product may be a satisfactory substitute for the original amino-acid of the diet. If the available

glucose were reduced as a result of the observed starch methylation the reduction would not amount to more than 0.002%. Such levels of destruction are probably small compared with the natural variations from one wheat crop to another. With the vitamins of wheat the dangers of significant destruction are very much greater. Their concentrations are so low (for example, 1 part of riboflavin per million of a typical wheat) that, if the unidentified fraction alone of the methyl bromide reaction products were the result of a selective reaction with one or another vitamin, its destruction might be complete. This possibility has, however, been tested experimentally and no evidence of vitamin-B loss was found under the conditions of methyl bromide fumigation (Michael et al., 1980).

3.2.5 Penetration, sorption and desorption of methyl bromide in wheat, flour and maize

The toxicity of fumigants to insects is reduced at low temperatures so that larger quantities are needed to obtain control as conditions become colder. Tests of fumigants have shown that the toxicity of certain materials can be improved when they are mixed. One of the fumigants already mentioned which is extensively used to control insects in stored products, methyl bromide, is not very effective at freezing temperatures (Dumas and Latimer, 1982) but addition of another compound like acrylonitrile greatly increases toxicity, especially at low temperatures (Dumas and Bond, 1977). Concentrations of acrylo-

nitrile used were much below the flammability range and the methyl bromide, being a fire suppressant, further increased the safety of the mixture. Tests made on insects in a 525-L chamber, where no commodity was present to interfere with penetration and loss of fumigant through sorption and residue formation, showed that insects could be effectively controlled over a range of temperatures down to -12.2°C. (Dumas and Bond, 1977).

Since diffusion and penetration of gases are slowed down as temperature drops more fumigant is sorbed and retained in the commodity for longer periods of time at low temperature. Furthermore, components of a mixture may separate to move and penetrate at different rates, particularly at low temperatures, so that the quantity of each component may vary in different parts of the system.

Studies were made on a 70:30 methyl bromide-acrylonitrile mixture to determine the penetration, sorption and desorption of mostly methyl bromide during fumigation and to establish the qualities of residue by wheat, flour and maize. The Dumas and Bond (1977) method was applied for the laboratory fumigation and determination of the residues. The length of time required for the methyl bromide-acrylonitrile mixture to penetrate a layer of wheat at 25°C to the same concentration on both sides of the commodity is shown in Table 3.2. (Residue Methodology as in Chapter 4).

Table 3.2 Penetration and sorption of methyl bromide and acrylonitrile in wheat treated in 6-L desiccator for 8 h at 25°C

Dosage mg/l	Quantity of wheat wt, g	Penetration time[a], h		Quantity of fumigant, mg			
		CH_3Br	CH_2CHCN	CH_3Br applied	CH_3Br sorbed	CH_2CHCN applied	CH_2CHCN sorbed
2	200	5	6.7	12	0	5.1	3.2
5	200	3	5.2	30	1.8	12.9	11.0
10	200	4	3	60	4.1	25.7	18.1
4	1500	2	8	24	13.7	10.3	9.7
10	1500	6	8	60	27	25.7	21.6

[a] - average time for fumigant to reach equilibrium above and below commodity.

Samples drawn from above and below the wheat at various times and analyzed in the gas chromatograph showed that sorption of fumigant, as indicated by a decrease in concentration in the free space, was a function of the amount of wheat present with other conditions constant. In the treatment with 10 mg/l the total quantity of methyl bromide in the free space dropped from 60 to 55.9 mg in the dessicator containing 200 g wheat and to 33 mg when 1500 g of wheat was present.

The rate of penetration of methyl bromide decreased in maize, flour and wheat as shown in Table 3.3 when a 10 mg/l concentration of the fumigant mixture was applied at 25°C. At 0°C and 108 mg/l mixture the order of each methyl bromide penetration was maize, wheat and flour.

Table 3.3 Penetration and sorption of methyl bromide and aerylonitrile in wheat, flour and maize for 8 hr in 6-L desiccators

Commodity	Dosage mg/l	Temperature °C	Quantity of commodity wt, g	Time to penetrate,h		Sorption quantities, mg	
				CH_3Br	CH_2CHCN	CH_3Br	CH_2CHCN
Maize	4	25	1500	3	6	0	0
	10	25	1500	3	9	15	19
	108	25	1500	3	7	182	126
	45	0	1500	1	7	79	63.5
	108	0	1500	1	8	150	147
Wheat	10	25	1500	5	9	20	14
	108	0	500	8	8	38	148
	108	0	1500	3	7	109	170
Flour	10	25	500	2	6	21	14.9
	10	25	1000	3	4	39	21
	126	0	500	3	8	398	240
	108	0	1000	7	8	500	130

Table 3.4 Volatile bromide lost during heating (expressed as ppm)
(Source: Daft, 1987)

Material	Initial residue (before heating)	Final residue (after heating)	Volatile residue trapped	Volatile bromide + final residue	% of initial residue accounts for
Milled wheat	72	27	40	67	93.0
Gluten	100	54	38	92	92.0
Starch	49	12	33	45	91.8

Related data on sorption of methyl bromide by a commodity treated at 25°C with 10 mg/l of the fumigant mixture showed that flour took up the largest quantity of methyl bromide equal to 39 mg/kg, wheat about half this amount at 13.3 mg/kg and corn absorbed the least at 10 mg/kg. At 0°C and 108 mg/l mixture, sorption on wheat and corn was somewhat similar for all these commodities with about half the total amount applied being taken up. In the flour nearly all the methyl bromide was absorbed at this temperature. Methyl bromide desorbed completely from commodities in a few hours and the residue level was found to be minimal.

3.3 THE NATURE AND SIGNIFICANCE OF ETHYLENE DIBROMIDE RESIDUES IN WHEAT

When wheat is fumigated with ethylene dibromide, several types of residual contamination, depending on the subsequent treatment of the wheat, takes place. Compared with methyl bromide (Section 3.2), the rate of airing is considerably slower and the ethylene dibromide can be retained unchanged for a considerable period. Whole wheat was still found to contain an appreciable amount of residual bromide after fourteen days of airing under ideal conditions. This contamination is due to insufficient airing. However, when the wheat is milled, the residual bromide concentration fell to zero in 48 hours of further airing. There is also contamination brought about by the product of reaction between ethylene dibromide and the

wheat. At normal fumigation temperatures, the amount of such reaction is small, much less than that between methyl bromide and wheat. The reaction appears to occur mainly with the protein of the wheat and increases slightly with the time of airing. Ethylene glycol resulting from the decomposition of unaired ethylene dibromide, causes contamination after heating. About a half of the unaired ethylene dibromide residue breaks down to glycol during heating for half an hour at 180°C, and the remainder volatilizes (Table 3.4). Ethylene glycol reacts with wheat protein on heating. A possible site of such reaction is with the $-SCH_3$ group of the methionine constituent of the protein. (Daft, 1987).

Any reduction of the content of essential amino-acids due to reaction between the ethylene dibromide and the wheat protein during fumigation and airing is extremely small. Daft (1987) assumed a simple bimolecular reaction occurs when ethylene dibromide reacts with methionine content resulting in a residue which represents a reduction of less than 0.4% of the total methionine content as given for a typical wheat flour. The chemically bound glycol after heating shows that, again assuming that only the methionine constituent of the protein is involved in the reaction, a significant residue represents a reduction of 60% of the total methionine content. As the glycol determinations were made in this case (Daft, 1987) on milled wheat exposed to ethylene dibromide, under extreme conditions, such a reduction is

most unlikely to occur in practice. Reaction with amino-acid constituents of the protein other than methionine also may be possible.

The possible effect of ethylene dibromide fumigation on vitamins contained in the wheat has not been investigated. However, as there is no evidence of lost vitamin-B under conditions of methyl bromide fumigation (Section 3.2), it would seem unlikely that any loss would occur with the less reactive ethylene dibromide.

Toxicologically, ethylene dibromide is a fairly toxic material when administered orally as a single dose to mammals. It has been reported that ethylene dibromide is toxic to human beings when ingested in large quantities especially after consuming unaired grains. However, cooking and baking reduces the risk of contamination (Chapter 2) which reoccurs when farm animals are fed on unheated or unprepared animal feed. The detrimental effect of incompletely aired ethylene dibromide residues in grain when fed to laying hens has been observed by Brown (1984), who found that a significant diminution in egg size occurred and in extreme cases, a complete cessation of laying occurred.

Ethylene glycol is a very much less toxic substance than ethylene dibromide itself. However, due to its chronic toxicity it should be omitted entirely from food preparations. The toxicological significance of any reaction product between the ethylene glycol and the protein constituents cannot be assessed, as the nature of

the reaction products has not been fully determined. Simple ethers and esters of ethylene glycol have similar toxic action to ethylene glycol, although the toxicity of the ethers to mammals is greater.

3.3.1 Effects of temperature, relative humidity and moisture content on sorption and retention of ethylene dibromide by maize and wheat

Certain physical and chemical relationships exist between wheat and ethylene dibromide when the latter is used as a gaseous fumigant (Section 3.3). As it will be seen in Chapter 4, ethylene dibromide does not react to any significant extent with wheat at a 9% moisture content. In this section, changes in the sorption and retention of ethylene dibromide by kernel wheat when the fumigation and storage of specific loads are carried out under varying conditions of temperature and humidity.

The extent at which the tissues of maize can be expected to react with ethylene dibromide as well as the sorption and retention of the latter at varying temperatures and moisture contents are discussed. Correlation of the retention of ethylene dibromide with changes in moisture content of wheat or maize, after treatment under specified conditions, are briefly mentioned.

Wheat and maize kernels both at a 9% moisture content were fumigated at different temperatures and humidities for 24 hr at a dosage of 16 mg/litre.

Subsequently portions of each load was stored in small open bottles at different temperatures and varying relative humidities (Chapter Four).

The sorptions of ethylene dibromide by both wheat and maize under identical conditions are approximately equal to one another at fumigation temperatures between 10°C and 21°C. Sorption at 32°C and above, however, is somewhat greater in maize than in wheat. Nevertheless, the sorptions of ethylene dibromide by both wheat and maize follow similar paths between 10°C and 32°C, varying inversely with temperature. These factors are illustrated in Figure 3.1 where the average sorption at approximately 40 ppm decreases as much as 10 ppm when the fumigation temperature increases from 10°C to 32°C.

Figure 3.2 indicates that a small difference occurs in the amount of ethylene dibromide retained in wheat when fumigated at 10°C and stored at 10°C and 32°C and when fumigated at 32°C and stored under identical conditions (Figure 3.3). This is due to the rise in moisture content at transition from 32°C to 10°C and to condensation of ethylene dibromide which volatilize rapidly at higher storage temperature. Both figures further indicate that retention is greater at low than at high storage temperature, as also found by Scudamore and Goodship (1982).

An examination of Figure 3.4 shows that increases in the moisture content in wheat, through sorption of water vapour from the atmosphere, at a high relative

Figure 3.1 Sorption of EDB by kernel wheat and maize fumigated at varying temperatures.

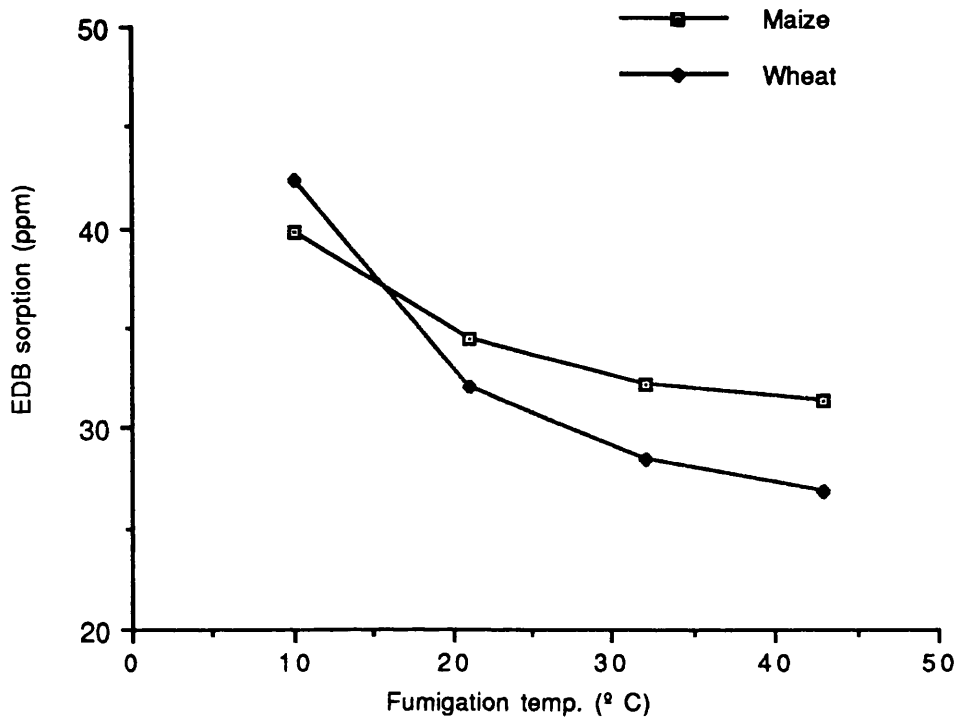


Figure 3.2 EDB recovered from kernel wheat fumigated at 10 º C and stored at 10 º C and 32 º C.

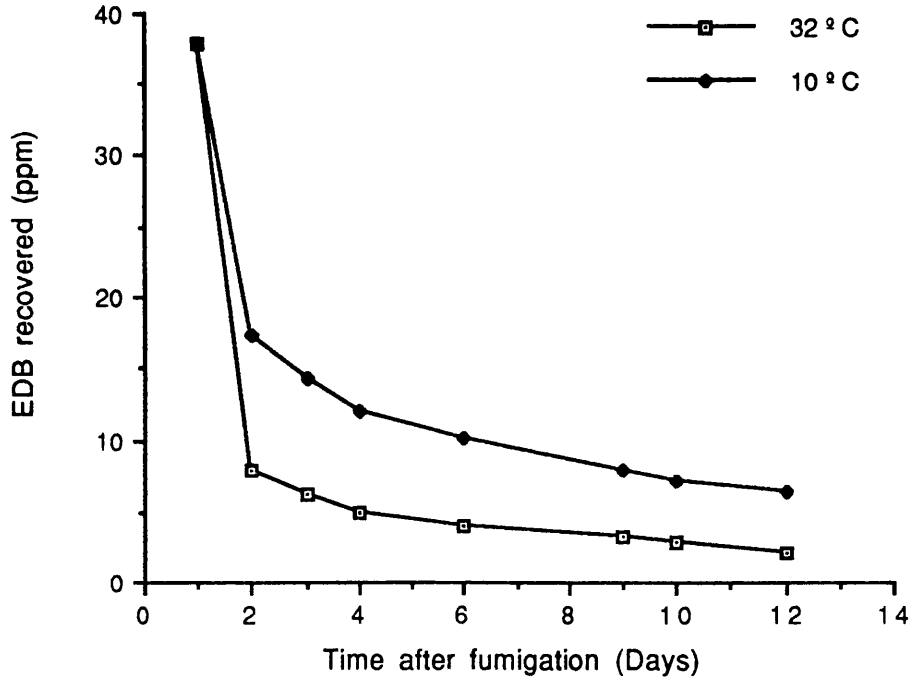


Figure 3.3 EDB recovered from kernel wheat fumigated at 32 º C and stored at 10 º C and 32 º C.

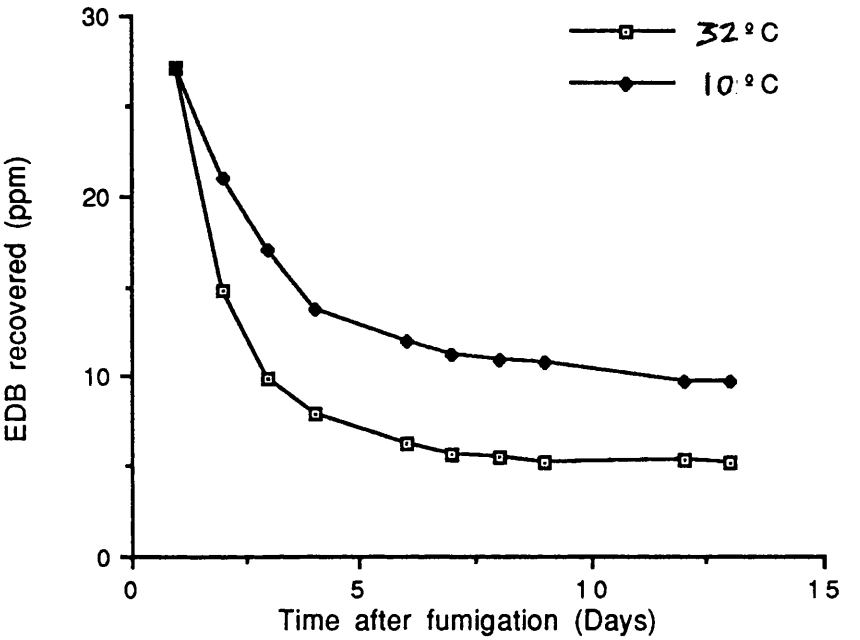
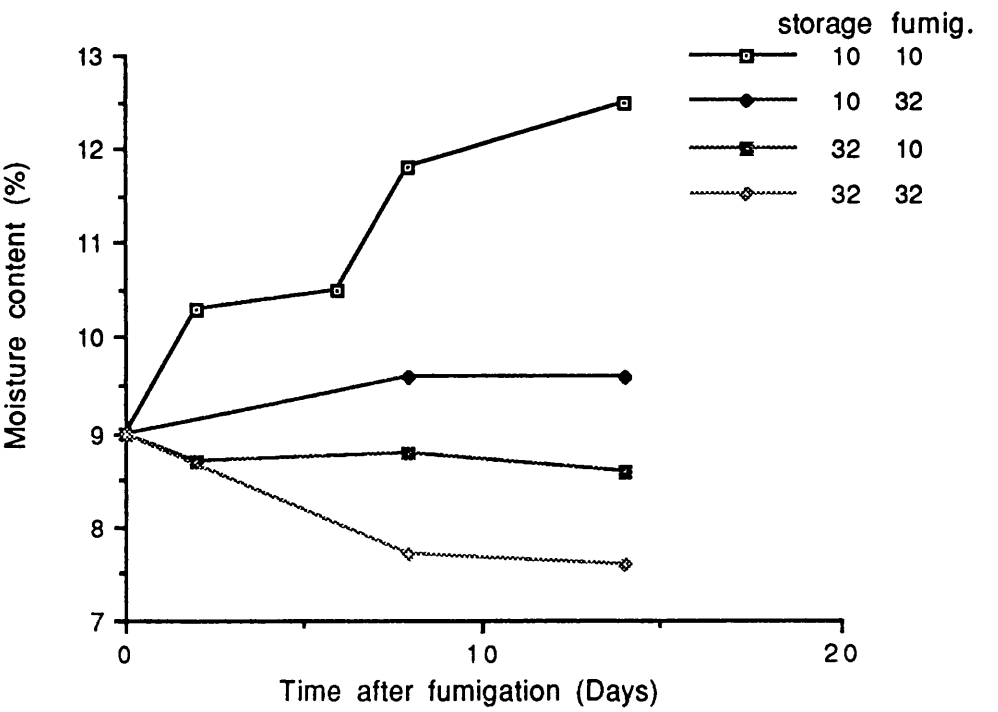


Figure 3.4 Changes in moisture content of kernel wheat fumigated with EDB at 10 º and 32 º C and stored at 10 º and 32 º C.



humidity, correspond with increases in the retention of ethylene dibromide in wheat when stored at 10°C (Figures 3.2 and 3.3). On the other hand, lower retentions of ethylene dibromide by wheat stored at 32°C and at a low relative humidity correspond with decreases in the moisture content.

Briefly, it may be said that (1) the retention of ethylene dibromide by wheat, characterized by higher sorptions at lower fumigation temperatures, falls rapidly during storage; (2) the retention is greatest where the factors of a high fumigation temperature, a low storage temperature, and an increasing moisture content within the wheat enhance one another; and (3) the retention of a 40% load of wheat at a 9% moisture content, fumigated for 24 hr at 16 mg/litre, at atmospheric humidity, and at 10°C, decreases to approximately 10 ppm after storage for 6 days at 10°C or 32°C.

An examination of the curves in Figure 3.5 indicates the similar characteristics in the sorption and retention of ethylene dibromide by kernel maize when fumigated under the same conditions as wheat (Figure 3.2). The sorption of about 3 kg load at a 9% moisture content, fumigated for 24 hr in a 10 litre chamber at 10°C, atmospheric pressure, and at a dosage of 16 mg/litre is, at 40 ppm, nearly equal to that of wheat. Similarly, retention decreases rapidly, amounting to no more than 10 ppm on the sixth day when stored at either 10°C or 32°C.

Although the moisture content on the seventh day

Figure 3.5 EDB recovered from kernel maize fumigated at 10 º C and stored at 10 º C and 32 º C.

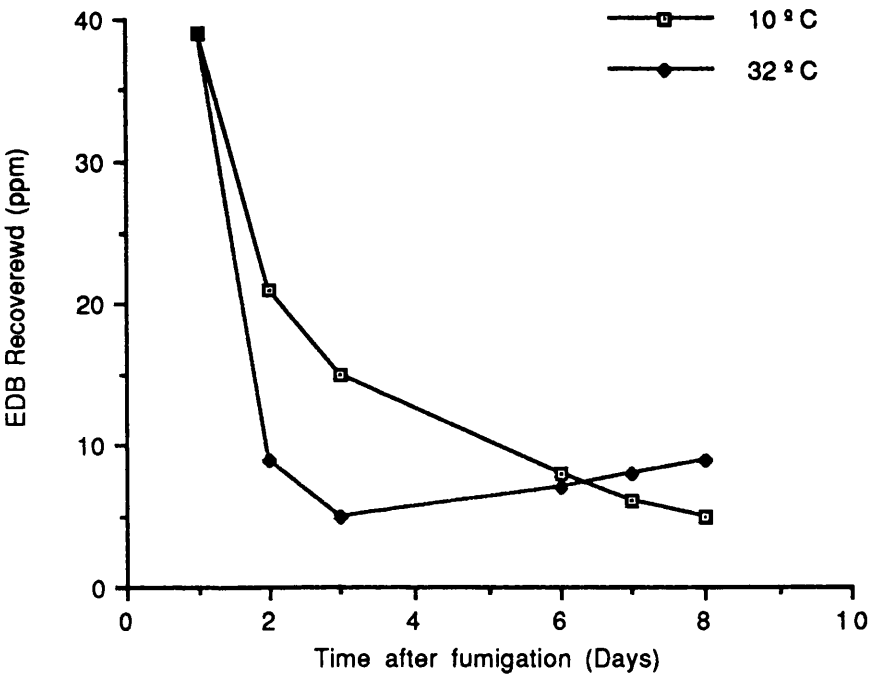
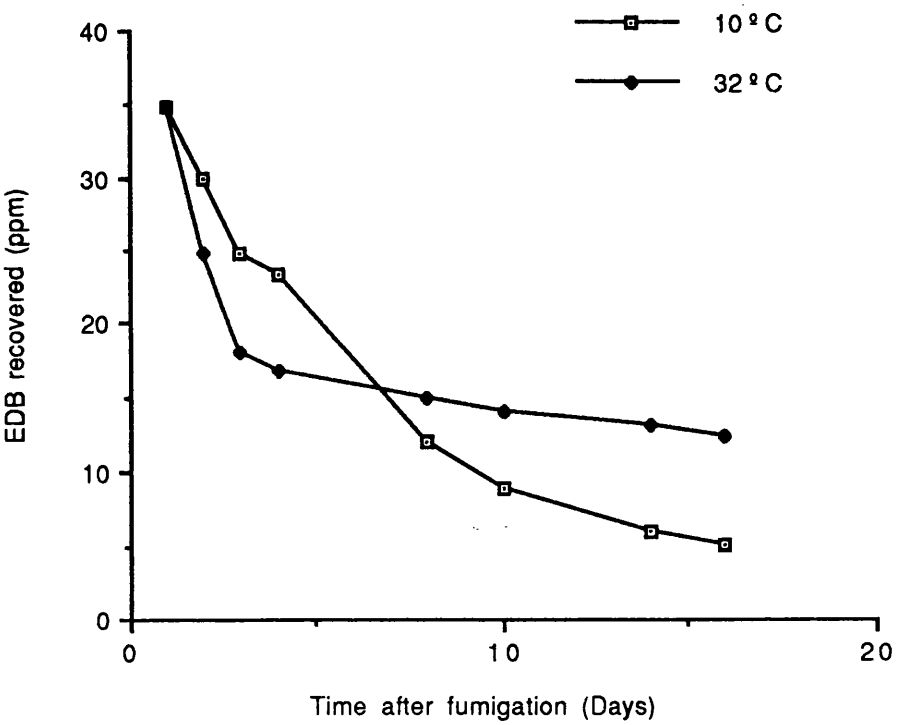


Figure 3.6 EDB recovered from kernel maize fumigated at 32 º C and stored at 10 º C and 32 º C.



increases to 12.7% at 10°C and decreases to 8.4% at 32°C (Figure 3.4), retention of ethylene dibromide in maize stored at 10°C at a high relative humidity appears to fall gradually below that in maize stored at 32°C at a low relative humidity. This feature, unlike the retention observed in wheat, is illustrated admirably in Figure 3.6.

An examination of the curves plotted in Figure 3.6 indicates that the sorption of ethylene dibromide by kernel maize fumigated at 32°C under the same conditions is, at approximately 35 ppm, not significantly more than that sorbed by wheat fumigated at 32°C (Figure 3.3). Nevertheless, the retention of ethylene dibromide in maize stored at 10°C, though greater for several days than that in maize stored at 32°C, becomes considerably less than the latter following the fifth day after fumigation. This fact is evident even though the moisture content on the fifteenth day increases to 13.6% at 10°C and decreases to 6.0% at 32°C (Figure 3.4), while the retention of ethylene dibromide decreases to approximately 7 and 13 ppm, at 10° and 32°C, respectively. This explains the similar results observed by Bielorai and Alumot (1975), who did not consider the effect of temperature on moisture content.

These facts serve to emphasize the effects that the temperature and the nonuniformity in the composition of maize have on the sorption and retention of ethylene dibromide. It may be said, therefore, that the retention of ethylene dibromide in maize is favoured by a high

fumigation and a high storage temperature, in agreement with Scudamore and Goodship (1982).

The curves in Figure 3.7 show the effect that an increase in moisture content has on maize. Wheat treated and stored under the same conditions gave similar results. Initial sorptions of ethylene dibromide are almost identical for both maize and wheat at 9% and at 15% moisture contents, respectively; and over a 2-week period, retention of the insecticide remains appreciable, particularly in maize (but also in wheat) at a higher moisture content.

The reaction between ethylene dibromide and ground maize appears to be somewhat greater than that determined with wheat under the same experimental conditions (Chapter Four). The curve in Figure 3.8 where 1.56 to 3.12 mg of ethylene dibromide in methanol are added to 25 g of ground maize kernels and sealed within glass stoppered flasks illustrates that the recovery decreases steadily from 98% during the first few days. It can be seen in Figure 3.8, however, that at equilibrium the recovery continues to remain above 90% throughout the 10-day period.

A knowledge of the extent of the degradation of ethylene dibromide in maize into products including ionized bromine, during a very long storage period, as well as the retention of that portion of the former which continues to remain in an equilibrium state within the maize is a necessary and useful tool. It has, however, been reported (Sinclair et al., 1964; Dumas et al., 1979;

Figure 3.7 The effects of the Moisture content in kernel maize on the sorption and retention of EDB

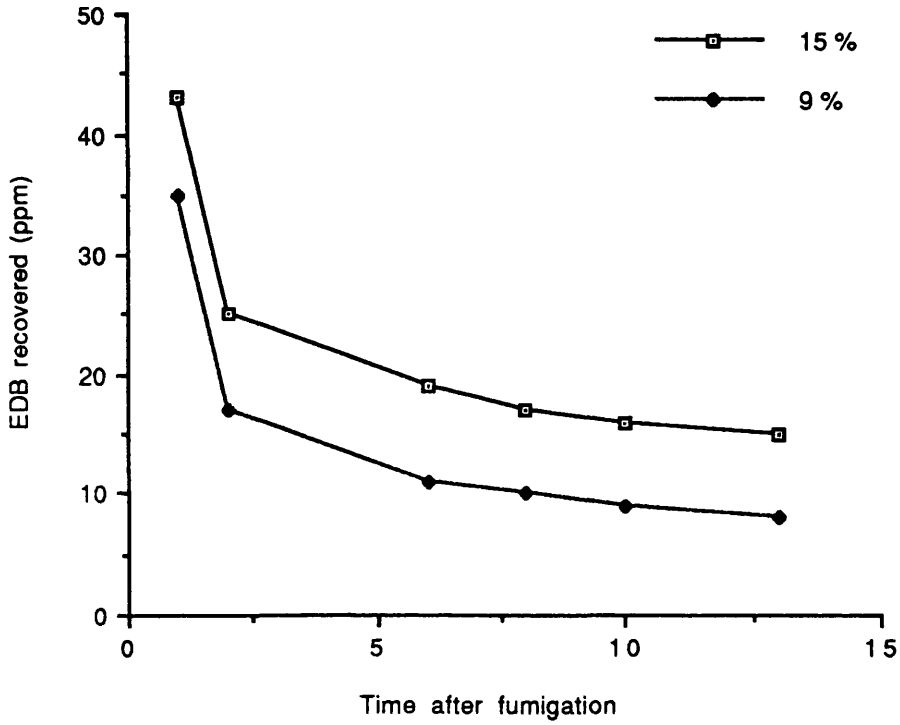
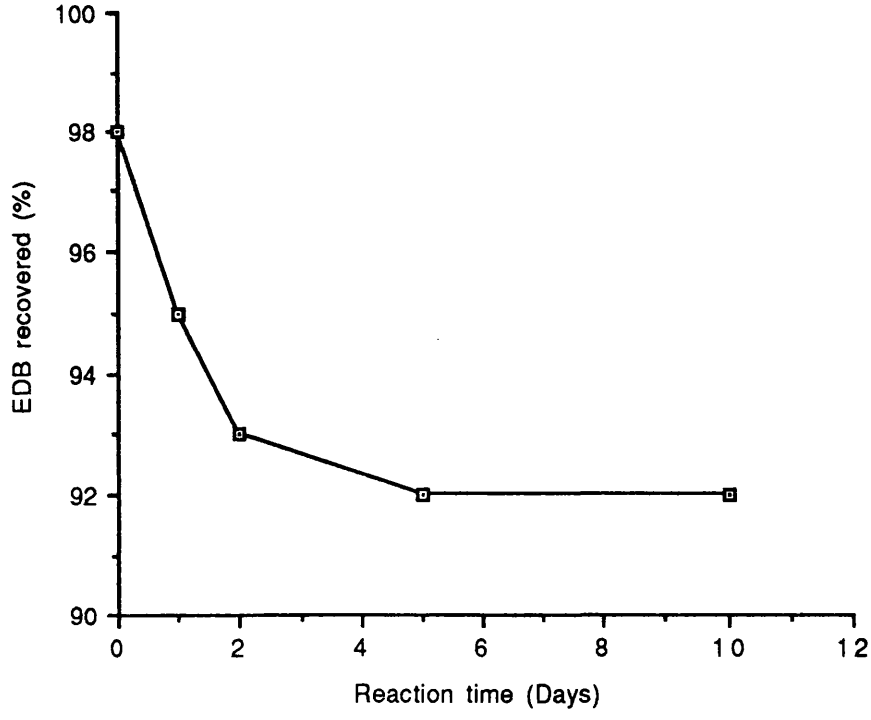


Figure 3.8 The recovery of EDB added in known amounts to ground maize kernels and determined after different reaction times at approximately 21 º C.



Daft, 1989) that residual concentration values obtained represent, virtually, a minimum in so far as the residues of pesticides are concerned. The degradation of ethylene dibromide in tissues of maize was found not to occur to any measurable extent. It may be said that under reduced pressure during or after the fumigation of kernel wheat under the specified conditions little effect remains on the sorption and none at all on the retention of ethylene dibromide.

3.4 SORPTION OF DOWFUME (EB-5) BY MAIZE FLOUR

This section deals with the sorption by maize flour of a mixture of three important fumigants, namely; ethylene dibromide, ethylene dichloride and carbon tetrachloride otherwise known as Dowfume EB-5. As earlier stated, Dowfume EB-5 is widely used as a bulk grain fumigant and contains 7.2, 29.2 and 63.6% (w/w) of the above fumigants respectively.

When the particle size of the grain is reduced, for example by grinding or milling, the amount of sorption increases drastically. The sorption thereby ascends faster and equilibrium is reached sooner than is the case with whole grain. The same effect can be achieved by raising the moisture content. Nevertheless, the increased gas uptake is not a simple or direct function of decrease in particle size or increase in moisture content, as is discussed below.

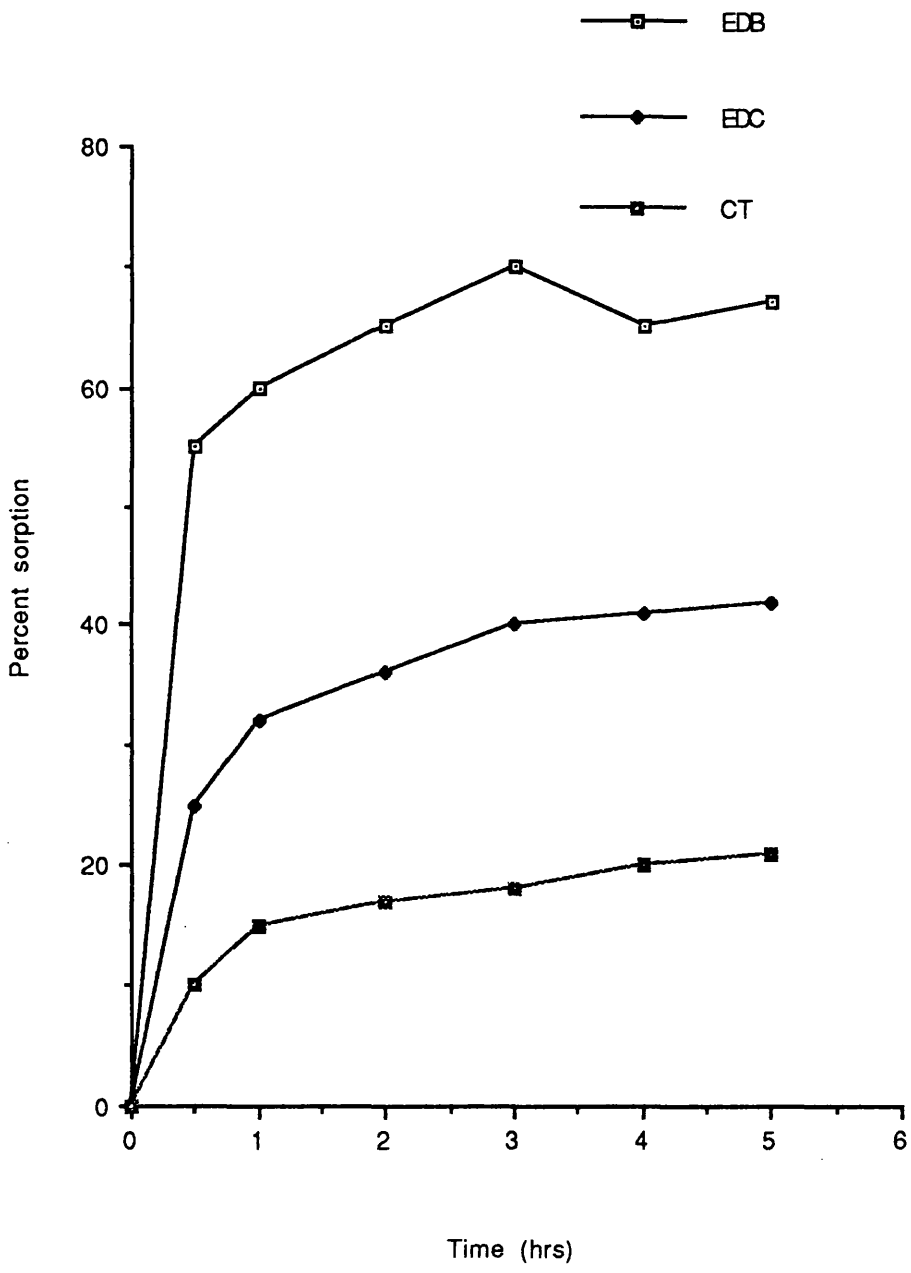
When the three gases are applied in a mixture, the

order of sorption by the maize flour is EDB > EDC > CT (Figure 3.9). This shows that ethylene dibromide is more strongly sorbed than the other two fumigants because of its marked affinity for flour. Relatively small differences in physicochemical properties may also induce marked differences in sorption characteristics. Maize flour, like other natural products, is a polyphase system composed of diverse combinations of polar and nonpolar substances such as water, carbohydrates, proteins, and lipids. Differences in constitution or composition would account for differences in sorption patterns. Grinding and milling of grains greatly multiply their sorption area and concomitantly create more sorption sites. The sites may be activated, all or in part, by additional moisture. The grinding or milling enables the physicochemical complex of grain endosperm to interact with ethylene dibromide, ethylene dichloride and carbon tetrachloride, as shown by their increased uptake in Figure 3.9.

Although the physicochemical nature of the protein-starch-lipid-water complex influences endosperm reactivity, a more exact delineation of the forces that affect sorption of fumigant gases by maize flour will be discussed in Chapter Five.

In conventional bulk fumigation of stored grain, liquid fumigant is applied at the top of a grain bin, the treated grain is covered with plastic sheeting, and the evolving vapours in their downward migration help to control mold and insect infestation. On the basis of

Figure 3.9 Sorption of EB-5 mixture by maize flour.



these and previously reported data (Berck, 1974), it is clear that components other than the grain itself - such as screenings, foreign matter, broken kernels, localized high moisture or low temperature areas, etc. - will compete with molds and insects for toxic gases. The heterogeneous grain bin components each with its own chromatographic character, assist in altering the vapour composition of multi-component mixtures after their application to the bin surface. Thus, the gases that reached the bottom consisted largely of carbon tetrachloride, small amounts of ethylene dichloride, and no ethylene dibromide, and insect infestations will not therefore be controlled at the bottom of the bin. The amount of fumigant residue remaining in or on the maize flour or its meal products after a prescribed treatment with multifumigant mixtures is of practical interest. This will be discussed further in the two analytical Chapters (Four and Five).

3.4.1 Effects of airing and influence of fat on grain at two different temperatures

Unchanged residues of insecticidal fumigants, except methyl bromide in some instances, persist in cereal grain several weeks or months after fumigation (see Chapters Four and Five). Efficient airing is therefore desirable to prevent harmful effects of residues, mainly on humans and animals consuming cereal grain as such. It was mentioned earlier (Section 3.3.1) that airing at low

temperatures is more efficient than at high temperatures. This section describes the effect of such airing on grains and the influence of fat on desorption and also airing at two different temperatures.

When wheat grains were fumigated with ethylene dibromide and carbon tetrachloride at 200 and 160 g/m^3, respectively, and aired at 32 and 14°C (Chapter Four), the results (Figures 3.10 and 3.11) showed that airing of grain proceeds more rapidly at low than at high temperature. With grain fumigated at room temperature, the intercept of the slow desorption term, indicating the firmly bound fumigant, is similar at both airing temperatures. The process of ethylene dibromide desorption (Figure 3.10) is similar to those of most other fumigants. However, the differences in desorption rate become evident after most of the loosely bound fumigant has disappeared, mostly after two or three days of airing. The desorption of carbon tetrachloride (Figure 3.11) is in general slower than that of the other fumigants and therefore the temperature effect appears less pronounced. It seems that the process of rapid desorption is slower and longer with carbon tetrachloride than with other fumigants. After airing for 10 days, the residues at 32°C were more than twice those at 14°C.

One of the possible causes of the unexpected slower desorption of residues at high than at low temperature could be better solubility and retention of the residues by the fat fraction of the grain, at the higher

Figure 3.10 Desorption of EDB from wheat aired at two temperatures.

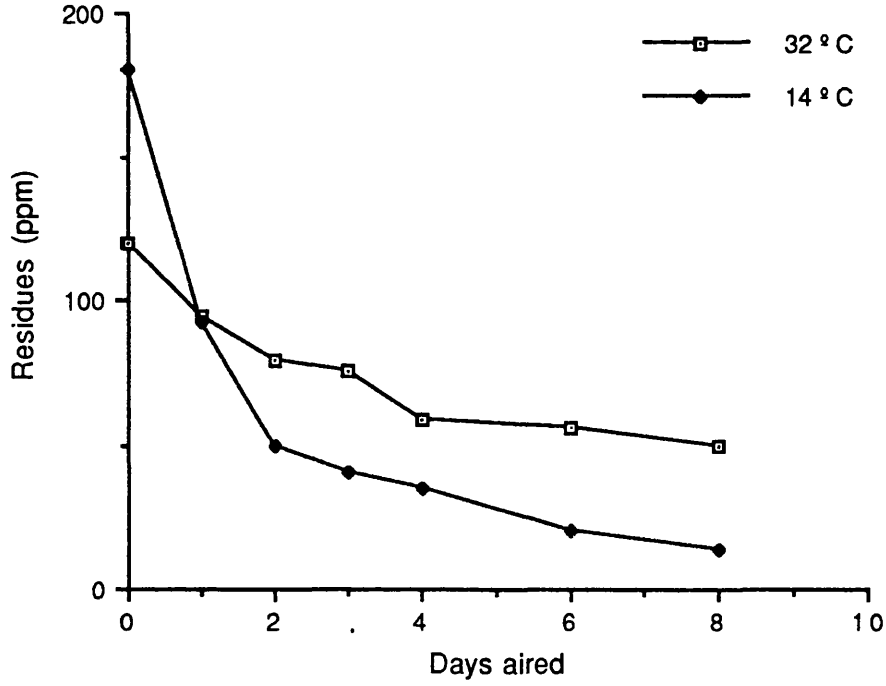
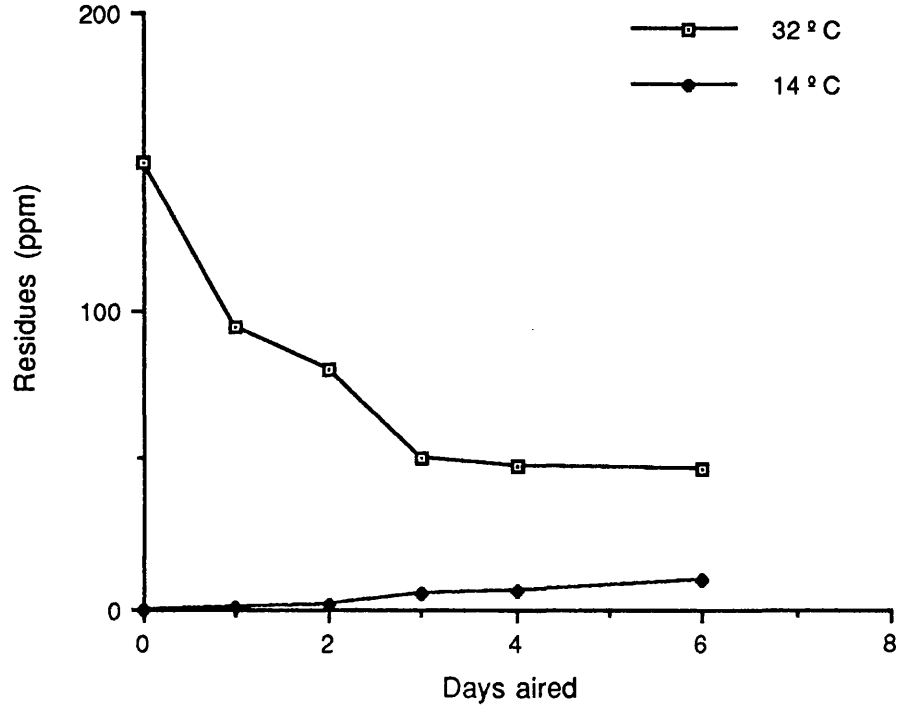


Figure 3.11 Desorption of carbon tetrachloride from wheat aired at two temperatures.



temperature. When experiments were conducted with defatted and fat-containing ground wheat fumigated with the fumigant mixture, the desorption process of ground grain was not similar to that of whole grain. The disappearance of the fumigants was more rapid at 32°C than at 14°C. The presence of fat did not result in longer retention of the fumigant. Therefore, fat is not the factor preventing desorption at high temperatures rather it is believed that probably the grain structure is the principle factor involved.

It seems that the fumigants are retained in whole grain in two forms: loosely and firmly bound. It is probable that the differentation into the two forms occurs during sorption. The relative amounts of each form initially sorbed, calculated from the desorption curves, depend on temperature during fumigation. Less firmly bound residues were found in grain fumigated at low than at high temperatures. When fumigation was carried out at one temperature and only the airing was conducted at different temperatures, the distribution of the loosely and firmly bound fumigants was similar (Figure 3.10). The loosely bound residues disappear very rapidly during the first and second days of airing. The desorption rate of the firmly bound residues increased several times by lowering the temperature, contrary to what had been expected from the physical laws of sorption. The firmly bound residues are probably retained by the whole grain more strongly at high than at low temperatures.

There are, therefore, two components of faster desorption at low than at high temperatures: (1) faster desorption of firmly bound residues when airing is carried out at low temperature and (2) lower initial levels of the above residues when fumigation is carried out at low temperature. The first component of temperature effect was distinct with ethylene dibromide (Figure 3.10), but less sharp with carbon tetrachloride, whose desorption is generally slower than that of the other fumigants. The first step of rapid desorption with carbon tetrachloride (Figure 3.11) seems to last several days, possibly influencing the levels of firmly bound residues.

3.4.2 Behaviour and regulation of fumigant residues in foods

This chapter examines ways in which developments in methods for detecting and determining the nature and magnitude of residues in foodstuffs occurring after fumigation have led to a better understanding of their behaviour and significance. As a result of this new knowledge, certain changes of approach on methods of regulating these residues have been proposed.

Fumigants are characterised by their considerable volatility in relation to other types of pesticides. When a foodstuff is exposed to a vapour or to a liquid which subsequently vaporises, some of the chemical is held by physical forces (sorption) and providing that no chemical reaction takes place, when the surrounding vapour is

removed the sorbed fumigant gradually dissipates into the atmosphere. This process may be relatively slow if solution in constituents of the food, for example in oil or fat, has taken place, or if diffusion is restricted by a surrounding bulk of material. This applies particularly to the use of liquid fumigants on bulk grains but to a lesser extent also to those which are applied as vapours, such as methyl bromide. However, it has for many years been accepted that after use in accordance with good practice no significant amounts of residues of unchanged fumigants such as ethylene dichloride, carbon tetrachloride, ethylene dibromide and methyl bromide would reach the consumer. On this basis some countries tend to exempt these compounds from the requirement for a maximum residue level in respect of the unchanged fumigant (Anon, 1979).

Developments in analytical methods in recent years have provided the means for detecting residues of these fumigants in raw foodstuffs such as cereals and animal feeding stuffs for weeks, months or even years after treatment. These methods are generally based on the analysis of solvent extracts of the commodities by gas chromatography using an electron capture detector (Chapters Four and Five) and are capable of detecting minimum levels of unchanged residual fumigant ranging from parts per million (ppm) to parts per trillion (ppt).

Although subsequent processing and cooking may destroy most of such residue (Daft, 1987) there are

undoubtedly instances where foodstuffs are consumed by man or fed to animals in the uncooked state, while evidence has been obtained of very small amounts of unchanged fumigants occurring in cooked foods (Heikes et al., 1986 and 1987, Daft, 1989). In these circumstances it has been felt that amounts of such residues should be reduced to a minimum at each stage by adherence to good practice in treatments and post-treatment handling.

The FAO/WHO 1979 (and subsequent years) Joint Meeting of Experts on Pesticide Residues took the view that it was desirable, in the absence of sufficient toxicological data to allow higher maximum level figures on the basis of an acceptable daily intake, to recommed limits for such residues in grains or grain products at the point of consumption by man at about the present lower limits of detection. However, in view of the evidence on behaviour of volatile insecticidal fumigant residues during storage and processing which had become available, the meeting felt it possible to recommend "guide-line" residue levels for other stages in the trade movement of grains, namely in raw grains at the point of entry into a country or when supplied for milling, and a second lower level referring to milled grain products to be subjected to baking or cooking. Those recommendations were made in the knowledge that residues in food as offered for consumption should not then exceed an amount close to the limit of determination by currently available analytical methods.

Table 3.5 shows the three levels set by the FAO/WHO Expert Committee for some commonly used fumigants. There was an additional provision made in respect of the upper set of figures. It was recognized that treatment might have been undertaken immediately before shipment or even on board ship and that in these circumstances, at the time of unloading, the residue levels could be changing rapidly due to the handling and movement through the air. The recommendations therefore stipulated that raw grains be discharged or freely exposed to air for 24 hours before sampling for determination of fumigant residue levels. This policy was adopted in order to eliminate the possibility of rejection of a consignment on the basis of high residue figures which shortly afterwards could be well below the maximum residue level, whilst at the same time recognising the value of some measure of control at a central point of distribution. (Anon, 1983).

Table 3.5 FAO/WHO guideline residue levels (ppm)

Commodity	Carbon disulphide	Carbon tetra-chloride	Ethylene dibromide	Ethylene dichloride	Methyl bromide
Raw grains	10	50	20	50	50
Milled grains for baking or cooking	2	10	5	10	10
In bread or cooked grain products	0.5	0.05	0.1	0.1	0.5

As another example of the impact of analytical method development on the subsequent examination of foodstuffs for residue for enforcement purposes, reference can be made to the identification and estimation at different types of bromine-containing residues which can arise as a result of the fumigation of produce with bromide compounds or alternatively by take-up of bromine from soil.

In national maximum residue schedules such as those of the United States, which have long been used as a pattern in many other parts of the world, levels for "inorganic bromide" ("ionic bromide or bromide ion") in particular commodities appear which, in many instances, have been set according to amounts which have been shown in trials to occur as a result of fumigation with methyl bromide or ethylene dibromide according to good practice. Two important points can be made about these maximum levels and their determination. Firstly, the analyses are almost invariably carried out by methods which determine total bromide. In the absence of additional analyses for organic bromine compounds it is by no means certain, especially after fumigation with ethylene dibromide, that the bromine determined is present in the ionised forms.

With the availability of the Gas Chromatographic methods (Chapters Four and Five) for determining small amounts of unchanged residual fumigants, assays can now be carried out to find by difference how much of the total bromine content is in ionic form.

The second point concerning maximum levels for ionic bromide in stored grains is that while they are partly designed to avoid excessive residues due to bad practice and in the case of methyl bromide fumigation to give an indication of the presence of other fumigant reaction products, it is not at present possible to determine whether ionic bromide is present as an addition product from fumigation or has been taken up by the plant from soil. Unless there is a considerable body of data available on the average levels of "naturally" occurring bromide in grains it is impossible to judge whether a certain bromide level can be ascribed to fumigation; thus such maximum levels are of limited value in controlling bromide arising from specific uses.

In the case of cereal grains such as wheat and maize there is abundant evidence that unfumigated produce seldom has a bromide content exceeding 10 ppm. In these cases the present levels of 50 ppm provides a means of regulating the use of bromine-containing fumigants on grain in storage.

The examples described are typical of a problem which is currently receiving a great deal of attention in field other than pesticide residues, namely the tendency to set maximum residue levels for impurities in isolation from consideration of the analytical and sampling methods available to enforce them.

In the present rapid state of development of analytical chemistry, the weight of technical opinion is

against the rigid specification of methods of analysis, lest improvements in technique prove difficult to adopt quickly. Nevertheless, methods to be used in the fields under discussion must be capable of producing unequivocal results and some form of intercomparison or collaborative method-testing is imperative. Providing that this requirement is met, the principle of equivalence of methods is probably better, scientifically and practically, than attempting to adopt one official method, on which agreement in the international field may prove to be unattainable in all but a few cases.

3.4.3 The fate of methyl bromide inside and outside fumigation premises

Methyl bromide has been chosen as a good example of a fumigant likely to escape to the surrounding environment because of its high volatility and acute toxicity. Restrictions on the use of liquid "spot" fumigants in flour mills have brought about the need for alternative procedures to control insects that infest the mills. One effective method for achieving the degree of insect control that is needed to satisfy health standards is to apply the fumigant, methyl bromide, in a general fumigation treatment to the entire mill facility. This procedure, although more costly than the spot fumigation treatments, can be effective if conducted according to recommendations.

In carrying out a general fumigation of a building, sufficient gas to kill the insects must be liberated into the free space and then maintained at the toxic level for a defined period of time. After the treatment, the residual gas remaining in the building is dispersed into the atmosphere outside the mill and into the environment. The normal procedure for aeration involves either opening doors and windows to allow the gas to diffuse or the operation of exhaust fans to blow it outside the mill. Thus, the residual fumigant can be quickly diluted to low concentrations by mixing with the outside atmosphere. The rate of the dispersal and diluting process is thought to be rapid because of the great amount of space into which the gas is liberated.

However, the question of dispersing the toxic gas like methyl bromide and other fumigants into the environment, where they might pose a hazard to human beings, plants and animals is a subject of great concern. One of the main questions is whether the gas does disperse as rapidly as has been assumed or whether it flows out of the building in plume-like currents without appreciable dilution to creat potential hazards to human beings and other members of the environment. This concern has pointed to the need for information on the levels of fumigant that occur in the atmosphere in and around buildings during a fumigation treatment.

According to Bond et al., (1988), the concentration of the fumigant inside the mill was found to be lower than

the original one and further dilution occurred rapidly to reduce concentrations to even lower levels as these gases escaped to the atmosphere either through leakage or open structures. The fumigant was carried a considerable distance from the site of application at concentrations above the threshold limit value of methyl bromide (5 ppm) and at a point 25 m from the exhaust point the concentration was only a little lower than that found at 14 m distance. Dumas et al., (1982) found that the fall in concentration of the fumigant that occurs during treatments of bulk grains points to the possiblity that large amounts of the fumigant escaped to the outside atmosphere and into the environment during the treatment period. This may mean that because a large amount was lost through leakage and sorption during the treatment, any hazard posed inside the mill may have been reduced but the one outside the mill may have been greatly increased. Moreover, the sorbed residual fumigant poses a great health hazard when such grains or their by-products are consumed. Because of the variety in structures, environmental conditions and other parameters of the treatment, the extent of hazard to human beings living or working in the vicinity of flour mills during treatment will only be guaranteed if carefully controlled procedures are followed.

CHAPTER FOUR

FUMIGANT RESIDUE ANALYSIS

4.1 HEADSPACE ANALYSIS OF METHYL BROMIDE IN MAIZE

4.1.1 Introduction

As a result of regulatory actions that have been taken in most countries to remove, or are in the process of removing, liquid fumigants from the ranks of approved pesticides, the use of alternative safer insecticidal compounds is expected to increase. With the severe restriction in the use of the widely used liquid fumigant ethylene dibromide in 1984 by most developed countries (Page and Avon, 1989), public and regulatory attention has been directed to other fumigants such as methyl bromide to control stored grain insects. A ready penetration into foodstuffs such as grain in silos or bins, coupled with a high toxicity to insects makes methyl bromide an excellent fumigant in most tropical countries where insect infestation is abundant.

To assure a food supply that is free of residues of methyl bromide above the established maximum residue levels, a method is needed which possesses the requisite sensitivity, selectivity, speed, and cost effectiveness for routine analyses of this hazardous compound. There are many modern methods for the analysis of fumigant residues in grain (Heikes, 1987; Scudamore, 1987; Daft, 1988 and many others). These methods involve soaking the

food commodity for a 24 - 72 hour period before extraction. This not only involved an extensive soaking period but also a long unnecessary sample preparation time, of which are unsuitable for methyl bromide due to its high volatility.

De Vries et al. (1985), Page and Avon (1989) and Norman (1991) reported analyzing for methyl bromide in food ingredients by the headspace method. This method determines volatile fumigants in food samples by analyzing the vapour phase that is in thermodynamic equilibrium with the sample in a closed system. It is used predominantly for the determination of trace concentrations of volatile fumigants and other industrial compounds in samples which are difficult to analyze by conventional gas chromatographic means. This method avoided the necessary long soak period and the need for drying the extracts obtained by the acetone/water soak methods described above. It also resulted in increased sensitivity in the detection of methyl bromide. The headspace method by Norman (1991) was checked and modified wherever possible and used to extract gaseous methyl bromide residues in laboratory fumigated and fortified maize grains in this study. Although this study will portray the headspace method as the best method so far for residue analysis, other available methods will be covered in this chapter to ascertain their overall validity in fumigant residue analysis.

4.1.2 Experimental method

A Hewlett-Packard model 5890 gas chromatograph equipped with a ^{63}Ni electron capture-detector and a 30 m x 0.32 mm iD (1.0 μm film) DB-5 fused silica capillary column with a splitless injector purchased from Burke Electronics Ltd (U.K.), was used in this study. The operating conditions for injector, oven and detector were 200°, 40° and 270°C respectively. The carrier gas was pure nitrogen purchased from B.O.C. Glasgow with a flow rate of 4 ml min^{-1}. The recording was made using a gas chromatography compatible PC computer model HP 900/300 Series.

The headspace vials were 120 ml and 30 ml (Chrompack Ltd) fitted with teflon-coated silicone septa (Phase Sep, U.K. Ltd) and crimp aluminium caps (Alltech). SGE gas-tight syringes supplied by Burke Electronics Ltd, were used to sample the headspace gas. Methyl bromide gas standard (99.5% pure) was obtained from BDH Chemicals Ltd, England. All other chemicals and reagents were Analytical grade obtained from Rathburn Chemical Ltd, Scotland and were used as such. Deionized water was used instead of tap water to minimize impurities and interfering peaks.

The principle of headspace sampling techniques is very simple and straightforward. The sample (solid or liquid) is placed in a glass vial of appropriate size and closed tightly with a rubber septum crimped in place by an aluminium cap to prevent the dissipation of the volatile gas into the surrounding environment. The vial is

carefully thermostated until equilibrium is established. The gas phase is then sampled by gas-tight syringe for manual (or automated) gas chromatographic analysis.

Since commercially fumigated grains were unavailable at the time of this study, laboratory treatment of maize grains was carried out with 16 mg l^{-1} concentration of methyl bromide at 22°C in the dark, equilibrated for 24 hours and then aired for about 2 hours before analysis. About 4 g of the aired grain sample was added into a vial containing 17 ml of deionized water, 4 g of sodium sulphate and a magnetic stirrer. The vial was immediately sealed with a crimp aluminium cap with a teflon-lined septum. The vials containing methyl bromide standard solution in acetone and samples were placed into a water bath at room temperature and left to equilibrate for 4 hours. The headspace from the standard solutions and the sample were sampled using a gas-tight syringe and quantified using a gas chromatograph with an electrom capture detector (GC-ECD) operated as specified above. Mehtyl bromide residues were calculated by comparing standards and sample peak areas.

4.1.3 Results and discussion

In developing a method to determine volatile insecticidal methyl bromide in maize or other cereal grains, it was first necessary to determine the chromatographic conditions best suited for such a volatile compound so that methyl bromide would be eluted in a reasonable time with acceptable peak shape. Figures 4.1

and 4.2 represent methyl bromide standard and spiked sample chromatograms respectively, determined using the gas chromatographic conditions specified in Section 4.1.2. In Figure 4.1, the standard peak of methyl bromide (about 400 $\mu g\ kg^{-1}$) eluted at 0.247 minutes while in Figure 4.2, the spiked sample peak (about 355 $\mu g\ kg^{-1}$) eluted at 0.255 minutes. All chromatograms in this project are direct reproductions from the original trace and are reported here as such.

When the grains were fumigated and analyzed almost immediately after only a 4 hour airing period, the average mean residue ranged from 5 to 45 $\mu g\ kg^{-1}$ (ppb). This shows that methyl bromide dissipates very fast from both the adsorbed and the absorbed levels and that in a longer period of aeration there will be little or none of the residue remaining in the grain. This makes methyl bromide a preferred insecticide compared with other liquid fumigants like ethylene dibromide which involves a long period of association with the foodstuff.

Table 4.1 shows the recovery of methyl bromide from a sample of maize grain spiked at the 10 ng/ml level. The mean percentage recovery was found to be 89% which is quite satisfactory in this study given the conditions of the apparatus used and the high volatility of methyl bromide.

Figure 4.1 Chromatogram of headspace of methyl bromide standard. Methyl bromide eluted at 0.247 minutes.

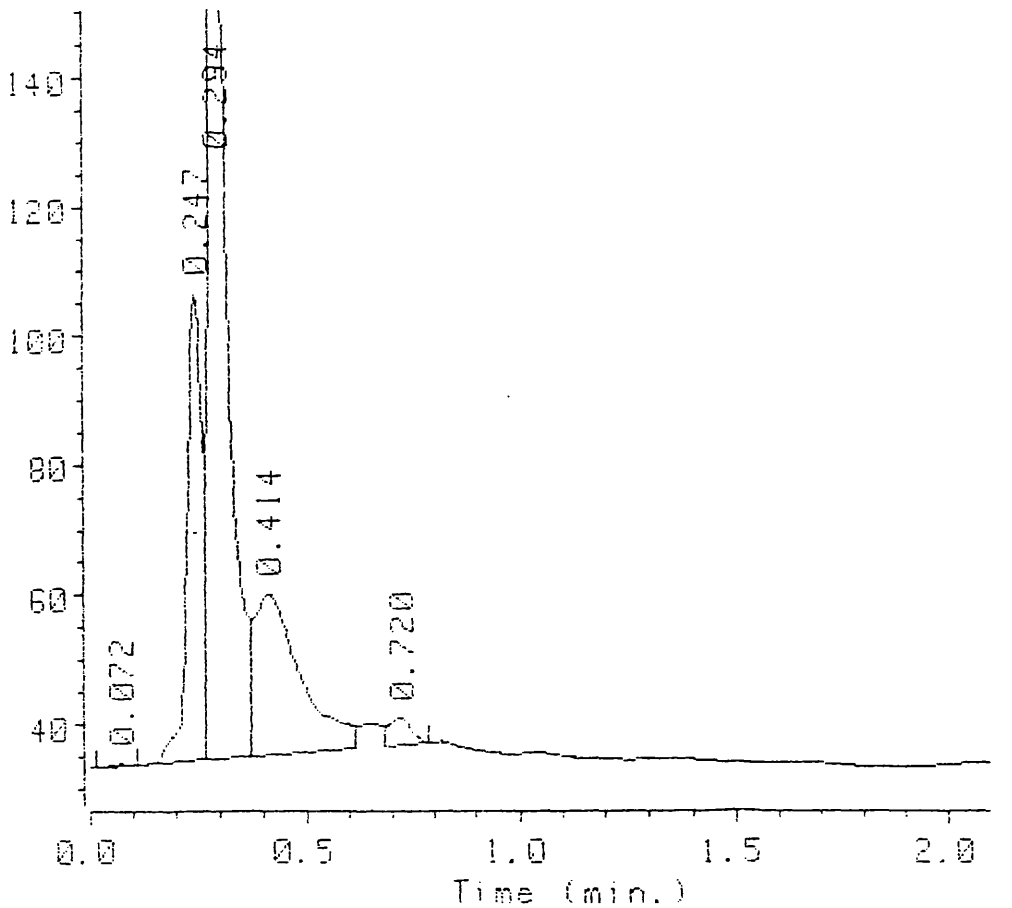


Figure 4.2 Chromatogram of headspace of from maize sample spiked with methyl bromide (retention time; 0.255 minutes)

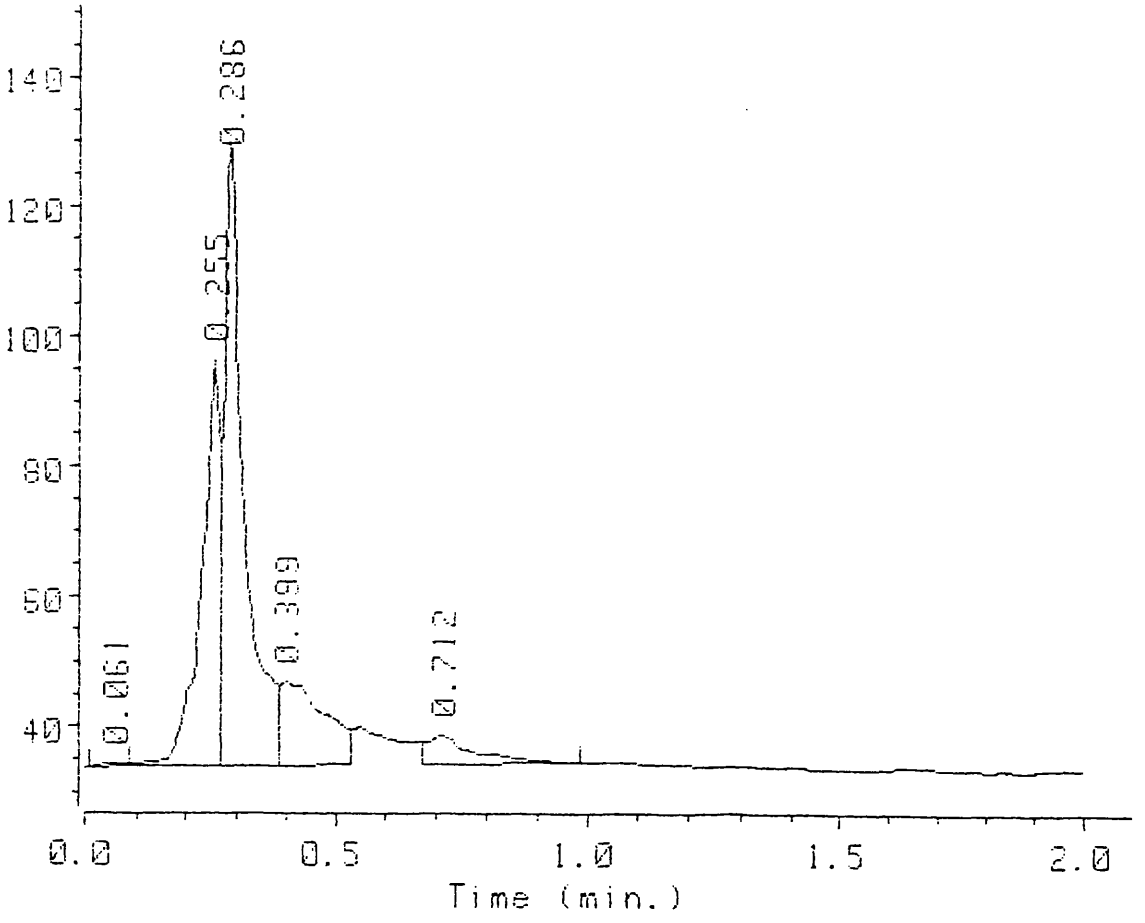


Table 4.1 Recovery study of methyl bromide in laboratory spiked maize grains

Treatment	Methyl bromide ($\mu g\ kg^{-1}$) Applied	Found	% Recovery ± SD[a]
1	400	355	88.8 ± 0.63
2	200	178	89.0 ± 1.25
3	100	89	89.0 ± 0.86
4	50	45	90.0 ± 1.33
	Mean[b] % Recovery		89.2 ± 0.54

a - standard deviation

b - mean of 4 replicate injections

Norman (1991) reported that absorbed fumigant (aged residue), which is much more difficult to extract will require a higher temperature to reach equilibrium than adsorbed volatiles (recently fumigated). Therefore, the reduction in the headspace concentration in a sample spiked with a standard of equivalent amount would be due to the absorption/adsorption of the fumigant by the commodity and also due to dissipation of the fumigant to the surrounding environment. For simplicity, room temperature (22°C) was chosen in this work as the headspace equilibration temperature because temperatures above room temperatures will require thermostatted stirring baths. In another study by Page and Avon (1989), it was found that temperatures above 40°C would require a

heated syringe to eliminate condensation of the water in the syringe during sampling.

To carry out static or equilibrated headspace analysis, an equilibrium of the analyte must be obtained between the condensed phase (liquid and/or solid) and the gas phase. The time required to reach this equilibrium depends on a number of factors including the sample particle size. Page and Avon (1989) blended the grain sample to obtain a finely homogenized representative sample. In this work, whole maize grain was used to avoid loss of the volatile methyl bromide. Although slow release of methyl bromide was observed in whole grain analysis, equilibration time was increased from 1 hour (Page and Avon, 1989) to 4 hours. This period of equilibration was found to be enough to release all the methyl bromide.

The coefficient of variation between methyl bromide fumigant and the grain sample is very high (Daft, 1986) and to be able to determine the truest mean residue level in large volumes of stored grains, sample portions in excess of 1 g as proposed by De Vries (1985) is needed. 4 g of the maize sample was used in this work and it was found to be sufficient.

The high volatility of methyl bromide resulted in a headspace method which is straightforward, rapid and efficient to carry out. The method is capable of detecting methyl bromide at the 0.4 ppb level based on 2^4 signal to noise on the chromatogram. The method also

prevents the introduction of nonvolatile materials into the column or injector and hence overloading of the detector is minimized. According to the work reported here, it is highly unlikely that any harmful residual methyl bromide will remain in food products milled from such grains if the starting ingredients are held and aerated for an appropriate time.

4.2 ANALYSIS OF ETHYLENE DIBROMIDE IN WHEAT BY PURGE AND TRAP METHOD

4.2.1 Introduction

In response to, and in conjunction with, the suspension of registration of fumigation products containing ethylene dibromide (Anon, 1984a) and the establishment of new maximum residue levels for residues of ethylene dibromide (EDB) in grain and grain-based products by the Environmental Protection Agency (EPA) and other international monitoring bodies, a world-wide survey began of EDB residues in foodstuffs and other products.

There are many methods used nowadays to determine ethylene dibromide in various food products (Clower, 1980; Rains and Holder, 1981; Clower et al., 1986; Konishi et al., 1986; and many others). Most of these methods gave unsatisfactory results which resulted in low recoveries of EDB in a number of fortified foods. This and many other factors prompted the development of a more generally applicable and sensitive method which is capable of

detecting EDB residues in very low concentrations and give high recoveries from the fortified samples. A method by Heikes (1985) was modified, checked and used in this work. This method is known as the Purge and Trap method and it involves purging the sample with an inert gas and trapping the fumigant in a trap-tenax TA tube.

4.2.2 Experimental method

Ethylene dibromide residue levels were determined with a Hewlett-Packard model 5890 gas chromatograph equipped with a ^{63}Ni electron-capture detector operated at 270°C and a 30 m x 0.32 mm iD (1.0 μm film) DB-5 fused silica capillary column held at 50°C with a splitless injection system operated at 200°C and a nitrogen carrier gas (purchased from B.O.C., Glasgow) with a flow rate of 4 ml min^{-1}. The integrator used was a Hewlett-Packard compatible computer model 9000/300 series. The residue levels were confirmed by mass spectrometry using positive electron impact and the direct inlet technique.

Ethylene dibromide (pesticide grade) standard was purchased from BDH Ltd Poole, England. Hexane, acetone and methanol, all high quality pesticide grade were purchased form Rathburn Chemical Co. Ltd., Scotland. All other chemicals and solvents were reagent grades or analar and were used as such.

Tenax TA (80 - 100 mesh) was purchased from Jones Chromatography, Mid Glamorgan, (U.K.). The Tenax TA (Figure 4.3) was washed thoroughly in turn with hexane, acetone, and methanol, dried with nitrogen between washes,

conditioned for 2 h at 340°C under a nitrogen stream (20 ml/min), then stored in a glass container with teflon-lined screw cap. The nitrogen (GC quality) was purchased from B.O.C., Glasgow. All the apparatus was assembled as shown in Figure 4.4.

The principle applied in this experiment is that the samples in water are extracted (sparged) with a nitrogen stream while being stirred vigorously in a water bath at 100°C. Ethylene dibromide collected on the adsorbent Tenax TA and eluted with hexane is determined by gas chromatography fitted with an electron capture detector (GC-ECD) and confirmed with GC/mass spectrometry (MS). Thermal desorption of the Tenax TA could have been the best method of elution but it was not available at the time of this experiment and therefore hexane elution which equally gives reliable results was used instead.

About 10 g homogeneous frozen wheat sample was accurately weighed into a 500 ml double-neck, round-bottom flask containing 250 ml deionized water, 1 ml antiform B, and stirring bar. Using a universal adaptor, a gas dispersion tube was placed into the angled side neck of the flask to a depth of about 5 mm above the stirring bar. The assembly of Purge and Trap apparatus was completed by addition of a Liebig condenser with a second universal adaptor and trap tube (Tenax TA tube) at the top as illustrated in Figure 4.4.

The assembled apparatus was placed in a boiling water bath positioned above a hot plate with a magnetic

Figure 4.3 Tenax TA trap tube: A, Pasteur pipette with shortened tip; B, glass wool plugs; C, 150 mg Tenax TA

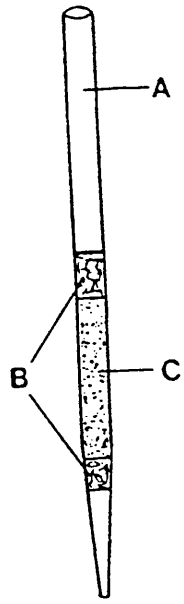
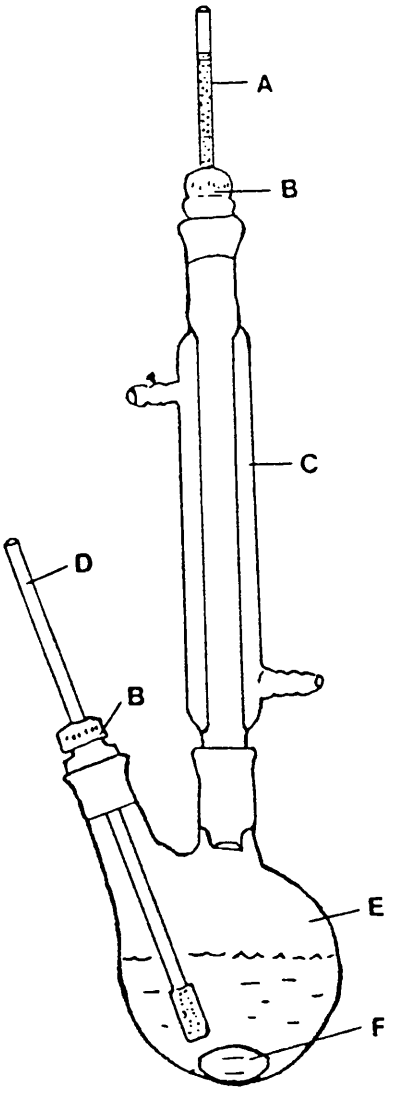


Figure 4.4 Purge and trap apparatus: A, fumigant trap tube; B, universal adaptors; C, 20 cm Leibig condenser; D, gas dispersion tube; E, 500 ml round-bottom, double-neck flask; F, magnetic stirring bar



stirrer. Nitrogen flow was adjusted to 100 ml/min through a gas dispersion tube and stirred vigorously to obtain a strong vortex.

After 1 hour, the Tenax trap tube was removed from the universal adaptor and eluted with at least 3 ml hexane into a suitable volumetric flask. The eluate was diluted to volume (or as required) with hexane and EDB quantified using GC/ECD. The presence of EDB was confirmed with GC/MS as described above.

4.2.3 Results and discussion

The retention time of ethylene dibromide standard was 1.269 minutes and that of the sample was 1.235 minutes under the above GC conditions with a capillary column as shown in Figure 4.5 and 4.6. A few impurity peaks were observed on the gas chromatograms but they did not interfere with the ethylene dibromide peak which was clearly resolved. The wheat used in the experiment was fumigated in the laboratory about one month prior to analysis and kept in the freezer packed in amber glass jars sealed with aluminium foil-lined caps. All analyses were performed with deionized water since tap water was found to contain several purgeable compounds which appeared as interfering GC peaks. Procedural blanks using deionized water however, were free of GC responses with GC-ECD. Purge and Trap method removes more than 90% of the ethylene dibromide residue from the sample once some important parameters were met. Some of these parameters include preventing frothing by using a silicone emulsion

Figure 4.5 Chromatogram of ethylene dibromide standard. (Elution at 1.269 mins.)

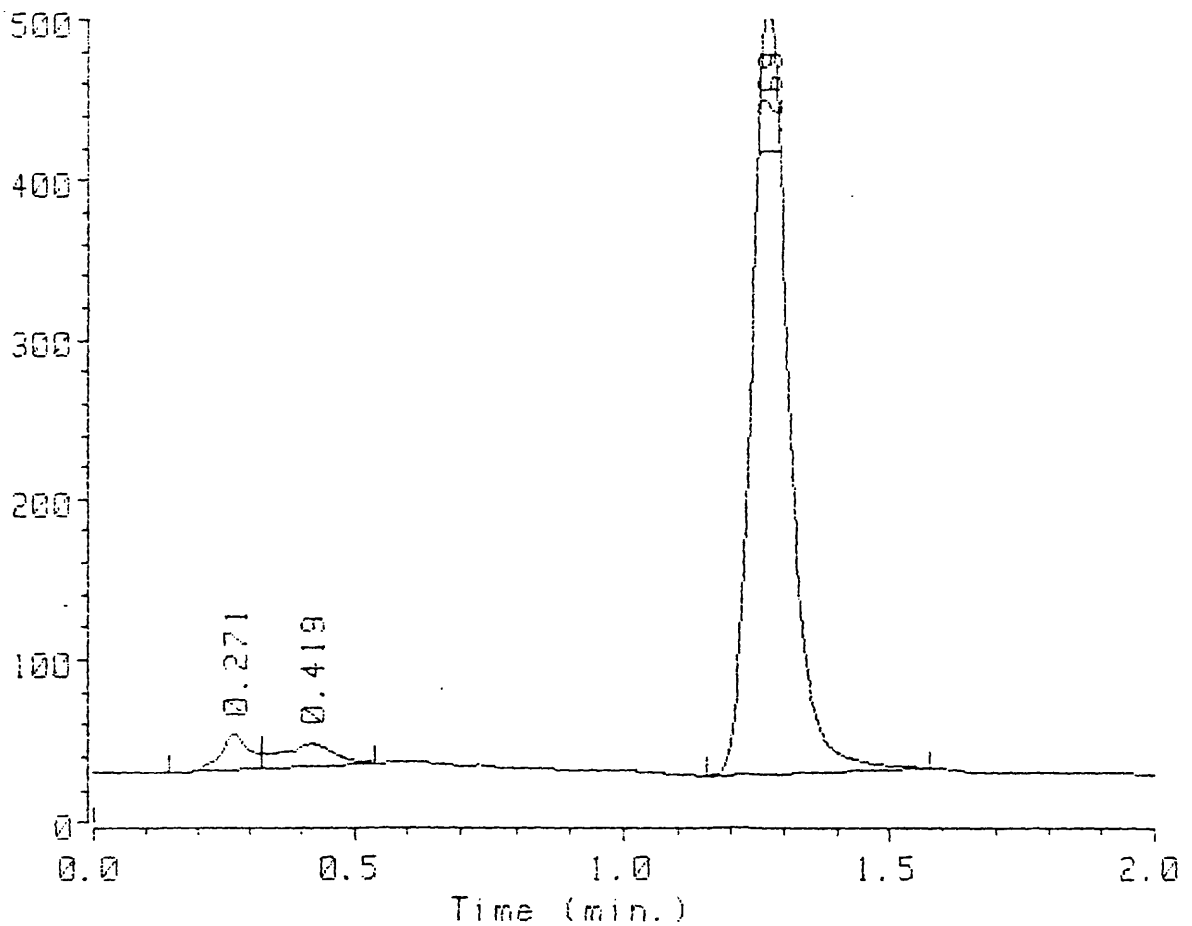
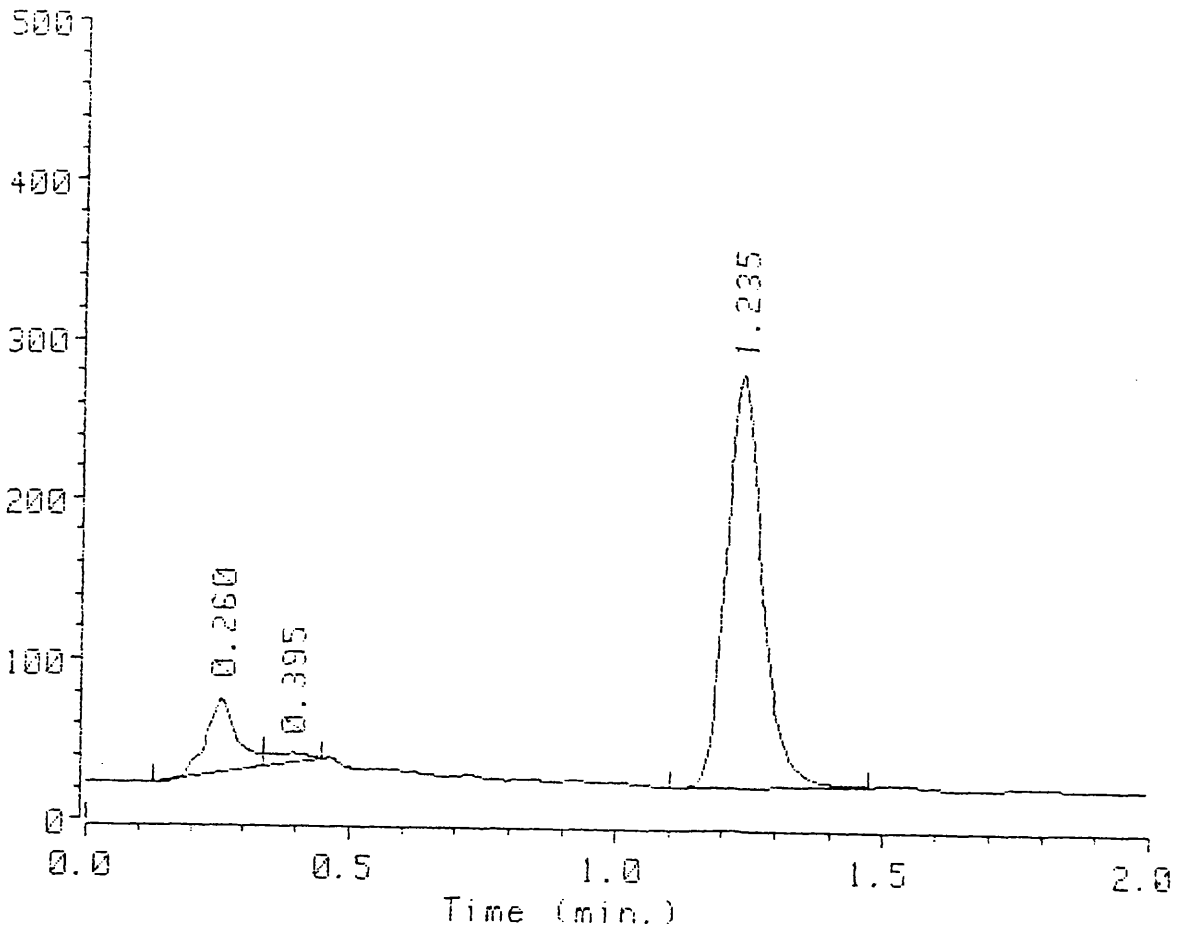


Figure 4.6 Chromatogram of ethylene dibromide from laboratory fumigated wheat sample. (Retention time; 1.235 mins.)

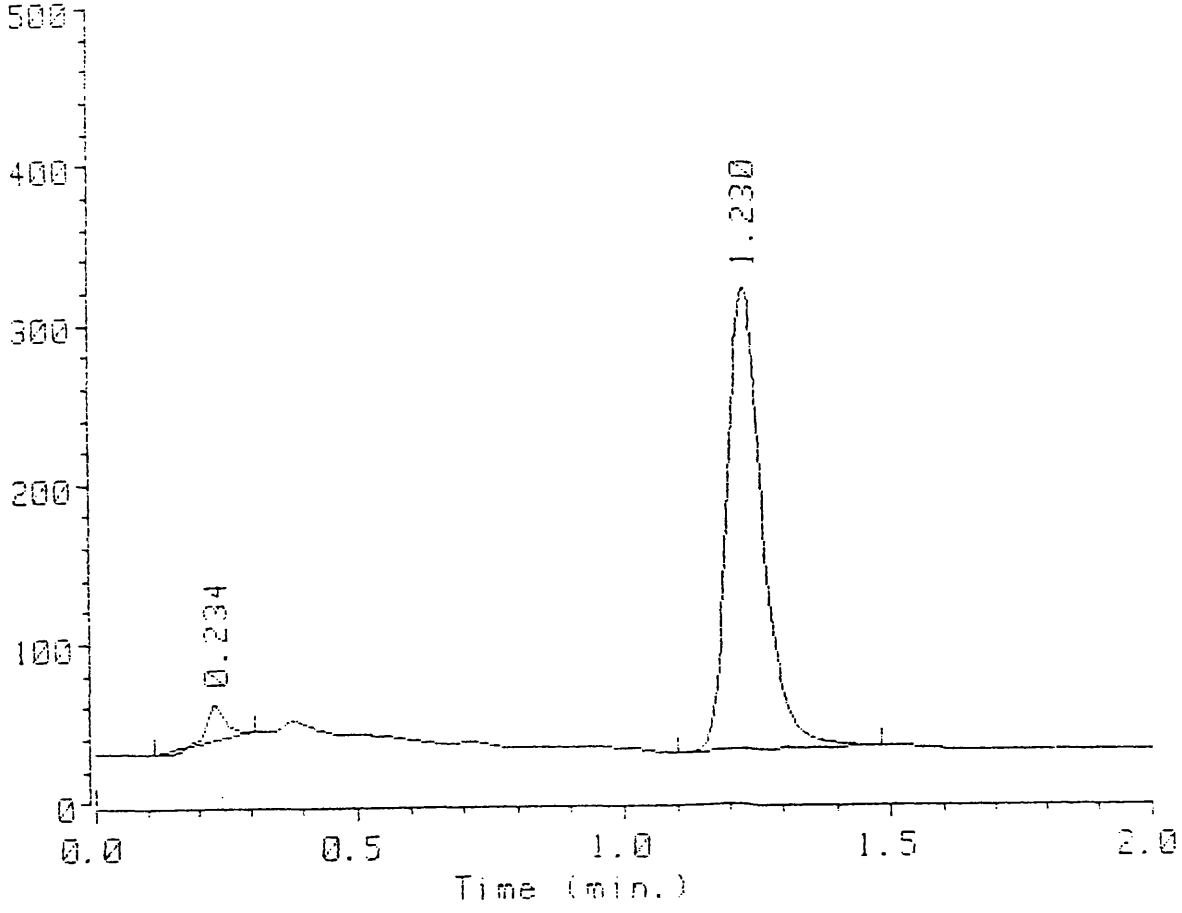


antifoam and by magnetic stirring. Inserting the gas dispersion tube in the angled side neck of the round-bottom flask will assure that the nitrogen will be released just at the edge of the stirring vortex. The bubbles are pulled beneath the water surface and dispersed throughout the mixture rapidly, increasingly yielding significantly high recoveries.

The wheat sample used in this work was fumigated with ethylene dibromide 30 days before the analyses. After airing and storage in the freezer, residues of ethylene dibromide ranging from 0.09 mg kg^{-1} (5.7%) to 0.18 mg kg^{-1} (20.7%) were observed after the extraction. This is the absorbed residue which is associated with the food constituent and will be passed on to the consumer. Although the amount here is small compared to the MRL of 5 mg kg^{-1} in raw grain, it is a clear signal that this compound is retained in food and can stay intact for a long time and can gradually accumulate in human tissues if repeated doses are frequently consumed.

Table 4.2 shows recovery of ethylene dibromide from a wheat sample fortified with 10 mg/ml of ethylene dibromide spiked standard in hexane. Figure 4.7 shows the resulting chromatogram. Ethylene dibromide elutes at 1.230 minutes. The recovery level was satisfactory although the average recovery was 85%±32.66 SD. A total of four duplicates was used in this analysis. All the resulting chromatograms of the samples (Figure 4.6) and the spiked sample (Figure 4.7) were compared with equal

Figure 4.7 Chromatogram of ethylene dibromide from fortified wheat sample (10 ng/ml). (Retention time; 1.230 mins.)



volumes of standard extracts (Figure 4.5). A blank (control) sample extract was also analysed to confirm the results.

In this work, a gas chromatograph equipped with an electron capture detector and a DB-5 capillary column (Section 4.2.2) was used to achieve 0.5 ppb as the detection limit.

Table 4.2 Recoveries of ethylene dibromide from fortified wheat grain

Replicate analysis	Amount Applied ($\mu g\ ml^{-1}$)	Amount Found ($\mu g\ ml^{-1}$)[a]	% Recovery ± SD
1	0.87	1.00	115 ± 2.16
2	0.87	0.49	56 ± 0.82
3	0.87	0.50	57 ± 1.83
4	0.87	0.97	111 ± 2.50
	Mean Recovery		85 ± 32.66

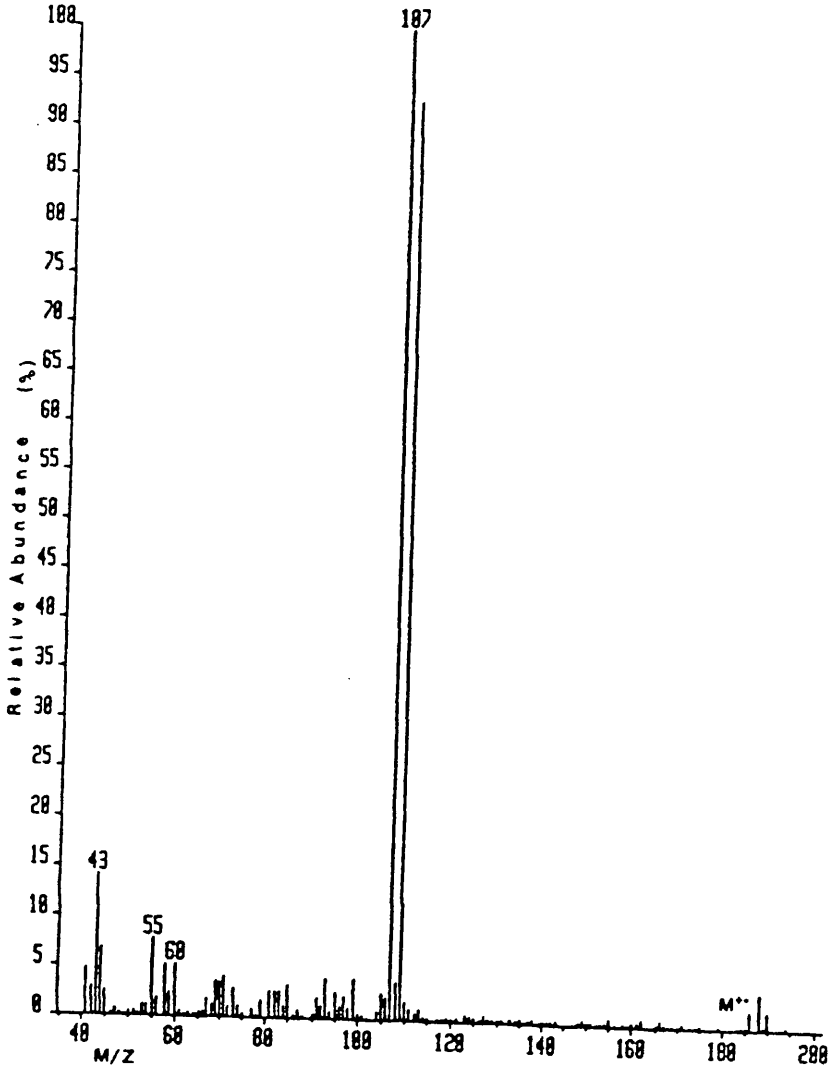
a - mean of 4 replicate injections

Techniques for environmental analysis using capillary columns have been developed rapidly in recent years. In this study, the capillary column was used for ethylene dibromide determination with satisfactory and acceptable results. The low column temperature (50°C) enabled the efficient separation of highly volatile materials from EDB. When a packed column was used at 150°C (Heikes, 1985) many interfering peaks appeared near

the ethylene dibromide peak. The detection limit of EDB on the capillary column in this study was 0.5 pg (0.5 ppb x 1.0 μl). This level was 50 to 100 times more sensitive than levels obtained by Heikes (1985) and Clower (1986) with a packed column. Heikes (1985) also found that the fat content of the wheat sample has little effect on extraction efficiency of sparging. At 100°C, fats are liquified and, with vigorous stirring, form an emulsion with water and nitrogen. A greater dependence of extraction rate therefore appears to be due to the mixing of the sample.

The adsorbed ethylene dibromide was removed from the Tenax TA trap tube with 3 ml of hexane instead of the 1 ml used by Heikes (1985). Tenax TA (Figure 4.3) was chosen in this study over other absorbents because of its low affinity for water, and the trap tube can be reused after thorough washing and conditioning as described under experimental method. Mass spectrometry confirmations were performed from the above concentrated eluate with further dilutions. Figure 4.8 shows an electron impact mass spectrum of ethylene dibromide (about 700 pg) purged and trapped from the wheat sample. The electron impact full scan spectrum of EDB exhibited a base peak isotope cluster at M/Z 107/109 representing the EDB ion. Because of the maximum sensitivity level required, M/Z 107 and 109 were selected as the ions to be monitored for the MS/EI analysis.

Figure 4.8 Electron impact mass spectrum (70 eV) of ethylene dibromide purged and trapped from wheat sample



To establish the presence of EDB in the sample, both ions M/Z 107 and 109 had to show a concurrent maximization at the retention time of EDB at least nine times greater than the average background level. Also, the ratio of the isotope intensities had to fall within the range obtained for the standards. The mass spectrum (Figure 4.8) was considered specific enough for the mass spectral confirmation of ethylene dibromide because M/Z 107/109 represents the EDB ion which is a structurally significant fragment ion.

In conclusion, this purge and trap method is suitable for the rapid determination of ethylene dibromide in wheat grains and other foodstuffs. It gave good recovery of the fortified samples although not good reproducibility. It is not labour intensive and the use of highly specialized equipment is not required. Elution of the adsorbent Tenax TA results in a relatively clean and concentrated extract suitable for both low level GC quantitation (0.5 ppb) and mass spectrometry confirmation analysis. It is however, early to conclude that this method is the best in fumigant analysis because there are many more methods to be compared in the coming section and in Chapter Five. In fact the headspace method (Section 4.1) is related to this method in that the eluate is in gaseous form in one stage or another.

4.3 STUDIES ON THE FUMIGANT RESIDUES IN WHEAT FUMIGATED WITH LIQUID BROMOMETHANE

4.3.1 Introduction

The use of methyl bromide as an insecticidal fumigant gas and its residues on fumigated maize grains was discussed in Section 4.1. The work on this section describes the study on the residues of methyl bromide as a liquid fumigant on wheat grain.

The sorption of bromomethane on wheat, barley and oats during fumigation and its subsequent loss during airing has been studied by Scudamore (1987b) after treatment with bromomethane alone or with a mixture with 1,1,1-trichloroethane. The extent of sorption of bromomethane and the amount of inorganic bromide formed from reactions of bromomethane with constituents of the grain samples were not affected by the presence of 1,1,1-trichloroethane (Scudamore, 1987b).

There is a continuing need for example in the United Kingdom and many other countries, for the use of liquid fumigants for the control of insect infestation on grain stored in silos or in bulk on farms (Bell and Rowlands, 1983). However, because of the increasing awareness of the harmful effects on man of many of the volatile halogenated hydrocarbons used as fumigants such as carbon tetrachloride, 1,2-dichloroethane, 1,2-dibromoethane and many others, bromomethane has been investigated as a possible alternative (Hole et al., 1985).

Bromomethane which is widely used as an insecticidal fumigant, is in general terms unsuitable for treatment of grain contained in many of the larger storage structures especially in the United Kingdom because of problems in achieving adequate sealing and acceptable distribution within a cereal bulk. However, bromomethane and many other halogenated fumigants are still being used in many countries all over the world and hence cannot be totally ignored as long as toxic residues keep on recurring in the environment and endangering the lives and comfort of its occupants. The use of bromomethane as a fumigant is rapidly declining in the United Kingdom especially in commercial situations and it is difficult to receive a constant supply of commercially fumigated samples for analysis. In order to get a true representation of the commercial (or aged-residue) samples, reliable methods were developed in the laboratory to treat grain samples. These methods were used in this project and found to conform fully to the agreed international fumigation standards which are normally followed during commercial fumigation. The influence of level of bromomethane treatment, commodity moisture content and temperature on sorption and subsequent airing rates has been further examined. The results of some preliminary work on the effect of variety or source on sorption and airing of the fumigants on wheat were discussed in Chapter Three.

4.3.2 Fumigation studies

Determination of moisture content of the wheat sample was carried out first. The whole grain (about 50 g) was weighed and placed in an oven for four hours at 113°C and weighed again. The difference gives the weight loss calculated as percentage loss. Moisture content was found to be 9%. Fumigation was carried out by treating three samples of wheat (50 g each) with 1.2 ml, 2.5 ml and 6.3 ml of bromomethane each at 25°C for 24 hours to get a concentration x time (CT) product of 240 mg h litre^{-1}, 500 mg h litre^{-1} and 1260 mg h litre^{-1} respectively. A 120 ml vial purchased from Chrompack Ltd, England, was used as a fumigation chamber and filled with wheat sample to about 75% of its size. After 24 hours fumigation, a headspace sample was taken and determined with gas chromatography/ electron capture detector (GC/ECD) as described above and the concentration compared with that of standard headspace. Concentration x time (CT) product was then calculated. The fumigated grains were first aired by passing air through them for about 30 minutes and then spreading them to air freely for another four hours before analysis. Residue determination was carried out using the acetone-water extraction method better known as the first official method of the Association of Analytical Chemist (AOAC) first described by Clower jr (1980).

4.3.3 Experimental method

This study was carried out on a Hewlett-Packard gas chromatography model 5890 equipped with a ^{63}Ni electron

capture detector and a splitless DB-5 capillary column. The operating conditions were 200°, 40° and 270°C for injector, oven and detector respectively. The carrier gas was nitrogen purchased from B.O.C., Glasgow, with a flow rate of 4 ml/min. The integration was done using a GC compatible Hewlett-Packard computer.

Technical grade acetone was purchased from Rathburn Chemical Ltd, Scotland, and was used as such. Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) both were purchased from BDH Chemicals Ltd and were of reagent grade. Deionized water was used throughout this study to avoid unnecessary impurities.

About 50 g of each of the wheat samples were immersed into a (5 + 1) acetone-water solution in a 250 ml volumetric flask at 20°C and left to soak for 24 hours with occasional swirling. After the extraction time elapsed, about 10 ml of the clear upper supernatant liquid was poured into a 25 ml graduated cylinder and closed tightly. 2 g of anhydrous NaCl was added and shaken vigorously for 2 minutes and allowed to stand until 2 layers formed (for about 5 minutes). About 5 ml of the upper clear liquid was again poured into a 10 ml graduated cylinder and 1 g of $CaCl_2$ (5 - 8 mesh) was added and shaken vigorously and allowed to stand for 30 minutes. Aliquotes of the upper layer were then taken for GC/ECD analysis as specified above.

4.3.4 Results and discussion

The amount of methyl bromide determined in the headspace sample of wheat fumigated with liquid bromomethane is summarised in Table 4.3. These results show that concentration x time (CT) product for bromomethane lie in general terms in the range of 50 to 2000 mg h litre^{-1}, the highest commonly occurring in pockets in grain at the bottom of the vial which in this case represent a silo or bin. The results would further suggest that at temperatures prevailing in several European countries, for example the United Kingdom, mean bromide levels within a given bulk would, in general, be considerably less than the guideline level of 50 mg kg^{-1} recommended by Food and Agriculture and World Health Organization (FAO/WHO) Committee on Pesticide Residue (CODEX) and proposed in the European Economic Commission (EEC) draft cereal directive (Anon, 1983).

The bromide residues shown in Table 4.3 above show lower levels than that recommended apart from the one obtained when the CT product was high. This amount doubled and could be attributed to the large amount of the fumigant applied during fumigation. Treatment of more sorptive cereals such as maize, or those with which bromomethane reacts more readily, needs careful monitoring especially at higher temperatures like those found in tropical countries such as Kenya, where the reaction rate of bromomethane would be greater. The commodity moisture content may also affect the amount of bromide formed.

The moisture content of the wheat used to obtain the residue levels in Table 4.3 was 9%. The bromide residue level increased with increase in moisture content.

In assessing the amounts of bromide likely to be formed under a specific set of conditions, temperature, moisture content and the variety should all be considered. The results for different wheat samples were reported to be similar suggesting that intra-varietal variation may be relatively small (Scudamore, 1987b).

4.3.5 Sorption of bromomethane from grains

After airing, the desorption rate is very high and loosely held fumigant desorbs very rapidly. Table 4.4 shows the residues of bromomethane left associated with the grain constituents after the aeration period. The fumigated grains were analysed almost immediately after fumigation and yet the results are significantly reduced. It is therefore, very possible not to find any residue in the grains after a long storage duration. This possibility cannot be ruled out for general health reasons and any grains or their by-products must be analyzed before consumption. The reduction in the residue level implies that the rate of airing at a specific time after treatment is more dependent on the time elapsed since treatment than on the amount of residue remaining at the time. This is probably related to the location of the remaining residue and how it is held within the individual grains.

Table 4.3 Residues produced by reaction of bromomethane with wheat grains

Replicate	CT product (mg h litre^{-1})	MB residue (mg kg^{-1})			
		1	2	3	mean
1	240	16	19	20	18.3
2	500	41	38	40	39.7
3	1260	103	103	98	101.3

Table 4.4 Residue of bromomethane detected in laboratory fumigated wheat grains

Treatment	bromomethane (mg kg^{-1})		
	Amount applied	Residue detected[a]	± SD[b]
1	2.77	0.10	± 0.020
2	5.77	0.21	± 0.043
3	14.53	0.53	± 0.029

a - Average of 3 replicates

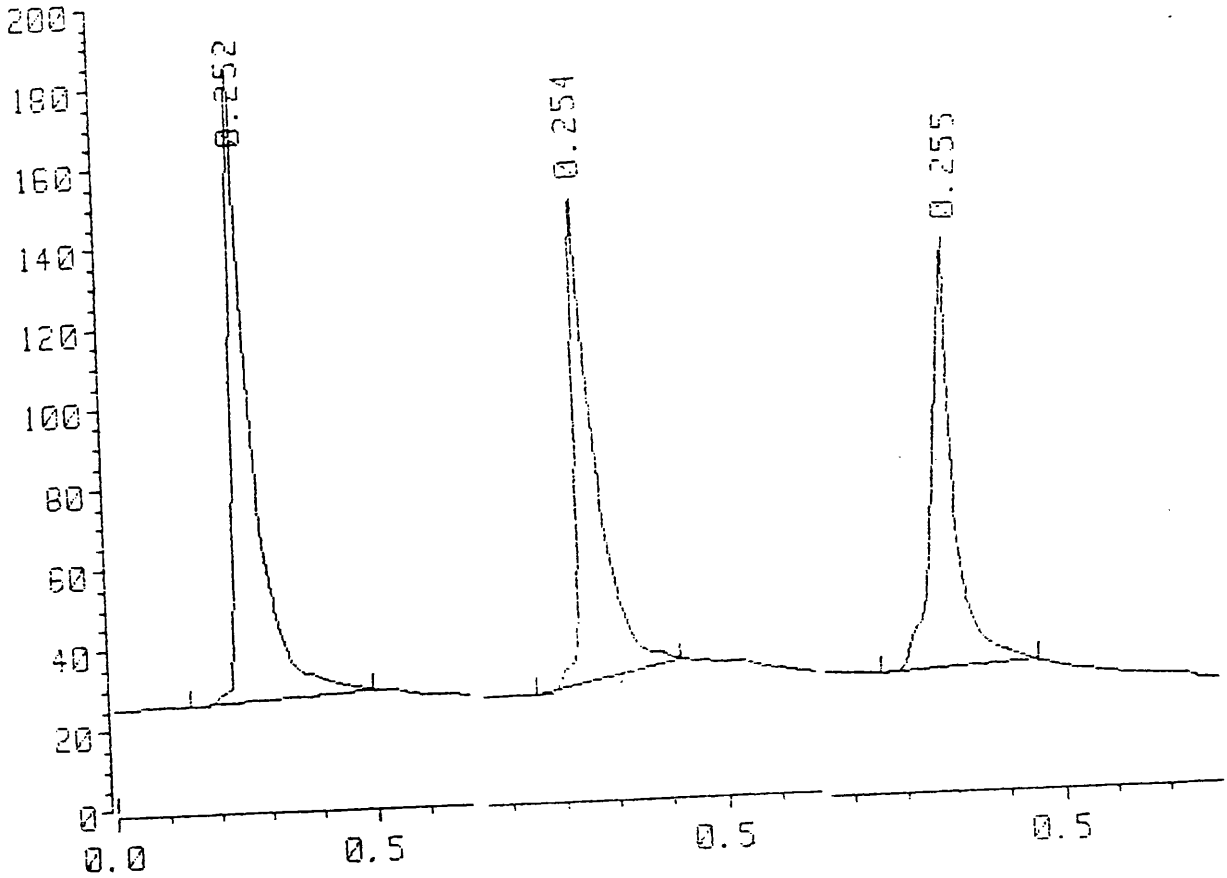
b - standard deviation

Figure 4.9 shows the chromatograms of bromomethane in standard solution and in wheat samples. A - represents the standard solution peak, B - is the headspace concentration peak of the fumigant during fumigation and C - represents the peak of the fumigant in the sample after airing. The chromatograms are clear and resolved with no interfering peaks. After only four hours airing an average mean of 0.28 mg kg^{-1} of the bromomethane was detected in the sample. It is probable that 92% of the fumigant dissipated away into the environment or was removed during the analysis.

Laboratory sorption or airing studies carried out under carefully controlled conditions represent idealised situations. In practice, during storage, a bulk of grain will be continually changing in temperature and moisture content depending on the environmental conditions prevailing and the use of aeration facilities, in addition to localised changes due to the activities of any insects present or growth of moulds in damp spots. The results given above from carefully controlled laboratory studies were used here to predict the fate of residues in wheat grains in field situations and have been found to generally reflect the actual situation reasonably and accurately.

In attempting to predict the fate of fumigants in a wheat sample, a full understanding of how the many parameters may influence the physical or chemical interaction of fumigants with the wheat is essential.

Figure 4.9 Chromatogram of bromomethane residues in headspace analysis; A, the standard peak; B, the headspace concentration during fumigation; C, the sample peak after aeration



Although residues of unchanged bromomethane in cereals have been shown to be transient (Scudamore and Heuser, 1970; Fairall and Scudamore, 1980) even at fairly low temperatures, inorganic bromide once formed by reaction is fixed. Amounts of bromide increase with higher moisture content, temperature and CT product and may also vary from cereal to cereal or variety to variety (Scudamore, 1987). From a knowledge of CT products for bromomethane achieved in commercial trials, laboratory data can prove useful in predicting which circumstances are likely to result in unsatisfactorily high bromide levels. The same circumstances may increase the possibility of tainting for example in bread making, or result in reduced germination of seeds in cereals destined for the seed market.

CHAPTER FIVE

ANALYSIS OF MULTI-FUMIGANT RESIDUES

5.1 STUDIES ON MULTI-RESIDUES IN MAIZE BY THE PURGE AND TRAP METHOD

5.1.1 Introduction

In Chapter Four, individual fumigant residues reacting with various grain constituents were discussed. It is true that these fumigants are sometimes applied in a mixture with other fumigants or compounds either to increase their toxicity, to aid in penetration and distribution or act as fire and explosion retardants. When a mixture of fumigants is used on grain stored for food consumption, it becomes difficult to account for all the compounds involved and analysis of their residues in food also becomes difficult to detect. Chapter Five will be dealing exclusively with the residues remaining in cereal grain (maize and wheat) after and during fumigation with six commonly used fumigants.

The method by which ethylene dibromide has been determined in maize by the purge and trap procedure (Chapter 4, Section 4.2) has prompted an expansion of this method to include several other important fumigants. There are many other volatile hydrocarbons (VHCs) found in different food constituents derived from grain or their by-products. Although the source of these residues could be from their use as fumigants, VHCs are also used in

large quantities in industries as chemical intermediates and solvents. Regulations allow various VHCs as indirect food additives from sources such as adhesives and components of coatings, plastics (polymers), and paper and paperboard used in food packaging (Heikes, 1987).

Heikes (1986) purged and trapped several of these volatile hydrocarbons and found that the method possesses all the desired criteria of simplicity and rapidity, and demonstrated sensitivity at levels less than parts per billion. This method was validated and used to study the multi-residues of several fumigants on maize whole grains.

5.1.2 Methodology

The principle in this study is the same as that reported in Section 4.2 (Chapter 4) except that the fumigants are collected on the adsorbent trap composed of Tenax TA and XAD-4 resin (Figure 5.1). The gas chromatography used and all the conditions are also similar and were adopted as such. The reagents and apparatus were again similar with a few modifications. The fumigant standards used to prepare the insecticidal fumigant mixture were methyl bromide (CH_3Br), carbon disulphide (CS_2), chloroform ($CHCl_3$), ethylene dichloride (EDC), carbon tetrachloride (CCl_4) and ethylene dibromide (EDB). They were all of the highest purity standards and purchased from BDH Chemicals Ltd, Poole, England. The fumigant mixture reference standard was prepared in glass

Plate 5.1 Laboratory fumigation of maize grain with a fumigant mixture

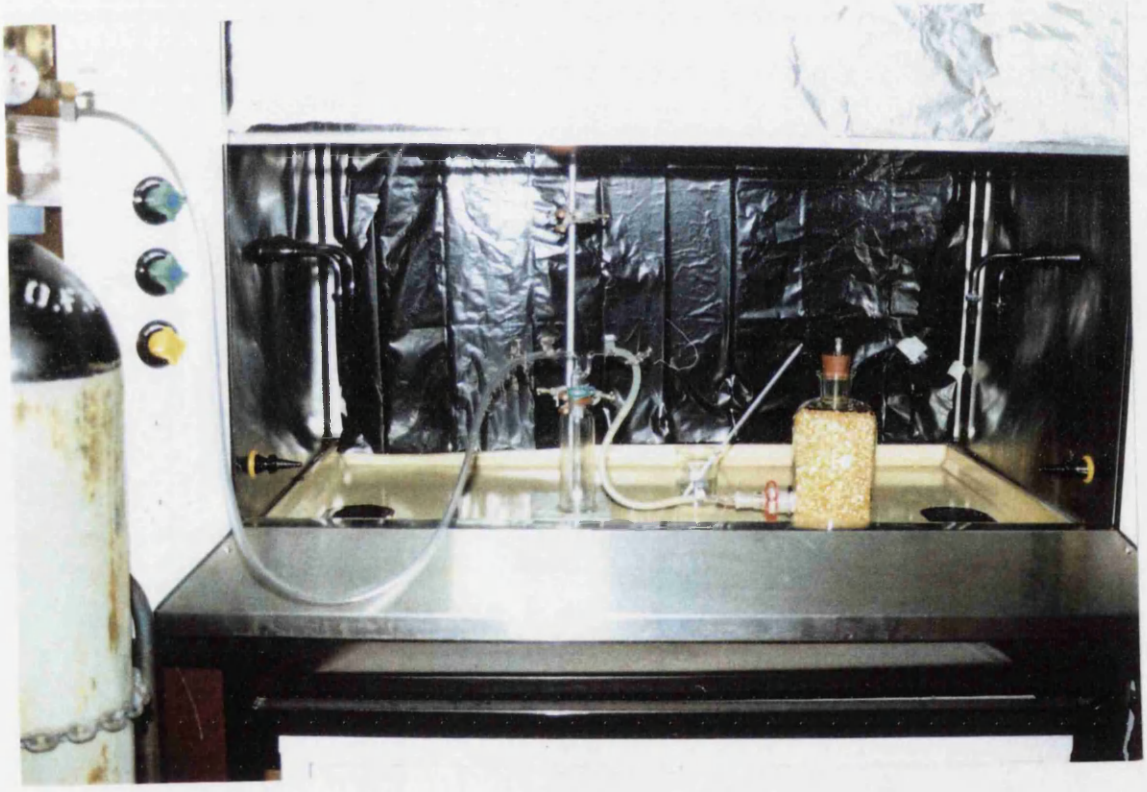


Figure 5.1 Fumigant trap tube; A, Pasteur pipette (6 mm id) with shortened tip; B, glass wool plugs; C, 200 mg conditioned XAD-4 resin; D, 100 mg conditioned Tenax TA

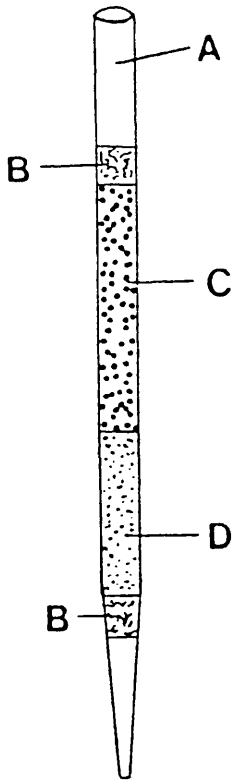


Figure 5.2 Fumigant mixture standard chromatogram with electron capture detector

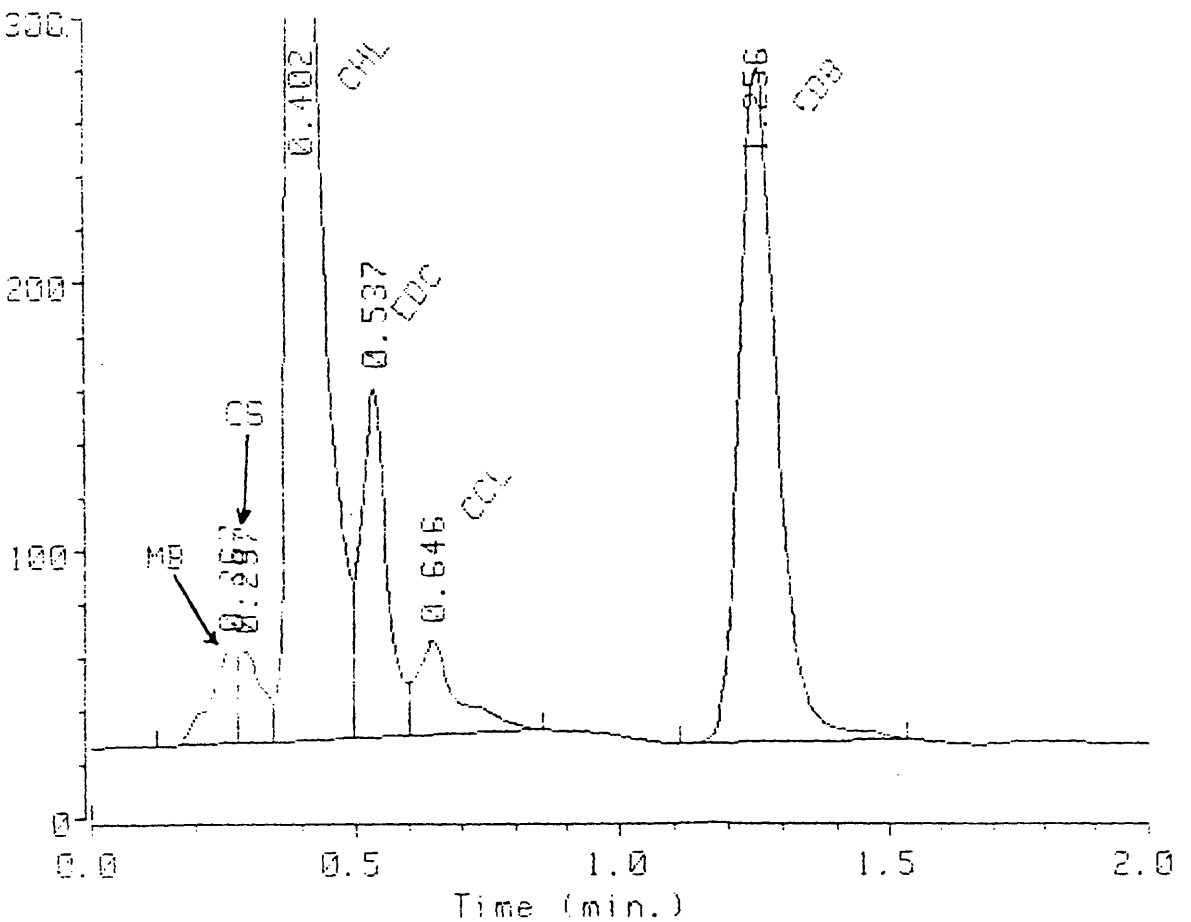
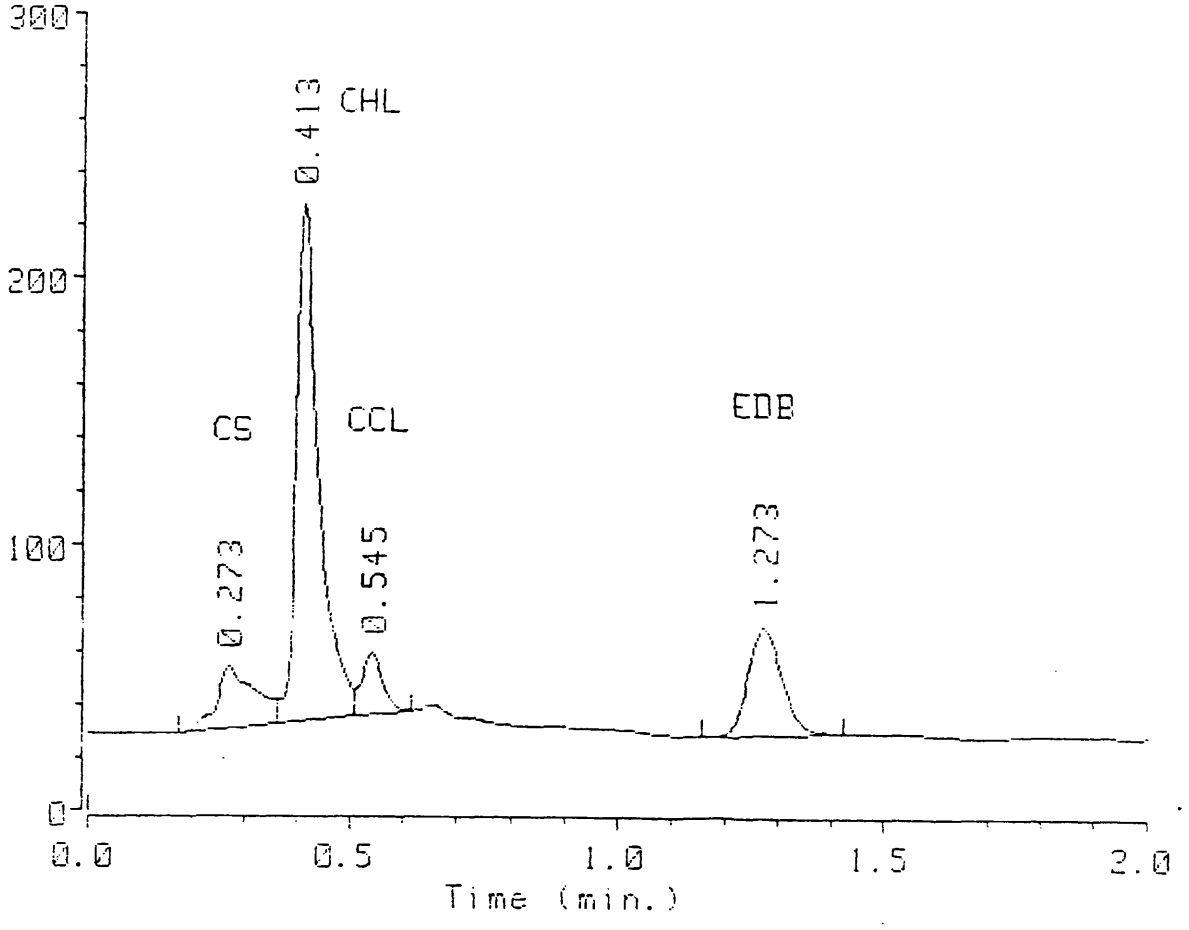


Figure 5.3 Chromatogram representing the sample extracted from maize grain after a long period of storage



distilled hexane (appropriate volumes given in Table 5.1).

The maize grain used was fumigated in the laboratory with 48 mg litre^{-1} concentration of the fumigant mixture mentioned above. A 2 litre bottle was used as a fumigation chamber and nitrogen gas at 15 ml min^{-1} was passed through the mixture which acted as a circulation media for fumigating grain. The assembled apparatus is shown in Plate 5.1. The extraction was carried out as per the procedure described in Section 4.2. The result was confirmed using a mass spectrometer with electron impact ionization.

5.1.3 Results and discussion

Figure 5.2 depicts a chromatogram (GC/ECD) of the six halogenated fumigants used in this study and for which quantitative data have been obtained using an electron capture detector. The analysis of these fumigants at low parts per billion and subparts per billion levels requires stringently clean reagents. This was checked by analysis procedural blanks to ensure the validity of the results. Some fumigants such as chloroform are nearly ubiquitous in the laboratory environments or as impurities in various reagents. This may have contributed to the large chloroform peak observed in Figure 5.3.

Several adsorbents were tested as potentials traps. Since Tenax TA had been used successfully in analyses of ethylene dibromide (Chapter 4; Heikes, 1985), it proved a natural starting point for development. Tenax TA

efficiently trapped fumigants that have longer gas chromatographic retention values like ethylene dibromide. It was found to be relatively inefficient for trapping the earlier eluting compounds such as methyl bromide, carbon disulphide and chloroform (Heikes, 1986). The amberlite resins effectively trapped those fumigants missed by Tenax TA. XAD-4 resin was most efficient in this respect and thus complemented Tenax TA in the duplex trap used in this study (Figure 5.1). Several lots of XAD-4 resin were examined, and all were found to be acceptable. A characteristic of XAD-4 in this application, however, is that it must be eluted fairly slowly with hexane to quantitatively remove the adsorbed fumigants. Because of its large mesh size, the XAD-4 resin allows hexane to elute with ease but with the duplex trap, Tenax TA is packed below the resin and thus retards the rate of elution.

The maize grain used in this study was fumigated 11 months before analysis and kept in a freezer at a temperature below 5°C. The results in Table 5.2 show that after such a long storage period, no methyl bromide was detected in the sample. Other fumigants were detected in insignificantly low levels ranging from 0.002 to 0.69 $\mu g\ kg^{-1}$ apart from ethylene dibromide which gave high residue levels. The overall picture depicts the low amount of multi-residue expected to be present in the grain after such a long time of storage. Figure 5.2 shows the mixed standard chromatogram and Figure 5.3 shows the sample

Table 5.1 Dilution scheme for mixed fumigant standards used with electron capture detector

Fumigant	$d^{20°C}$ (Lit)[e]	Amount to (50 ml)	Stock conc. (mg ml^{-1})	Working conc. (ng ml^{-1})
CH_3Br	1.73[a]	1.0 ml[b]	2.30	230
CS_2	1.26	1.0 ml[c]	2.52	252
$CHCl_3$	1.48	13.5 µl	0.4	40
EDC	1.26	1.0 ml	25.2	2520
CCl_4	1.59	20.0 µl[d]	0.06	6
EDB	2.18	20.0 µl	0.87	87

a - density, 0°C

b - pre-diluted 15 x

c - pre-diluted 10 x

d - pre-diluted 10 x

e - (Anon, 1987)

Table 5.2 Results of analysis of maize grain for multi-fumigant residues

Fumigant[a]	Residue detected (mg kg^{-1})	Standard deviation (SD)[c]	Coefficient of variation (CV, %)
CH_3Br	ND[b]	-	-
CS_2	0.02	0.01	50.0
$CHCl_3$	0.34	0.08	23.5
EDC	0.06	0.05	83.3
CCl_4	0.16	0.02	12.5
EDB	0.69	0.03	4.3

a - fumigant mixture

b - not detected

c - mean of 4 replicates

extracted from the maize grain. The representative peaks are labelled MB, CS, CHL, EDC, CCl and EDB for methyl bromide, carbon disulphide, chloroform, ethylene dichloride, carbon tetrachloride and ethylene dibromide respectively. The peaks were sufficiently resolved although a few insignificant shoulders were observed which were also present in the standard peaks. Table 5.3 shows the recovery of fortified samples. Recovery ranged from 98 to 112 per cent with a mean average of 101 ± 5.44 standard deviation (SD). This result indicates an acceptable recovery with good reproducibility.

Table 5.3 Recovery from maize grain fortified with a multi-fumigant mixture

Fumigant	Applied ($mg\ kg^{-1}$)	Found ($mg\ kg^{-1}$)	Percentage recovery (%)
CH_3Br	2.30	2.58	112
CS_2	2.52	2.50	99
$CHCl_3$	0.4	0.39	98
EDC	25.2	25.1	99
CCl_4	0.06	0.06	100
EDB	0.87	0.85	98

Interference-free, full scan mass spectra were obtained from these purged and trapped eluates. The spectra of these fumigants is shown in Figure 5.4 and they are identical to those of the respective standards.

In conclusion, this purge and trap method was found to be suitable for rapid analysis of multi-fumigant residues in whole grain, and provides both adequate recovery and reproducibility. The procedure is not labour-intensive, and does not require highly specialized equipment. Elution of the dual adsorbent trap resulted in very clean, concentrated eluates suitable for both low-level gas chromatographic quantification and mass spectrometric confirmation.

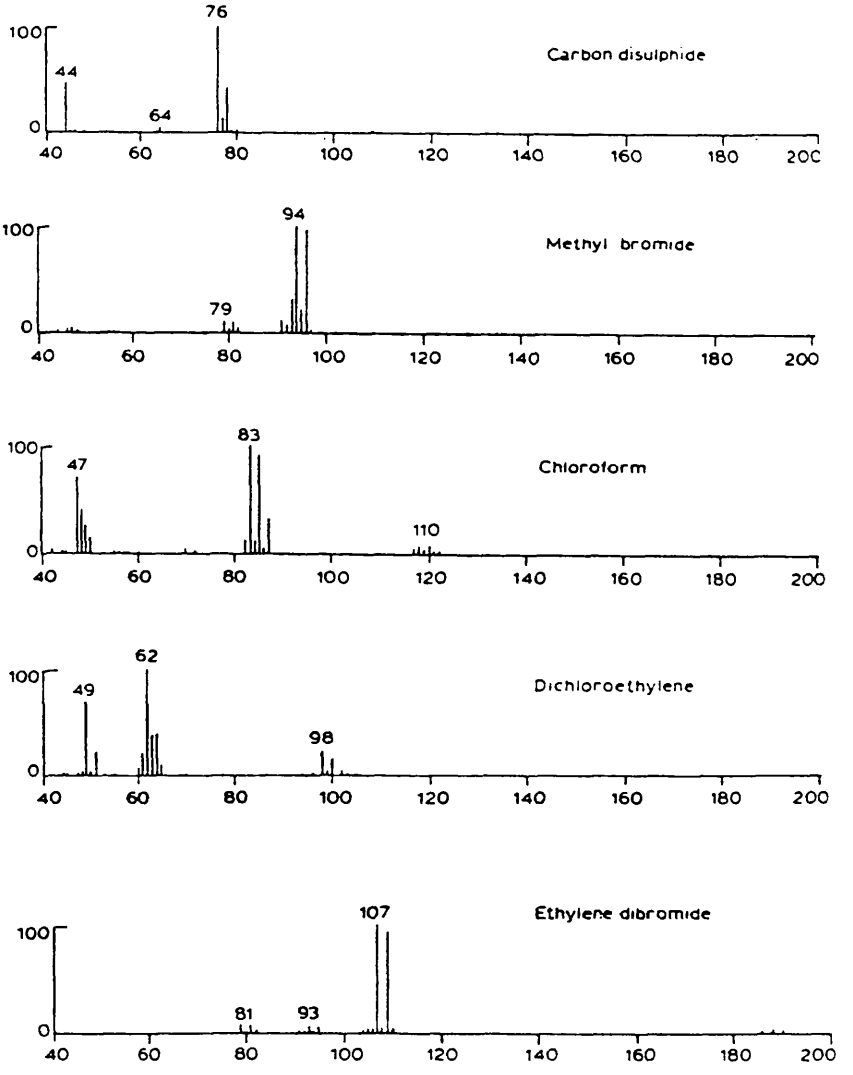
5.2 ANALYSIS OF MULTI-RESIDUES IN MAIZE BY AOAC METHOD

5.2.1 Introduction

The determination of fumigant residues in maize by the purge and trap method was reported in Section 5.1. Another equally effective method for fumigant analysis has been developed by a number of researchers (Berck, 1974; Clower, 1980; Daft, 1983b; Pederson and Cornwell, 1984) and accepted by the Association of Official Analytical Chemists (AOAC) as their official first and recommended method. This section will be devoted to discussing this method in respect of it being an alternative method of analysis to the other two common methods already mentioned, namely; headspace and purge and trap.

The AOAC method is suitable for both parts per millions and parts per billion levels. The current existing method has been modified wherever necessary and used to analyse the six fumigants of interest in stored maize grain.

Figure 5.4 Electron ionization spectra of fumigant multi-residues obtained by MS at 70 eV



5.2.2 Experimental

The maize used in this study was fumigated and aired as described in Section 5.1. The method of extraction, the apparatus and reagents are similar to those used in Section 4.3 (Chapter 4) except that phosphoric acid was added to the extraction solution to enhance recovery. The fumigant mixture consists of methyl bromide (CH_3Br), carbon disulphide (CS_2), chloroform ($CHCl_3$), ethylene dichloride (EDC), carbon tetrachloride (CCl_4) and ethylene dibromide (EDB) in their purest forms. The gas chromatography and the conditions were the same as those in Section 5.1. To enhance the ease of determination and reduce analyst exposure to toxic reference fumigants, relatively stable mixed-fumigant standards were used to measure both incurred residues and fortified samples. Fortification was carried out by injecting known amounts of fortified standards into samples already immersed in extraction solution. The volatile nature of fumigants precludes normal recovery studies in which known amounts of standards are added directly to grain samples before addition of the extraction solvent. The data obtained in this manner represent recoveries through the method but exclude the question of extraction efficiency (Clower, 1980).

5.2.3 Results and discussion

The fumigation of the grain with the six component fumigant mixture was carried out for 72 hours. The sample of the headspace gas was taken using an air-tight syringe

and analysed by gas chromatography as reported in Section 4.3 (Chapter 4) and Section 5.1. The concentration was determined after 1, 6, 18, 24, 48 and 72 hours of fumigation. The results are reported in Table 5.4.

Some individual fumigants migrated fast through the pile of grain and were detected in the headspace in large amounts. This was probably the case with methyl bromide which is very volatile and was least retained by the grain constituents while ethylene dibromide which migrated slowly was retained or absorbed by the grain constituents. Table 5.5 shows the mean average concentration of the fumigant headspace after a 72 hour period. The total concentration was less by about 5 ml m^{-3} with a mean coefficience of variation of 31.05. This means that an amount of the fumigant mixture has been adsorbed or absorbed by the grain. Sometimes, carbon tetrachloride and chloroform are used as eluting agents. These agents aid other fumigants to penetrate through the pile of grain in a silo. Also these chlorinated hydrocarbons are used for the improved surface treatment of bulk grain in a specific case where the properties of each component of the mixture are used to advantage. Methyl bromide has a high vapour pressure at normal temperatures, low sorption on commodities and excellent penetrative ability. Ethylene dibromide on the other hand, vaporises much more slowly, is heavily sorbed on grain commodities particularly at lower temperatures and consequently has little penetration power.

Table 5.4 Headspace concentration of fumigant mixture after 72 hours fumigation period

Fumigant	Concentration (ml m^{-3})						
	Initial	1	6	18	24	48	72
CH_3Br	5.0	4.8	4.3	4.3	4.5	5.0	5.0
CS_2	8.6	9.2	8.5	8.4	8.2	9.0	8.2
$CHCl_3$	10.4	9.7	9.1	9.1	9.3	9.6	11.5
EDC	9.0	8.7	8.9	8.4	8.7	9.7	9.6
CCl_4	4.0	3.7	0.0	3.9	0.0	3.9	0.0
EDB	11.9	11.7	11.5	12.8	0.0	11.9	12.5
Total	48.9	47.8	42.3	46.9	31.2	49.1	46.8

Table 5.5 Mean average headspace concentration after 72 hours period

Fumigant	Concentration (ml m^{-3}) (Lit)	(50 ml)	Standard deviation (SD)[a]	Coefficient of variation (CV %)
CH_3Br	5.0	4.7	0.33	7.02
CS_2	8.6	8.7	0.38	4.37
$CHCl_3$	10.4	9.7	0.91	9.38
EDC	9.0	9.0	0.53	5.89
CCl_4	4.0	1.9	2.10	110.53
EDB	11.9	10.1	4.96	49.12
Total	48.9	44.1		

a - mean of 4 replicates

Residue determinations of these mixtures were taken immediately after three days of fumigation and 6 hours of airing. Another analysis of the same grain was taken six and eleven months after fumigation. Prior to this analysis, grain was securely kept in a refrigerator with a temperature below 5°C. The results of these studies are reported in Table 5.6. The decreasing trend shows that the residue level decreases with time. The longer the grain was stored for the less the residue level.

Table 5.6 Residue levels detected in maize grain after a long storage period

Fumigant	Residue level (mg kg^{-1})[a]		
	3 days	6 months	11 months
CH_3Br	0.03	ND[b]	ND
CS_2	0.12	0.05	0.02
$CHCl_3$	0.15	0.08	0.07
EDC	0.08	0.05	0.02
CCl_4	0.26	0.15	0.05
EDB	0.53	0.50	0.43

a - mean of 4 replicates

b - not detected

This study indicates that maximum residues of methyl bromide in grain three days after fumigation will be

relatively low and that it dissipates away and none will be remaining after a further 24 hours of storage. Ethylene dibromide remains associated with grain constituents for a long time and can be detected after eleven months of storage. Other fumigants were also detected after this long period although at very insignificant levels. The recovery study on fortified samples showed very high recoveries throughout the mixture (Table 5.7). The recovery ranged from 82% ± 3.30 to 100% ± 0.61 SD.

Table 5.7 Recovery study of fortified maize sample with a fumigant mixture

Fumigant	Recovery (mean) (%)	±	Standard deviation (SD)
CH_3Br	82	±	3.30
CS_2	83	±	2.65
$CHCl_3$	95	±	1.26
EDC	89	±	1.36
CCl_4	100	±	0.61
EDB	98	±	0.95

a - mean of 4 replicate injections

Figures 5.5 and 5.6 represent the sample chromatograms of the fumigant mixture after 3 days and eleven months of storage respectively. The peaks were resolved with no interferences. The absence of a methyl bromide peak in Figure 5.6 indicates that there was no

Figure 5.5 Chromatogram obtained from maize sample after three days of fumigation

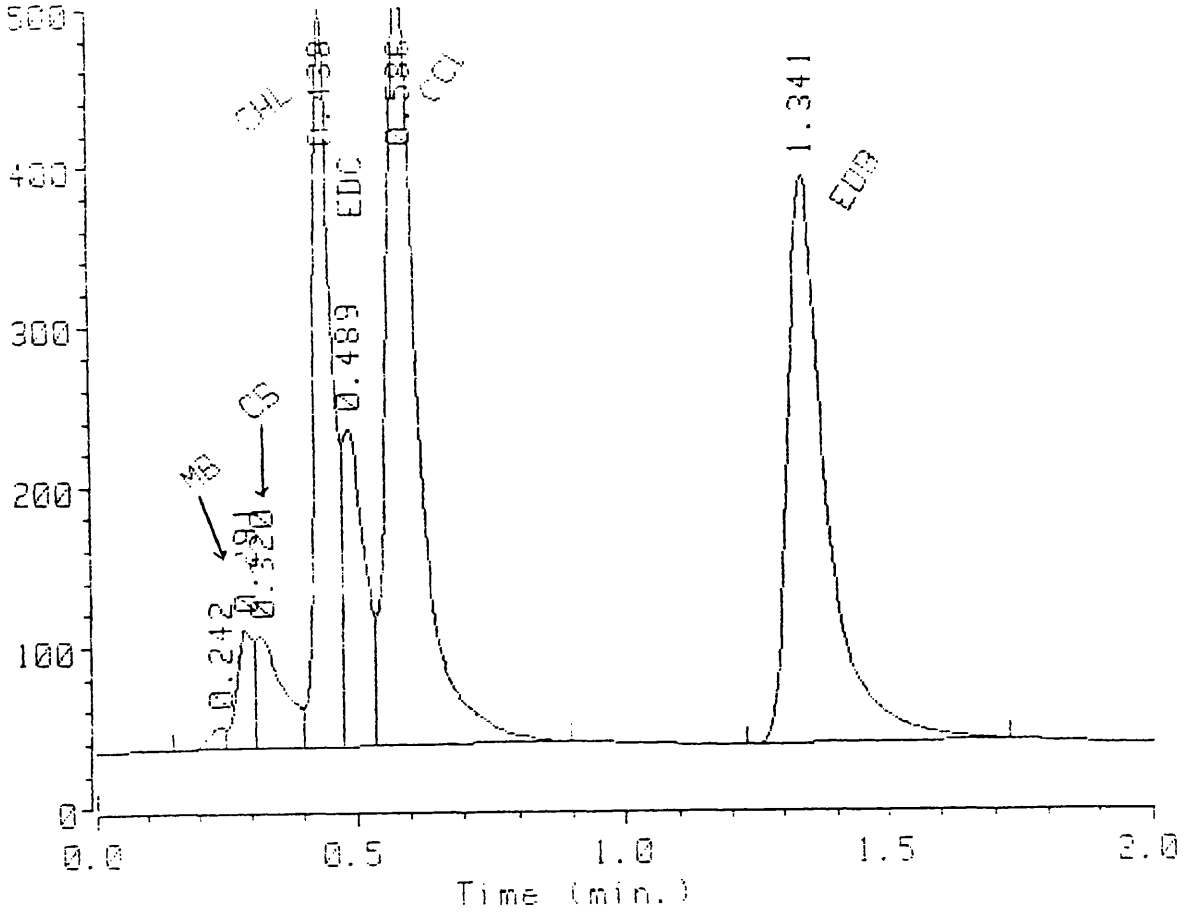
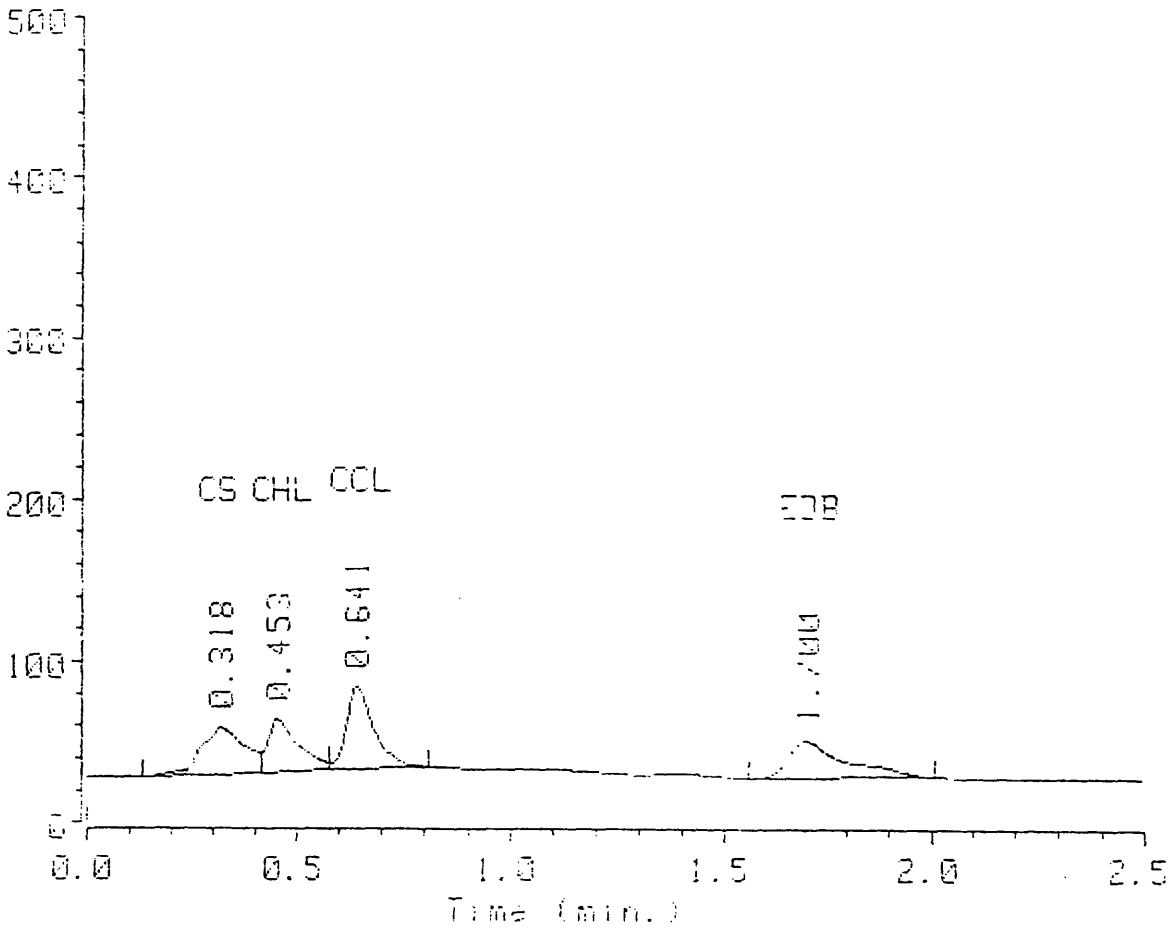


Figure 5.6 Chromatogram obtained from maize sample after eleven months storage period



methyl bromide left in the grain by the time of analysis. The reduction in size of the peaks in Figure 5.6 compared to those in Figure 5.5 could be attributed to the dissipation of the fumigants during the storage period. The shoulder observed near the carbon disulphide peak could not be explained but it could be the remains of methyl bromide residue or just other impurities present.

In conclusion, this method gave good recoveries in all cases although for methyl bromide and carbon disulphide the recoveries were less than for the other four fumigants. A single back-extraction of leached grain with isooctane permits quantitative analysis for the six fumigants by gas chromatography with electron-capture detector.

5.3 COLD SOLVENT EXTRACTION OF FUMIGANT RESIDUES IN WHEAT

5.3.1 Introduction

The Association's first official method of fumigant analysis has been discussed extensively in Chapter Four and in Section 5.2 of this chapter. While the study in Section 5.2 dealt with fumigant residues in maize grains, this section will draw attention to the effects of prolonged treatment and the aeration period on wheat grain fumigated with a mixture of the six common fumigants already mentioned.

With the advent of gas chromatography and other modern equipment, analytical methods become much more

sensitive and selective, and hence more attention has been focused on suitable methods for the extraction of residues from cereal grains and other food materials (Daft, 1983a; Scudamore, 1987a). A multi-residue method based on cold extraction of whole cereal grains and their by-products using a mixture of acetone and water was first described by Heuser and Scudamore (1969). This method was subsequently tested collaboratively by members of the United Kingdom Ministry of Agriculture, Fisheries and Food Panel on fumigant residues in grain (Anon, 1974) and later accepted by the United States Association of Official Analytical Chemists as a recommended method for the determination of fumigant residues in grains (Anon, 1980). More recently, several workers have examined this method in detail and have suggested modifications (Clower, 1980; Daft, 1983b; Scudamore, 1987a), usually in the gas chromatographic conditions or in the procedures to remove water prior to chromatography. Many other methods utilising other solvent mixtures have also been published by many researchers such as Greve and Hogendoorn (1979).

It is difficult to establish the efficiency of any method for extracting a range of volatile compounds from grain and other food commodities, but in this study recoveries of the six fumigants used have been determined from wheat whole grains after treatment with vapour under conditions attempting to reproduce known amounts of residues in a form similar to that occurring after commercial fumigation. Although this method of approach

avoids direct spiking of extract solutions, which often does little more than check the analytical procedure, the extraction and recovery can only be carried out on freshly fumigated samples, leaving aside the problem of how to determine the extractability of residues from well-weathered samples.

Another approach would be to use radio-labelled compounds for checking extractability, but this would not necessarily give unequivocal results as any unextracted label could be due either to firmly held residues of the parent compound or to reaction products or both. However, use of radio-labelling would quantify the unextractable residue. Scudamore and Heuser (1973) showed that the difference in results obtained in determining carbon tetrachloride residues in wheat and maize using a cold extraction procedure and a steam distillation method increased with length of time after fumigation. They reported that up to 7 days soaking in a mixture of acetone and water was required for samples stored for 6 months after fumigation, when results were considerably higher than by steam distillation. They further reported that grinding samples after admixing with dry ice improved the rate of extraction, apparently without loss of vapour, but did not increase the total amount of residue extracted.

With the introduction of recommended maximum residue levels for fumigants in a range of food commodities (FAO, 1978) and the tight legislation in the European Economic Community (EEC) and other countries, it

is important to use methods that can be relied on to extract efficiently any residue present in foodstuffs.

5.3.2 Sample preparation

Samples (50 g) of wheat were spread in thin layers on metal trays and stored in a room held at constant temperature (25°C) and relative humidity (60%) for several weeks to allow the moisture content to reach equilibrium. Moisture content was determined according to the procedure recommended in the International Standards Organization's Method (Anon, 1979).

Fumigation of the conditioned grains was carried out using the procedure illustrated in Section 5.1 except that a 1 litre rather than a 2 litre container was used. A mixture of the six fumigants was prepared from measured volumes of the individual compounds, heated slightly and the vapour passed into the chamber with the aid of a stream of nitrogen gas. To determine vapour concentrations of each compound, gas samples were drawn from the headspace at intervals and analysed using gas chromatography. Peak areas obtained after injection of the aliquots of the gas samples were compared with those obtained by injecting samples of the standard mixture in a similar manner and the concentration of each compound determined.

The concentration x time (CT product) was calculated from the mean gas concentration (mg litre^{-1}) during fumigation multiplied by the length of fumigation (h). The gas chromatography equipment used was similar to

that used in Sections 5.1 and 5.2. At the end of the fumigation period all free vapour was removed by air circulation through the chamber or container before removal of the fumigated grains. The fumigated grains were placed on trays in a room maintained at 25°C and 60% relative humidity. After aeration, the grains were kept in a freezer ready for analysis.

The cold extraction with acetone and water of the 50 g wheat sample was carried out using the AOAC procedure reported in Section 4.3 (Chapter 4). The direct injection of the wet extracts obtained thereafter was accomplished by diluting the extract with dry acetone and injecting into the gas chromatograph without drying. Dilution of 1 part of extract solution with at least 19 parts of acetone was necessary to avoid significant interference by water in the separation and detector response.

The removal of water by partitioning into isooctane was accomplished by shaking the extract with saturated sodium chloride solution, deionized water and isooctane as described by Daft (1983b). Aliquots of the isooctane were used for gas chromatography and diluted with dry acetone as necessary. Aliquots (0.5 - 1.0 μl) of the extracts were injected into the injection block (200°C) of a gas chromatograph operated as described in Section 4.3 (Chapter 4). Peak areas obtained were compared with those for known amounts of standard mixtures of the six compounds prepared by serial dilution of weighed amounts of each, and the residues calculated.

Recovery studies were accomplished using prepared spiked solutions added to the extraction solutions in the presence of the samples.

5.3.3 Results and discussion

The CT products achieved during fumigation were 1200 mg h litre^{-1} for 24 hours, 2400 mg h litre^{-1} for 48 hours and 3600 mg h litre^{-1} for 72 hours. These levels are believed to be typical of what might be achieved in commercial fumigations, although much higher CT products may occur in pockets within a bulk or in good gas-tight structures to obtain quantitative results with the cold solvent extraction methods used in this work, assumptions are made of how extracted compounds partition between the liquid phases present and about the composition of these aqueous, saturated salt solutions or organic solvent mixtures (Heuser and Scudamore, 1969; Clower, 1980; Daft, 1983b; Scudamore, 1987a). The recoveries of spiked amounts of fumigants are thus apparent recoveries under the conditions used and, as such, results may range from low values for compounds which cannot be efficiently recovered or extracted, to values greater than 100% where concentration effects have occurred. Clower (1980) reported that the anhydrous calcium chloride ($CaCl_2$) added to 10 ml portions of extract selectively sorbs a small amount of acetone but not the fumigants, thereby producing a more concentrated solution which results in higher recoveries. In this study, it was found that added amounts of fumigants were apparently recovered in excess

of 100% from aqueous solvent. This could be due to selective removal of water from the solvent mixture by the salt, so that the fumigant was concentrated in a smaller volume of solvent than that calculated as the total. With the removal of water, the fraction of the extract chromatographed was related only to the volume of organic solvent used (125 ml). Where a recovery much below 100% was obtained it could often be attributed to continuing reaction of the fumigant with the grain constituents.

Table 5.8 represents the results of apparent recovery of the six fumigant mixtures from spiked solutions and spiked extracts of wheat samples. Spiking acetone-water solution and proceeding with the AOAC procedure (1 in Table 5.8) gave good and reproducible recoveries, generally equal to those obtained by partitioning into isooctane (procedure 3). However, values obtained by direct injection of wet extracts (procedure 2) were slightly less than the other two values. The small differences in recoveries between the three procedures are in general not significant in these spiked solutions. When grain extracts were spiked, recoveries for fumigant-commodity combinations were similar to that obtained from spiked pure solvent mixtures.

Table 5.8 Mean recoveries of fumigant mixture from spiked extracts of wheat

Fumigant	Recovery (%) by procedure[a]		
	1	2	3
CH_3Br	85	89	90
CS_2	91	97	90
$CHCl_3$	110	97	112
EDC	103	100	97
CCl_4	99	94	100
EDB	98	99	98
Mean	98	96	98

a procedures: 1, extraction with acetone-water solution and salting-out with sodium chloride followed by calcium chloride; 2, direct injection of diluted wet extract; 3, partitioning into isooctane.

Extractions with acetone and water solutions tend to give recoveries of over 100% as reported previously (Clower, 1980; Daft, 1983b; Scudamore, 1987a) but the values in this study (98%) are not as high as those obtained by Clower (100-120%) or Daft (125-150%). A number of factors may affect the end result. For instance, acetone and water should be mixed prior to measuring out

the recommended volume of solvent, as a reduction of about 4% occurs on mixing the two solutions. However, the amounts of sodium chloride and calcium chloride used in the salting-out stages and the contribution of the water within the commodity structure are probably more important factors. Grain samples usually contain between 5% and 20% water and for a 50 g sample this would contribute 2.5 - 10 ml in addition to the 25 ml of water present in the extract solvent mixture, possibly affecting partitioning and salting-out equilibria (the moisture content of the wheat used in this work was 11.8%). A further factor to be considered is that most cereal grains swell considerably when soaked in acetone-water solution. If this is due to preferential uptake of water, the extracted fumigants would be concentrated in the solution surrounding the grain which is used for residue determination, resulting in high results. However, when soaked grains were removed from the acetone-water solution and the surface carefully dried, the subsequent weight loss on standing was rapid and the grain continued to smell strongly of acetone for several hours, suggesting that at least some acetone had been absorbed by the grain.

Table 5.9 shows the residues determined in samples taken after 14 days, 30 days and 60 days of storage, representing well-aged samples. To facilitate the assessment of the methods, the ratio of the result obtained by extraction with acetone and water solution

followed by salting-out with sodium chloride and calcium chloride (procedure 1) to that after partitioning into isooctane (procedure 3), has been calculated similarly, ratio values calculated from spiking recovery results presented in Table 5.8 are included for comparison. The results show that the ratio of the results for procedures 1 and 3 (see Table 5.8 for procedure) are generally lower than those for procedures 2 and 3. The overall reason is underlined by the factors discussed earlier, for instance, salting-out the water in the extracting solution. The residue levels in Table 5.9 indicate that the residues decreased gradually as the time of storage increased. The results also indicate that the extractant becomes less effective the longer the time elapsed since the original fumigation.

It may be concluded that the use of cold solvent residue extraction of wheat grain has a number of advantages in that it uses a minimum of apparatus, is simple and, although the analysis is spread over several days, operation is less tiring. Most importantly, losses due to volatility, and to breakdown by heat and of photo-labile compounds are kept to a minimum.

In developing, improving or using recovery methods it is essential to have a full understanding of the different effectiveness of solvent extractions with well-aged as opposed to freshly fumigated or spiked samples. In practice, except in laboratory experiments, most samples will be of unknown origin and any residues

Table 5.9 Residues of fumigant mixture extracted from fumigated wheat grains by three procedures

Fumigant	Time after fumigation (days)	Residue expected by procedures:[a] ($mg\ kg^{-1}$) 1	2	3	Ratio of results for procedures: 1:3	2:3
CH_3Br	14	0.04	0.04	0.01	4.0	4.0
	30	-	-	-	-	-
	60	-	-	-	-	-
CS_2	14	0.36	0.38	0.38	0.9	1.0
	30	0.11	0.20	0.24	0.5	0.8
	60	0.04	0.07	0.18	0.2	0.4
$CHCl_3$	14	0.34	0.41	0.42	0.8	1.0
	30	0.04	0.28	0.35	0.1	0.8
	60	0.16	0.21	0.20	0.8	1.1
EDC	14	0.08	0.09	0.12	0.7	0.8
	30	0.02	0.02	0.08	0.2	0.3
	60	-	-	0.01	-	-
CCl_4	14	0.51	0.56	0.57	0.9	1.0
	30	0.16	0.08	0.43	0.4	0.2
	60	0.14	0.24	0.46	0.3	0.5
EDB	14	0.87	0.91	1.23	0.7	0.7
	30	0.14	0.30	0.80	0.2	0.4
	60	0.09	0.16	0.43	0.2	0.4

a - see Table 5.8

present are likely to be well aged. On the evidence of the analytical work reported in the two analytical chapters covered in this project, the 48 hour extraction period recommended in the AOAC official first action multiple residue method for fumigants in grains might be expected occasionally to give low results and therefore a 72 hour or longer period is strongly recommended. Although both procedure one and three gave the same recovery, procedure one is more favoured in this project and hence recommended. This procedure is simple, less tiring and uses minimum apparatus.

CHAPTER SIX

ADVANTAGES AND LIMITATIONS IN METHODOLOGY AND EVALUATION OF FUMIGANT RESIDUES

6.1 INTRODUCTION

The last two chapters dealt with the methodology used for fumigant residue analysis. The methods applied, namely; headspace, AOAC and purge and trap extracted the residues from foodstuffs effectively and quantitatively. These methods are basically chemical methods and therefore are vulnerable to scrutiny and criticism especially when the treatment of the food may also include a diverse natural biosphere. To discuss the advantages and disadvantages of chemical methods for fumigant residue analysis without mentioning any other available methods will present an incomplete picture. Chapter Six of this project will be devoted to putting together the adequacies, advantages and limitations of chemical methods for fumigant residue assessment and will briefly compare them with some relevant biological methods, although these latter methods are not part of this project and hence not discussed in any detail.

Before the advantages and limitations of chemical (and biological) methods for analysis of fumigants and fumigant residues are reviewed, it is worth noting that results are generally a function of method (Berck, 1975). Thus, the current ability to identify and measure numerous

molecular species in the biosphere at nanogram and picogram levels stemmed from improved, validated and accepted analytical methods.

Maximum residue levels (MRLs) have in various instances been revised downwards and regulatory policy readjusted because of results obtained by assay methods of improved specificity and lower limits of detection. The contribution of improved methodology to environmental protection is reflected in the proliferation of new data and more specific toxicological back-up needed to answer increasing public concern about long-term (chronic) effects of trace amounts of pesticides and other chemicals in the biosphere.

The task of determining parts per million (ppm) and parts per billion (ppb) amounts of a particular compound is generally regarded as straightforward and commonplace. In order to get such amounts in correct perspective, 1 ppm is considered to be equivalent of the ratio 1 cm in ten million kilometres, and that 1 ppt (part per trillion) is one million times smaller. Quite apart from chronic hazards, nanogram (10^{-9}g) and picogram (10^{-12}g) amounts are in the range that would be encountered by insects, moulds, bacteria and miscellaneous plant and animal cells after application of a fumigant gas (Berck, 1974). At the same time, stretching analytical capabilities to attain ever-decreasing limits poses serious problems. Thus, Gunther (1980) pointed out that background values increase disproportionately, contamination becomes a major problem,

instrumental temperament is more difficult to control and the interpretive skill of the analyst is strained to the full.

6.2 ADVANTAGES OF CHEMICAL METHODS OF EVALUATION OF FUMIGANT RESIDUES

The advantages of chemical methods of evaluation are:

(1) Specificity;

Chemical methods enable exact and highly selective determinations of hundreds of different biologically active compounds, including some that mey be chemisorbed or conjugated, or that undergo gradual decomposition with time, temperature, light, metal ions and humidity. Mixtures of fumigant molecules can be resolved and the components measured effectively.

(2) Sensitivity;

Modern instrumentation such as a gas chromatograph with an electron capture detector, compartible computers for integration and narrow capillary columns increase the sensitivity of these methods drastically. The limits of detection by some methods have been shown (Chapters Four and Five) to be down to parts per trillion (picogram) levels. Low levels of detection are needed to assess physical and chemical binding, rate of extraction, volatilization and chronic hazards including carcinogenic potential and possible genetic damage (Berck, 1975a).

(3) Wide range of methods;

There are over 50 classes and subclasses of chemical methods yielding a potential of hundreds of different methods that could be applied in fumigant research and also be used to confirm the validity of particular data (Scudamore, 1988).

6.2.1 Limitations of chemical methods of evaluation

The limitations of chemical methods of fumigant residue assessment include the following:

(1) Artifacts;

Sometimes false positive results may be obtained due mostly to impurities and other extractable natural materials. The frequent use of pure standards and solvents only minimized this risk.

(2) Differences in method performance;

Different methods have different operation techniques and the results obtained do not always agree because of differences in extraction efficiency, substrate, moisture content etc.

(3) Interfering substances;

Many naturally occurring constituents (lipids, proteins, gums, etc.) in a sample may be co-extracted. These may interfere with or mask the determination of trace amounts of fumigant residues being sought. Interferences from polynuclear hydrocarbons in the air, or in the laboratory environment have been frequently observed. The problems posed by co-existence of interferences is much like looking for very small specific needles (residue sought)

that are buried within a very large heterogeneous haystack composed of thousands of other constituents (Berck, 1975). Attempts have been made to counteract or eliminate these interferences by special clean-up treatments of the extract in order to obtain maximum recovery of the fumigant residue being sought but mostly limited success is achieved especially when dealing with volatile chemicals like halogenated hydrocarbons.

6.3 ADVANTAGES OF BIOLOGICAL METHODS OF ASSESSMENT

There are fewer advantages than limitations in the biological methods of fumigant residues assessment. The main ones are:

(1) Direct function;

Biological methods are a direct measure of the physiological effectiveness to toxic molecules.

(2) Wide range;

A wide range of methods are available to determine dosage-mortality effects, such as LD_{50}, LT_{50}, ED_{50} (effective dosage, median, 50%).

(3) Operation cost;

The cost of operation in many instances is low, particularly if mass rearing of test species and ample trained personnel can be obtained.

6.3.1 Limitations of biological methods of assessment

There are many more limitations to these methods of assessment than there are with chemical methods. There are namely;

(1) Non-selective;

They are non-selective for specific fumigants. They are not suitable for determining the effects of differential uptake of individual fumigants of a multifumigant mixture, especially any change in composition.

(2) Environment-dependent;

They may be environment-dependent, and are affected by nutrition, rearing conditions, atmospheric composition (Berck, 1974). The dietary materials used in the rearing stage must be free from toxicants or fumigant traces of any kind (Felton, 1977).

(3) Differences in response;

Response may depend on the test species. Extrapolation of toxicological conclusions to another species or to higher animals may not be acceptable (Somers, 1969).

(4) Hidden effects;

Chronic (sublethal) doses may have obscure, hidden or delayed effects, which may not be detected by biological methods of evaluation.

(5) Interferences;

Oily or waxy substances that are co-extracted may be toxic to the test species, or may encapsulate the fumigant molecules. Thus, false positive or false negative (artifact) results may be obtained (Cook, 1968).

According to Berck (1975a), the limitation of a given method may be reduced or counteracted by using a variety of chemical and biological methods. At the same time, investigations are generally and sometimes advisedly

narrow. Interactions may pose problems that may not be readily resolved by computers or by mathematical models.

6.4 FACTORS THAT INFLUENCE EFFECTIVENESS, DISTRIBUTION AND PERSISTENCE OF FUMIGANTS

There are many factors that influence the effectiveness, distribution and persistence (concentration-space-time relationships) of fumigant gases and volatile liquids applied to the surface of grain held in bulk (Berck, 1965b, 1974). The storage environment has a major many-sided influence on insecticidal effectiveness of fumigants, and on the traces of fumigant residues that remain in or on the treatment commodity or substrate. In this regard, fumigant residues are mainly dependent on gas concentration, the nature of the substrate, its particle size and on temperature, moisture content and contact time (duration of exposure).

There are additional factors involved, such as temperature gradients, which promote differential air movement patterns and thus help to explain differences in fumigant residues due to sample location and time of sampling (Berck, 1974, 1975b; Scudamore, 1982; Daft, 1989). Thus, different intensities and direction of updraft, downdraft and lateral movement of air may be encountered depending on the magnitude and location that exists in a given situation. Under field conditions, significant diurnal differences in percentage relative humidity (% R.H), CO_2 and O_2 levels are found due to

differences in direction and velocity of air movement, as an integral part of the internal atmosphere or microclimate of a particular grain storage facility (Berck and Kalra, 1974; Dumas and Bond, 1977; Dumas, 1982). Some important factors mentioned above are discussed and summarised below.

(1) Effects of storage environment;

Measurement of fumigant residues as such would be meaningless if the results were not related to the storage environment and to the concomitant sorption affinities pertaining to a particular determination. Atmospheric composition also may influence the biological efectiveness of a given application. Thus, traces of volatile substances generated during storage of high-moisture grain (20-22.5% moisture content) can synergise (potentiate) the insecticidal effect of fumigant gases (Berck, 1966; Michael et al., 1980; Goodship et al., 1982).

The effects of storage environments on fumigant residue levels need much more research, as a direct corollary of the well documented fact that storage conditions have a primary influence on the kind and degree of infestation of stored products. Under good storage conditions, the bacterial, mould, mite and/or insect populations increase rather slowly or not at all. Losses due to infestation and costs of fumigation could be minimized by improving storage conditions. Use of less fumigant would also yield lower fumigant residues (Berck and Kalra, 1974; Scudamore, 1982; Daft, 1983, 1988).

Related to the effects of storage is the fact that health protection and regulatory agencies, such as the Food and Agriculture Organization and the World Health Organization (FAO/WHO) Panel on Pesticide Residues in Food, the Environmental Protection Agency (EPA) and other governmental food and drug administrations, are increasing their surveillance of stored food products not only for pesticide or insecticidal fumigant residues but also for mycotoxins, bacterial toxins, salmonella etc. Greater attention to consumer safety is thus broadening the scope of inspection services to encompass possible toxic effects of storage environments.

(2) Sampling effect;

Pesticide residues in general and fumigant residues in particular, may vary considerably in a given sample. The residue levels are influenced by the size, storage, and method of preparation and processing of the sample. The stability of the fumigant molecules that are volatile, or are unstable to light, heat, moisture, and metallic ions must be ascertained beforehand. Regarding sample size, the smaller the subsample, the larger the variability, especially when the substrate is not homogeneous in composition or physiochemical properties. Several kilograms of maize grains may differ considerably from the rest from the same silo or farm, even though it could be assumed that the particular fumigant or fumigant mixture had been uniformly applied. However, it could be tacitly assumed that several kilograms of subsample is a

representative of a sack or a carload of stored grain. The gas-air composition of a several litre bin after fumigation may vary considerably (Berck, 1974), as is also the case for CO_2, O_2 and water vapour (Berck and Kalra, 1974), with differences that were related to sample location, time of sampling and temperature gradients.

(3) Chromatographic behaviour of stored wheat;

The ability of a packed or coated chromatographic column to separate different molecular species by selectively retarding their passage through the column is the nucleus of gas chromatography. The possibility that stored wheat could behave in a similar manner as a chromatographic column towards fumigant gases was first advanced by Berck (1956). The concept was based on differences in the rates and amounts of fumigants observed to migrate downwards after application of a fumigant mixture to the top of a bin containing wheat grain. The wheat kernels were regarded as a heterogeneous, coarse-grained solid support, coated with natural stationary liquid phases with surface-active properties, capable of differential sorption of fumigants applied to the surface of the wheat pile. Differential sorption of fumigants and different affinities of cereal grain to fumigants were demonstrated by many researchers (Berck and Gunther, 1970; Bielorai and Alumot, 1975; Dumas, 1980 etc.; also see Chapter Two, this thesis).

Moisture content and column temperature are the principal factors in bringing about differences in

retention time, whereas the column packing is also the test sample. The peaks can be displaced to longer retention times when the temperatures are lowered. The response to increased temperature (comparative increase in retention by the substrate) is used as an index of chemisorption and varies with the nature of the substrate (Scudamore and Goodship, 1982).

(4) Differential uptake and fumigant residues;

When wheat is fumigated with a mixture of fumigants, a differential uptake of the components is visibly observed (Chapters Four and Five). There is considerable interest in methyl bromide, a remarkably effective fumigant for stored grain. Table 6.1 to 6.3 shows some of the data obtained when methyl bromide was applied in the gas phase to various grain commodities (Chapter Four, Sections 4.1 and 4.3). Table 6.1 shows the differences in uptake of methyl bromide by different wheat fractions stored at 35°C. These and related data show that the percentage uptake varies not only with the type of product but with the storage period. In Table 6.1, wheat 1, 2, 3 and 4 stands for the ground level of wheat, e.g. fine, less coarse, coarse and whole respectively.

Table 6.1 Percentage (%) uptake of methyl bromide by wheat fractions at 35°C

Commodity	Moisture content (%)	% uptake, days	
		1	3
Flour 1	13.6	8.2	13.6
Flour 2	13.0	13.6	27.1
Wheat 1	8.7	11.1	16.1
Wheat 2	6.1	39.0	71.8
Wheat 3	12.0	12.0	29.3
Wheat 4	14.1	40.6	76.9

Table 6.2 shows the percentage (%) chemisorption of methyl bromide by flour, starch and gluten at two temperatures and two storage periods (Chapter Four, Section 4.3). The amounts of the commodity used were the same on a dry weight basis. In this instance the nature of the commodity rather than the moisture content as such has a major role. For gluten, its greater chemical (covalent) binding forces would explain its greater affinity for methyl bromide. The percentage uptake was further increased when the exposure period was increased. Only a minor increase in uptake was obtained by flour and starch, respectively (Scudamore, 1987b).

Table 6.2 Percentage (%) chemisorption of methyl bromide by flour, starch and gluten

Wheat product	Moisture content (%)	Temp (°C)	% chemisorption	
			1 day	3 days
Flour	13.6	24	1.8±0.17	4.8±0.26
		35	7.0±0.13	9.9±0.21
Starch	8.7	24	7.7±0.18	12.0±0.34
		35	13.1±0.14	16.1±0.22
Gluten	6.1	24	32.9±0.40	47.8±0.36
		35	41.8±0.25	77.9±0.30

Table 6.3 shows percentage (%) chemisorption of methyl bromide by whole maize kernels compared to the same kernels ground coarsely, both at 13% moisture content and held at three storage temperatures for one and three days. respectively (Chapter Four, Section 4.1). When the particle size of the kernels was reduced by coarse grinding, chemisorption was increased due to the increased availability of sites for chemical action. Similarly, increase in both temperature and exposure time also increased percentage chemisorption.

Table 6.3 Percentage (%) chemisorption of methyl bromide by whole vs coarse ground maize, 13% moisture content

Commodity	Temp (°C)	% chemisorption[a]	
		1 day	3 days
Maize,	4	0.0±0.00	18.0±0.38
Whole	24	12.2±0.22	30.4±0.77
kernel	35	27.8±0.30	61.7±0.65
Maize,	4	4.6±0.24	24.1±0.21
Coarse	24	19.6±0.30	39.6±0.34
ground	35	46.4±0.39	76.6±0.25

a- mean of 4 replicates

The purpose in citing the foregoing research on residues of methyl bromide in stored grain serves as a dual purpose; a) to demonstrate that absence of free (unchanged) methyl bromide, carbon disulphide, carbon tetrachloride, ethylene dichloride or ethylene dibromide in a product after fumigation does not mean that residues are absent. The parent molecule may be altered, as is encompassed in the definition of the term fumigant residue discussed in Chapter One; and b) it also helps to explain the fact that fumigant residues need not be toxic, or they may be of low mammalian toxicity after interaction of the fumigant with the food commodity. In either case, toxicological support would be a prerequisite, to ensure that the conclusion on mammalian safety is correct.

CHAPTER SEVEN

GENERAL CONCLUSIONS

The main objectives of this thesis, as mentioned in Chapter One, were 1) to develop a method or methods of fumigant residue analysis, 2) to determine the accumulation of the residues in foodstuffs with special reference to grains and to check the extent of such accumulation, and finally 3) to study the effects and fate of these fumigants on the environment and also their metabolism and reaction with the constituents of the food.

To be able to develop a methodology suitable for these fumigants, it was necessary to critically review the existing literature pertaining to the subject in general. Although conclusions have been drawn at the end of most chapters, especially the analytical ones (Chapters Four and Five) in the form of results and discussion, it was found necessary to draw a general conclusion covering the above objectives of the whole project and to point out areas which would benefit from further research.

The field of fumigants and fumigation covers a large number of volatile chemicals that are used in everyday life. However, as described in Chapter Two, it is very difficult to study all the listed fumigants used as insecticides hence just a few common successful ones have been chosen and discussed in this project.

A lot of research has been carried out in recent years concerning fumigants, their environmental fate and residues in foodstuffs. However, the bulk of the literature is relatively old and limited and in much need of further updating, reviewed and expanded. More effort should be given to this very interesting field of research. Those specializing in the determination of fumigant residues at the moment represent only a small percentage of the work-force of professional chemists. However, they have contributed substantially to the advancement of the state-of-the-art of these trace organic analysis.

Because the statistical possibilities of finding suitable, small molecular weight compounds, that have not been tested already are remote, the fumigants that are now being used are the only ones that are likely to be available in the foreseeable future. This means that the future of fumigation techniques is dependent on a very small number of chemicals. Furthermore, the continued acceptance of these few chemicals is dependent on whether or not the necessary research will be carried out to provide the information needed for them to meet modern health standards.

There is a general need for pesticides to protect, preserve and maintain the food supply. From this point of view, it should be abundantly clear that pesticides are in many cases an indispensable part of modern food protection practices, enabling us to obtain new standards of food

production and quality, and to greatly improve human health and happiness. However, like virtually every other development of modern science and technology the use of these chemicals has sometimes been abused, generally following the adage that if a little is good, a lot will be even better.

In Chapter Three, the environmental fate of these fumigants especially in foodstuffs, which is one of the most common areas where these chemicals are used in large quantities and frequently ignored in terms of their residue effects, is summarised. Although it is generally believed that these gases dissipate into the atmosphere and are diluted in the process such that there is no significant risk to the environment as a whole apart from the residues likely to be found in foodstuffs and the air around the fumigation premises, accepted levels of these hazardous fumigants should be set and frequently monitored.

Pesticides interact with the environment with possible adverse consequences for man and other non-target species even at low levels. Volatilization can be a major vehicle for the movement of these chemicals from treated areas. The vapours resulting from volatilization of some pesticides can cause adverse effects to man via inhalation exposure at sites of application or biological effects on non-target organisms at some distance from the treated site. Methyl bromide, for example, a very volatile and toxic insecticidal fumigant, should not exceed 15 ppm in

human exposure with average levels of less than 5 ppm during each working day. However, if these chemicals are used carefully and the proper instructions followed, there will be no appreciable risk to man or organisms. There is no evidence of any serious environmental pollution problem so far although vigilance is still called for.

In the field of analytical method development, data supporting the adequacy of a given method must be accumulated and presented when the method is first described. In residue methodology, the evidence should meet two important premises; 1) that the method removes the residue from the substrate quantitatively, and 2) that the steps subsequent to extraction provide an accurate measurement of the residue extracted. The latter requirement is the easier to fulfil, the former being met only by vigorous experimentation. The development of analytical methods for fumigant residues present an even greater challenge to the analyst than is normally the case because the transitory nature of the compounds severely limits the handling operation to which they may be exposed without loss. Studies to determine the recovery of a compound through a method are worthless if losses occur during processes exterior to the method itself. This in turn forces the analyst to perform recovery experiments which the conditions do not necessarily duplicate the actual residue situation.

Researchers have varied widely in their efforts to prove the effectiveness of their methods. Proof that the

compound of interest can be recovered through the method without loss is the simplest to obtain, and this information is fully discussed in this thesis (Chapters Four and Five). Recovery studies of volatile compounds usually require the addition of the fumigant standard just prior to analysis, in a system with minimal exposure to the atmosphere. Even when experiments were performed in this way, losses could be partially blamed on the fortification step rather than on the method itself. This uncertainty is not beneficial to the widespread acceptance of a given method. Minimal assurance has been sought in this project that the method or methods used would effect the removal of any residue resulting from fumigation, in addition to recovery of standards. This involved laboratory fumigation of samples and comparison of results obtained by the specified method or methods with results obtained by some more rigorous extraction. Often, the more rigorous treatment was merely a repeated analysis on the same sample or by introducing a more prolonged period of time for the extraction step. The advantages of this type of experiment over simple recovery tests lies in the more realistic state of the fumigant-food relationship. In the recovery test, there is usually very little contact between fumigant and food. In the analysis of a fumigant product, the residue is bound to the substrate in some way. Similar results obtained by the described analytical method and by the extended analysis procedure do not prove conclusively that the extraction technique is adequate,

but the additional information provided is certainly useful. The residues extracted from laboratory fumigated grains were equivalent to what could be expected in a commercially fumigated sample having in mind the handling it is likely to receive.

Chapters Four and Five dealt exclusively with method development and validation of some existing methods such as headspace, the Association of Analytical Chemists first official method (AOAC) and the purge and trap method. A method for determination of fumigants in cereal products has to be based on two main steps; 1) extracting the fumigant residue from the grain with a suitable solvent and removing interfering substances from the extract before injection into the gas chromatograph; and 2) finding a suitable column packing material for separating the different components and the solvent, and devising a sufficiently sensitive detection method. To establish the validity of a method, it is necessary to establish; 1) that complete extraction of aged residues occurs; 2) that the standard error of analysis is acceptably low; 3) that an acceptable (100%) recovery is obtained from spiked samples, and 4) that the same result, within reasonable acceptable limits, is obtained by the different methods followed.

In Chapter Four, the headspace method was found to give good recoveries, with a highly volatile fumigant like methyl bromide. It was capable of detecting methyl bromide at very low levels and also prevents the

introduction of non-volatile materials into the column or injector point hence overloading of the sensitive electron capture detector is minimal. Equally both the purge and trap method and the AOAC method also extracted the residues to very acceptably low levels with good recoveries and reproducibilities. The headspace method was however found to be excellent in analysis of very volatile compounds like methyl bromide while the other two methods are perfectly suitable for either single or multi-residue analysis. It is therefore recommended as discussed in Chapters Four and Five that these three methods of fumigant residue analysis should still be used with some further improvement when appropriate. For instance, the 48 hour extraction period recommended by the AOAC method was found to yield low results in this project and therefore a 72 hour or more is strongly recommended.

During the analysis of fumigated grains, methyl bromide was found to dissipate very fast from both adsorbed and the absorbed levels and that in a longer aeration or storage period, there will be little or none of the residue left in the grain. The amount which might be left associated with the food is almost certainly decomposed, with the formation of inorganic bromide.

Ethylene dibromide (EDB) on the other hand, heads the less volatile group of the stored grain fumigants. According to the results obtained from both analytical chapters; 1) EDB is very strongly sorbed by grains and their by-products; 2) there is very little decomposition

of the EDB or reaction with the grain material at ordinary temperatures and 3) loss of unreacted fumigant during the airing period is very slow. Residues of unreacted fumigant (EDB) appeared to be the chief toxic hazard in treated foodstuffs. EDB is mostly used in tropical countries where ambient temperatures are relatively high and its disadvantages are therefore less marked. The toxicity of fumigants to insects is reduced at low temperatures so that larger quantities are needed to obtain control as conditions become colder and that is the reason why fumigation is less important in cooler countries. Since diffusion and penetration of gases are slowed down as temperature drops more fumigant is sorbed and retained in the commodity for longer periods of time at low temperature. Whereas at ordinary temperatures a very small amount of reaction takes place between EDB and the grain especially wheat protein, on heating, a substantial proportion of the sorbed EDB undergoes decomposition to ethylene glycol and inorganic bromide, the remainder being lost by volatilization.

In studying the nature and amount of the residues which may be present in foodstuffs after commercial-scale fumigation with EDB, analytical procedures are required which will differentiate between unreacted EDB physically sorbed on the material and the water-soluble inorganic bromide which may result from the decomposition of the EDB or its reaction with the grain material. The amount of EDB used as a fumigant has become less and less worldwide.

High levels of EDB pollution might not be a major problem because of its volatility. However, its elimination is recommended because of its carcinogenic activity, and thus it is necessary to monitor closely EDB residue levels in food.

One of the most important factors governing the choice or rejection of any particular chemical as a fumigant is the extent of its chemical reactivity with constituents of the food or commodity being subjected to treatment. Some fumigants are not only unstable, and decompose when sorbed by a commodity, but also react chemically to form new compounds which can be either degradation or addition products with the constituents in the fumigated materials. A high proportion of the sorbed methyl bromide for example, reacts chemically with the wheat constituents with the formation of water-soluble bromide and methylated products (Chapter Three). At the end of the fumigation period any undecomposed methyl bromide would either be rapidly desorbed or undergo ultimately complete decomposition. In fumigated wheat flour the end-products appear therefore to consist almost entirely of inorganic bromide, O-methyl, S-methyl, dimethyl sulphonium, and N-methylhistidine derivatives. There is no reason to believe that these products would differ if the wheat were exposed to methyl bromide as the whole grain or as the milled product. The total sorption and consequent rate of decomposition would, however, be higher in milled wheat grain than in whole grain (Chapter

Four). The end-procucts may be toxic and remain in the commodity as residues. In order to establish the maximum residue levels, data on the fate of the fumigant reaction products are therefore very important and will have to be collected.

Environmental fate analysis (Chapter Three) indicates that residues decreased with increase in temperature, humidity, aeration or exposure time and increased with increase in moisture content. Residues may also vary from cereal to cereal or variety to variety. Sorption of fumigants is greater in maize than in wheat and also slightly more rapid loss of residue has been observed from maize than wheat. Another important result from this analysis is that the rate of disappearance of residue is seen to be similar whether the fumigant has been applied initially as a liquid or a vapour, an important factor when predicting the behaviour of fumigants.

Although grinding causes a substantial reduction in residue level and further elimination of the fumigant will occur during processing and cooking, it seems likely that residues of fumigant and their breakdown products detectable by the highly sensitive analytical methods already described, will frequently be present in treated grains. The problem is even more serious in animal nutrition, since, in contrast to human food preparation, the feed for animals is not normally subjected to baking or cooking processes, which are known to reduce the

residues to negligible levels. This calls into question the position previously accepted that after use in accordance with good agricultural practice no significant residue of the unchanged residue will reach the consumer. This assumption has resulted in a number of countries exempting some fumigants from the requirements of a maximum residue level (MRL) or alternatively setting a zero tolerance.

Methyl bromide and other less volatile fumigants like ethylene dibromide are believed to dissipate away after aeration or a long storage period, a position supported here by the fact that essentially no residues derived from the two treatments was reported in the two analytical chapters of this thesis. However, the repeated application of these toxic chemicals after re-infestation had occurred as soon as the toxic concentration of the gas had dissipated, has been found on occasion to leave toxic residues in food. Therefore this possibility cannot be ruled out on general health grounds and any grain or their by-products suspected of being treated with fumigants must be analysed before consumption.

Further studies aimed at following batches of grain from commercial treatments, through storage and processing to the point of ingestion are required to test the correctness of these proposals. This information will also provide guidance on appropriate levels of residue to be employed in any long-term feeding trial which may be required to determine the toxicological significance of

such residues.

To rely on a particular pest control method in order to reduce the risk of contamination while reaping maximum benefit has been of great concern for centuries. As discussed in Chapter One, production of food to feed the growing world population is clearly the primary world problem, both now and in the future. By conservation projections, the world needs to double total food production by the year 2000. The farmers need to maximise the yield and quality of all crops without being concerned about any toxicological risk. In order to do this, the farmer must be provided with the best control techniques possible. These techniques need not only be chemical methods as portrayed in this project nor biological ones which were sympathetically mentioned in the text but a kind of an integrated, comprehensive approach to the use of various control methods that takes into account the role of all kinds of pests in their environment.

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