THE EFFECT OF METHYL BROMIDE UPON *SALMONELLA PULLORUM*

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Theodore Augustus Maag

1960


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THE EFFECT OF METHYL BROMIDE UPON *SALMONELLA PULLORUM*

By

Theodore Augustus Maag

A THESIS

Submitted to the School of Graduate Studies of Michigan
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1960

This work is

respectfully dedicated

to

MY FAMILY

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INTRODUCTION

After an outbreak of an infectious disease in a poultry house, disinfection of the house and premises is generally recommended. Especially is this needed when young birds are being introduced as replacements. During the time interval between the disposal of the old birds and the placing of new birds in a house, disinfection is again advised to prevent the exposure of replacement birds to a heavy microbiological infection.

In disinfecting any poultry house the major problems are the porous surfaces, the residual organic matter and the dust which remains even after cleaning. Many poultry houses have dirt, or porous, rough concrete floors. Therefore, a desirable disinfectant for poultry houses should: a. have superior penetrating activity, b. applied easily, c. kill micro-organisms in presence of soil and organic matter, d. be non-toxic to poultry, and e. be non-toxic to man. There are other important attributes of a disinfectant such as being non-corrosive to equipment, lingering or residual effect, dosage level, etc. which could also be included.

The objective of this investigation was to ascertain the effectiveness of gaseous methyl bromide as a disinfectant against a poultry pathogen.

REVIEW OF LITERATURE

Gaseous Disinfection

Introduction

Phillips (1957) briefly reviewed gaseous disinfection and stated that vapors were used in Biblical times. The burning of incense, such as frankincense and myrrh, was associated with purification. During medieval epidemics, the materials on which messages were written were held in smoke in the belief that this procedure would prevent the spread of disease. The first authenticated report of scientific disinfection with an aerosal was recorded by Lister who sprayed phenol in operating rooms with the specific objective of controlling microbes.

Early use of gaseous disinfectants took two directions. Air purification was the object in one and the other was terminal or complete sterilization of the air and all objects exposed within an enclosed space. Air purification involved substances, such as the glycols, which were directed toward aerosol sanitation. Harry (1956, 1957) presented an excellent review of air purification as related to poultry rearing industry. Terminal sterilization with vapors was first used in sick rooms to kill infectious disease agents. This practice began in the late decades of the nineteenth century and has continued into this century. Some countries made routine fumigation of sick rooms mandatory whenever an infectious disease occurred. Present knowledge of the

actions and limitations of the various disinfectants used for these fumigations makes it doubtful if total disinfection was thus obtained.

The early gaseous disinfectants were sulfur dioxide and chlorine. These gases were extremely damaging to many objects which was a definite disadvantage to their use. Formaldehyde was discovered in 1894 as a bactericidal agent and was the first practical gaseous disinfectant. It came into general use because of its effectiveness and general safety for personnel and equipment. Numerous papers had been written concerning formaldehyde as a disinfectant. Nordgren (1939) reviewed the early literature on gaseous disinfectants and Phillips (1957) and Sykes (1958) have summarized published research of more recent date.

Formaldehyde

Formaldehyde gas is generated either by vaporizing paraformadlehyde or by heating a solution of the gas in a water mixture called formalin. Formaldehyde is stable only at temperatures above $80^{o}C$ when in high concentrations. Paraformaldehyde is solid polymer of formaldehyde existing as a mixture of polyoxymethylene glycols. When heat is applied to paraformaldehyde depolymerization occurs with formaldehyde and water vapor resulting. Formalin is the most common source of formaldehyde. It exists in a 35 $\pm$ percent concentration as a hydrate. Commercial formalin is stabilized with 8 to 15 percent methanol to prevent formation of the solid polymers. One of the most common methods used

to volatilize formaldehyde gas is by the heat of the oxidative reaction produced in mixing two parts of formalin and one part potassium permanganate.

When used as a disinfectant, formaldehyde is a surface disinfectant. Formaldehyde has little activity in penetrating even the most porous substances. Cracks, crevices and pores are penetrated with difficulty. On a smooth surface, microorganisms may be easily protected by a covering of organic matter. The poor penetrability of formaldehyde is due to polymerization upon all surfaces with which it comes in contact. These polymers are extremely obnoxious in that they may linger for several days and slowly break down to formaldehyde gas. Formaldehyde, because of its instability, must be used at a temperature above $20^{o}C$ with the relative humidity 60 to 80 percent. Only relatively low concentration can be maintained in the atmosphere. At room temperature only about 3 ounces can be maintained per 1000 ft^3 of space. Sykes (1958) reviewed the activity of formaldehyde against bacterial cells and observed that the reaction was reversible. The inactivated cells are apparently in a state of suspended animation; but that certain "neutralizers" plus a normal nutrient medium will revive these bacteria. Perhaps this phenomenon will explain the conflicting evidence concerning the effectiveness of formaldehyde on organisms that have been exposed to the gas.

In conclusion, formaldehyde disinfection is only effective if several varied conditions are accomplished. There are also adverse side effects present in many types of applications.

Ethylene Oxide

Ethylene oxide was used first as an insect fumigant by Cotton and Roark (1928). Schrader and Bossert (1936) applied for patents in Germany during 1928 and in the United States in 1929. In the patent applications it was stated that the alkylene oxides were capable of killing pests and germs of all kinds. Later, ethylene oxide was widely advocated as a microbicidal agent in the sterilization of spices, foodstuffs and other common organic products by Griffith and Hall (1938, 1940a, 1940b, 1940c, 1943), Kirby, Atkins, and Frey (1936), Yesair and Cameron (1938), and Hall (1938). Phillips and Kaye (1949), Phillips (1949), Kaye (1949), and Kaye and Phillips (1949) introduced the practical field of gaseous sterilization as it is used today. These four publications by Phillips and Kaye reviewed the earlier published data on ethylene oxide as a bactericidal gas and presented extensive original data substantiating the earlier work.

Phillips (1957), Phillips and Warshowsky (1958), Sykes (1958), and Phillips (1958) reviewed extensively the various uses of ethylene oxide as a sterilizing agent. Textiles, plastics, paper, wood, oil paintings, medical and biological preparations, plaster of Paris bandages, medical instruments, arteries, animal feeds, eggs, bacteriological media, soil, airplanes, trucks, delicate electronic instruments, and analytical balances are some of the various materials sterilized. All species of bacteria, fungi and viruses are affected by the lethal action of ethylene oxide.

The boiling point of ethylene oxide is 10.8 C. It has an ether-like smell, is highly explosive, generally safe to use on most

types of objects and is relatively non-toxic to humans. This gas has extremely good penetrating power and does not require special conditions in order to be reactive. To suppress the explosiveness of ethylene oxide a mixture of 10 percent ethylene oxide and 90 percent carbon dioxide is commonly used. This dilution lowers the concentration of the gas and increases its cost considerably.

Kaye and Phillips (1949) found that only extremes in humidity were important factors in the sterilizing activity of ethylene oxide. At 28 percent relative humidity ethylene oxide is most active, while at 65 percent it is one-fourth as active and at 100 percent but one-tenth as active. At complete dryness of a product such as would occur in lyophilization, the action of ethylene oxide is inhibited. They advanced two theories as to the role of humidity in the sterilizing action of ethylene oxide. First there must be a mechanical role in that some moisture must be present within the cell to dissolve the ethylene oxide and permit its contact with the cellular protein. Too much moisture causes a dilution effect upon ethylene oxide before it comes into contact with the organism. A second theory postulates that ethylene oxide reacts with and adds onto groups within the protein molecules, such as hydroxyl, sulfhydryl, amino, or carboxyl groups which contain a labile hydrogen. To do this the labile hydrogen would have to ionize before the ethylene oxide could react with them.

Hawk and Mickelsen (1955), Bakerman, Romine, Schricker, Takahashi, and Mickelsen (1956), Windmueller (1956), Windmueller and Engel (1956), and Windmueller, Ackerman, and Engel (1956) reported

research concerning the adverse effect of ethylene oxide on the diets of experimental animals.

The availability of thiamine, riboflavine, pyridoxine, niacin, and folic acid is adversely affected. Pantothenic acid, biotin and vitamin B_{12} remained unaffected. Ethylene oxide fumigated casein, as a protein source, for rats gave no growth but this condition was rectified by addition of supplemental methionine and histidine in the diets. It appears that some chemical reaction inactivates various nutrients in the diets without imparting toxicity.

Several workers have used ethylene oxide to sterilize media for the cultivation of microorganisms and insects without adverse effects on the subsequent growth of several different species (Hansen and Snyder 1947, Wilson and Bruno 1950, Judge and Pelczar 1955, and Barlow and House 1956). Sykes (1958), however, reported that he has found that some lots of serum sterilized with ethylene oxide were less capable of supporting the growth of some sensitive bacteria.

Beta-propiolactone

Hoffman and Warshowsky (1958) were the first to report the bactericidal activity of beta-propiolactone vapor. Their report shows that beta-propiolactone vapor is 50,000 times more effective than methyl bromide and 40,000 times more effective than ethylene oxide and 25 times more effective than formaldehyde vapor for a 90 percent kill of bacteria.

Phillips (1958) and Phillips and Warshowsky (1958) briefly reviewed beta-propiolactone as a gaseous sterilizing agent. They make this statement:

> "The present authors feel that the compound possesses almost all of the advantages of formaldehyde, and avoids two of its disadvantages, slow action and extreme persistency as a vapor, following use. It is believed that beta-propiolactone should in the future replace formaldehyde in many applications where it is now used, and should find additional applications where the disadvantages of formaldehyde have limited its use."

Beta-propiolactone has a relatively high boiling point (155 C) hence low vapor pressure at ordinary temperatures. This gas is similar to ethylene oxide, methyl bromide, and formaldehyde in that it is also bactericidal by virtue of its alkylating ability. It is active at a low concentration so it is easy to obtain sufficient vapor concentration in an atmosphere to be bactericidal. This gas, like formaldehyde, requires a high humidity (70 percent or above) and an effective concentration can be maintained in an area without it being hermetically sealed. Beta-propiolactone does not penetrate porous materials easily but is rapid in its inactivating action, two hours of exposure being adequate compared to formaldehyde which needs ten or more hours in order to be effective. Unlike formaldehyde, beta-propiolactone does not polymerize on surfaces and fumigated areas are readily aired free of gas. This gas is lachrymatory but less irritating than formaldehyde.

Phillips in a personal communication (1959) states:

> "...we (workers in the field of gaseous sterilization at Fort Detrick, Frederick, Maryland) would believe from our own work, that beta-propiolactone would be more effective than methyl bromide in the control of poultry diseases."

Methyl Bromide

A general review - Le Goupils (1932) demonstrated the toxicity of gaseous methyl bromide for insects. It quickly came into extensive usage by the plant, grain, and food industries as an effective agent against insects and rodents. The advantages of methyl bromide over other fumigants then in use was its high penetrability and low toxicity to most plants at a low concentration, dormant stock and seeds, low residual toxicity, freedom of damaging chemical action on equipment and materials, and wide range of effectiveness at different temperatures. Richardson and Johnson (1935), Mackie and Carter (1937), Phillips and Easter (1943), Steinweden (1945), Latta, Richardson and Kindler (1946), Phillips (1955), Dow Chemical Corporation manual and Michigan Chemical Corporation manual have reviewed methyl bromide, the methodology of its uses and effects. These are but a few of the many publications which have dealt with methyl bromide and its uses in the plant agricultural field and insect control. Through its use in the fumigation of greenhouses, nurseries, and buildings to control insects and rodents, methyl bromide was also found to be highly lethal to many other organisms. These included weeds and other plants, insect larvae and other phytopathogenic and free-living invertebrates, as well as fungi, bacteria, and viruses which are pathogenic to plants.

The New Jersey Experiment Station in 1939 reported that 2-1/2 lb of methyl bromide per 1000 ft^3 of soil for 2-1/2 hours did not affect the root knot nematode Heterodera marioni. Hawkins (1939)

eliminated the white fringe beetle Pontomorus leucloma from potting soil by a treatment with 40 ml of liquid methyl bromide per yd^3 of soil. Taylor and McBeth (1940 and 1941) presented the first evidence of methyl bromide's wide range of activity in soil when the gas concentration was maintained through the use of gas-tight cover over the soil. These workers used methyl bromide to kill Heterodera marioni and innumerable species of free living nematodes in the soil. They also reported that bacteria and fungi were killed in the treated soils. Taylor and McBeth noticed that less damping off occurred with plants grown upon the treated beds. These workers used 80 ml/M^3 of soil in vault fumigations and 1-1/2 lb/200 ft^2 for fumigation under a gas-tight cover. Livingstone (1940) used methyl bromide to control the larvae of the white fringed beetle in the soil. An anonymous article in Florist Exchange and Horticulture Trade World in 1940 reported the use of methyl bromide to control grubs of the Asiatic beetle in azalea beds.

Mark (1941) used methyl bromide and ethylene oxide in controlling spoilage organisms of dates. Salle and Korzenovsky (1942) reported that methyl bromide in two hours exposure at an unstated concentration did not kill bacterial spores. Godfrey and Young (1943) reviewed soil fumigation for plant disease control through part of 1942. They used methyl bromide at 2-1/2 ml of the liquid per ft^3 of soil in gas-tight containers and killed nematodes and fungi pathogenic for plants. In other trials using a gas-tight cover, methyl bromide killed Sclerotinia sclerotiorum buried eight inches in wet potting soil at 53 F with a dose of 2 lbs/yd^3. When the soil was dry,

Sclerotinia was not killed but the damping off organism was killed. Baerwald (1945) patented a method of sterilizing foodstuffs such as sausage with methyl bromide and ethylene oxide. Baerwald stated that 1, 2, or 3 g of a methyl bromide snow (50 percent methyl bromide, 50 percent water mixed and frozen then ground) would completely sterilize a heavily contaminated sausage wrapped in pliofilm or cellophane. After sterilizing the package the methyl bromide would diffuse out of the wrapper leaving no toxic residues. Johnson (1945) inactivated the virus of wheat mosaic disease using gaseous methyl bromide.

Whelton, Phaff, Mrak, and Fisher (1946), fumigating freshly inoculated slants for 24 hours at room temperature, found the following concentration of methyl bromide necessary to effectively kill: bacteria (Bacillus, Escherichia, and Pseudomonas) 5 ml of the liquid per 1 of air, fungi (Aspergillus and Penicillium) and yeasts (Endomycopsin, Zygosaccharomyces, Saccharomyces, and Hanseniospora) 1 ml/1. Ethylene oxide mixture (20 percent ethylene oxide in ethylene dichloride) was approximately five times more effective than the methyl bromide in this trial.

Newhall (1946) reviewed methyl bromide as a fungicide and nematocide. Using gallon crocks with no vapor seal, Stark and Lear (1947) obtained control of the damping off organisms with methyl bromide. Christie (1947), using methyl bromide without adequate means to confine the gas, found that nematodes but not fungi were killed in their trials. Newhall and Lear (1948) recommended methyl bromide for

soil fumigation as a result of experimentation in which they showed that methyl bromide killed fungi and was easily administered with good penetration through the soil with great effectiveness. Phillips (1949) working with ethylene oxide and methyl bromide found the latter bactericidal but less so than ethylene oxide.

Lear and Mai (1950) killed golden nematode cysts on used bags tied in bundles of fifty or in soil, 10 lb per paper sack, when fumigated with methyl bromide. The gas was confined by means of either gas proof flexible plastic tarpaulin, a copper lined fumigation chamber, or a 55 gallon metal drum with a removable cover. Lear (1951) in a review presents some new data on the effectiveness of methyl bromide as a soil fumigant.

Swank (1951) reported that methyl bromide killed damping off and root rot organisms in the Rhizoctonia, Pythium, and Fusarium genera. Lear (1952) advocated methyl bromide fumigation of bags and machinery on the farm as a method of controlling the spread of the golden nematode of potatoes. A lethal exposure consisted of 23 lb for 16 hours or 46 lb of methyl bromide per 1000 ft^3 for two hours. The temperature needed to be, at least, 60 F. According to a report by Stover and Koch (1952) muck soil fumigated with 2 lb of methyl bromide per 100 ft^3 was free of all tobacco pathogens. A dosage of 1 lb/100 ft^3 killed the damping off organism and all weeds. The work of Wensbey (1953) states that methyl bromide is more effective against fungi and bacteria than against actinomycetes. This work was done utilizing a

L. D. $_{90}$ (90 percent of the organisms killed) as the endpoint. Soil moisture did not affect methyl bromide's activity. High organic content soils slowed up the action by combination with methyl bromide and slowed penetration by the gas.

Trickel (1952) used methyl bromide in controlling molds on cheese. He found that ethylene oxide required 16 hours and methyl bromide 8 hours for complete kill of all Penicillium roqueforti, using 5 lb of the gas per 1000 ft^3 at 74 F. Methyl bromide did not kill P. roqueforti in 20 hours with a 10 lb dose at 40 F. At 60 F dosages of 2, 3,4, 5, and 10 lb/1000 ft^3 of fumigated volume killed completely P. roqueforti in 20, 12, 8, 6, and 4 hours respectively. Trickel studied various factors as they might affect the activity of methyl bromide to kill molds. The pH of the media upon which the molds were exposed, moisture content of the atmosphere, negative pressure, formation of inhibitory substances in the media upon fumigation, and varied susceptibility of the spores and mycelium were not found to be factors which altered the effectiveness of methyl bromide. Also surveyed in this paper was the activity of methyl bromide to penetrate various plastic films. Trickel also studied the disinfectant properties of methyl bromide on various organisms. Methyl bromide killed Saccharomyces cerevisiae, Candida krusei, Aspergillus niger, Geotrichum candidum, and Cladosporium herbarum at 10 lb/1000 ft^3 for four hours of 5 lb after eight hours of exposure, but Streptococcus lactis, Escherichia coli, Serratia marcescens, and Mycoderma sp. were not inactivated during these same exposure conditions.

Using pathogenic plant fungi Munnecke and Ferguson (1953) effectively fumigated these fungi in soil within flats stacked under a plastic cover. The lethal dosage necessary was found to be 4 lb/ 1000 ft^3. Mac Lachlen, Monro, Radicot, and King (1953) found that neither 50, 100, nor 150 mg/l of methyl bromide when used for eight or sixteen hours killed Corynebacterium sepedonicum. McKeen (1954) found that methyl bromide was more effective against fungi than against bacteria and actinomycetes.

The bacteria had a greater range of variability than fungi in their susceptibility to methyl bromide. Some fungi were killed at 3/5 lb of methyl bromide per 100 ft^3 of soil. Other fungi had a minimum lethal dose of 2 lb/100 ft^3 of soil except for some sporeformers which survived at dosages as high as 4 lb/100 ft^3 of soil. To completely inactivate non-sporeforming soil bacteria dosages from 10 to 20 lb of methyl bromide per 100 ft^3 were needed.

McKeen fumigated soil for a 24 hour period at temperatures between 70 and 80 F. The moisture content of the soil in these experiments did not influence the number of organisms killed.

Munnecke and Lindgren (1954) fumigated 225 flats of soil under a plastic cover with 4 lb of methyl bromide per 100 ft^3 and studied the concentration and the fungicidal activity of the gas throughout the pile. Methyl bromide rapidly penetrated the soil throughout all flats. The gas rapidly diffused out or was absorbed in the soil in the first few hours until about 1-1/2 lb of methyl bromide per 100 ft^3 remained in the air. Upon fumigating 24 hours

Verticillium alboatrum frequently survived but other fungi did not.

Newhall (1955) gave a review of soil disinfection using methyl bromide.

Phillips (1959) in a personal communication concerning work that his group had done with methyl bromide and ethylene oxide states:

> "We did not publish on this (methyl bromide) compound because it was not particularly promising to us. It is only about 1/10 as effective as ethylene oxide for example. We did measure its activity at various temperatures against B. subtilis (B. globigii) Staph. aureus, E. coli and the T-2 phage of E. coli. It killed all of these organisms, but slowly and as with other alkalating agents there was not the very marked difference in rate of kill between spores and vegetative agents as is found with other types of disinfectants."

Action against animal pathogens - Swanson and Taylor (1943) first used methyl bromide against nematodes parasitic for mammals. They fumigated sandy loam plots of 63 ft^2 each with 1 lb of methyl bromide. These plots were covered with glue coated kraft paper for 22 hours after fumigation. The soil normally contained free living nematodes of several genera and was artificially contaminated with several other free living forms. The plots were also contaminated with the larvae of several parasitic species cultured from calf feces: Haemonchus, Ostertagia, Trichostrongylus, Oesophagostomum, Cooperia, Bunostomum, and Nematodirus. All the represented genera were inactivated in the fumigated plots but remained active in the unfumigated plots. In addition it was noticed that moving protozoa were present in the control soil but none was present in the fumigated soil. These workers only checked the soil to a depth of 12 in.

Andrews, Taylor, and Swanson (1943), using similar conditions and dosage as above, killed Ascaris lumbricoides embryos within the eggs, the infective larvae of Strongyloides and Oesophagostomum, immature larvae within kidney worm eggs, coccidia, and the earthworm as the intermediate host of the swine lung worm. They suggest methyl bromide as a means of cleaning up animal runs or other small lots used in extensive growing operations. Cram and Hicks (1944) fumigated sewage, a mixture of digestion sludge and sand, at a temperature of 61 to 68 F at the same dosage as used in the preceeding work. Ascaris lumbricoides eggs with only slight development were killed, but eggs with more mature embryos were not inactivated.

Boney (1948) considered methyl bromide to have possible economic value as a fumigant in the control of cecal coccidiosis of chickens. He found that methyl bromide inactivated Eimeria tenella under practical conditions in soil plots or in plots covered with cane pulp litter. These plots were enclosed under wet Sisal Kraft paper and fumigated for 13 hours at a temperature of 65 F. Fifteen-hundredths to 0.3 ml of liquid methyl bromide per ft^2 (approximately 1 lb/1000 ft^2) was found to be lethal to E. tenella. In practical type of fumigations involving small brooder houses, E. tenella was inactivated. The dosage used was 2 lb/1000 ft^3 for a house sealed completely within a gas-tight cover or 3 lb/1000 ft^3 in a house sealed with masking tape and wet newspapers only. The fumigation period was 13 hours at 65 F. Clapham (1950) fumigated plots ten yards square for 24 hours with 25 g of methyl bromide using a covering of rubberized ballon fabric. She effectively controlled

Heterakis gallinae, Ascaridia lineata (galli), Colinus virginianus, Trichostrongylus tenuis, Syngamus trachea, Capillaria and Histomonas meleagridis. In addition to these parasites all other life in the soil of the fumigated plots were killed, even at the 9 inch depth which was as deep as soil samples were taken. These plots were not plowed and had been part of a pasture for several years. They were rough with plant growth typical of a mediocre pasture. Birds reared upon these fumigated plots were kept essentially free of parasites for a period of a year. The source of recontamination seemed to be from bordering unfumigated plots and wild birds. Clapham advocated methyl bromide as having excellent possibilities for fumigation of bird runs once a year as a control measure of many poultry parasites.

Kolb and Schneiter (1950) and Kolb, Schneiter, Floyd, and Byers (1952) showed that gaseous methyl bromide is germicidal and sporicidal for Bacillus anthracis upon exposure for 24 hours in the presence of moisture at a concentration of 3.4 to 3.9 g/l. This is equal to a concentration of 211 to 248 lb/1000 ft^3 of methyl bromide. Kolb and Schneiter used six different strains of virulent organisms from different animal species. When the organisms were placed upon filter paper strips and exposed to methyl bromide in presence of moisture for one hour spores survived in 17 of the trials. However, no survival was evident at 24 hours of exposure. In the absence of moisture, some spores survived in 13 of their tests regardless of the length of exposure time. Liquid methyl bromide was ineffective in

killing spores of B. anthracis. In another series of experiments using excessively dehydrated spores, the action of methyl bromide seemingly was not impaired. Saiki (1952) found that methyl bromide would kill Vibrio cholerae, Bacillus dysenteriae, and Bacillus typhosa on Endo agar plates, dry and wet gauze, and in peptone water when these were fumigated in a sealed room. Schmittle (1955) reported that methyl bromide inactivated Newcastle disease virus (NDV) on feed sacks at a concentration of 5 lb/1000 ft^3 at 25 C for 21 hours, but was ineffective at 4 C. Using large desiccator jars as fumigation chambers, NDV was killed in 21 hours at 4 C with 8 lb dose, at 25 C with a 2 lb dose, and at 30 C with a 1 lb dose per 1000 ft^3.

Edgar and King (1955) stated that methyl bromide was effective in killing Aspergillus fumigatus on agar plates, and Ascaridia galli and E. tenella in a small amount of water within a Petri plate. These organisms were fumigated with 1 ml of methyl bromide per a one qt fruit jar used as a fumigation chamber. This dosage was approximately equivalent to 105 lb methyl bromide per 1000 ft^3 of fumigated area. In a field type experiment, a methyl bromide impermeable plastic cover was placed over used litter, and in the litter were buried Petri dishes containing the above mentioned organisms. A dose of 1 lb/100 ft^2 inactivated all the organisms. Used litter was fumigated in the 1 qt jars at the rate of 1 ml liquid methyl bromide in each. This litter contained viable coccidial oocysts, two species of nematode eggs, mites, fungi and several species of bacterial contaminants. All

these organisms were killed upon fumigation. In summary, Edgar and King stated that methyl bromide may prove practical in freeing heavily contaminated premises of undesirable disease agents.

Whelton et al. (1956) reported that approximately 540 lb/1000 ft^3 were necessary to inactivate Bacillus, Escherichia, and Pseudomonas species. No explanation can be given as to why this high concentration was needed for inactivation unless the fumigation was done in such a way as to allow the gas to escape.

Manns and Schmittle (1956) patented the use of methyl bromide for inactivation of Salmonella pullorum, coccidia, and the virus of Newcastle disease in used feed bags. The minimum stated amount needed for this purpose is 5 lb of methyl bromide per 1000 ft^3 for 24 hours at 70 F. Table 1 is a summarization of their results which show dosages needed of methyl bromide per 1000 ft^3 at various temperatures and exposure times with the three different organisms.

Schmittle (1959) stated that methyl bromide at 50 lb/1000 ft^3 will not penetrate tightly packed bales of bags and enact sterilization. If the bags are fumigated in an unbaled or loose bale form with proper distribution of methyl bromide, sterilization will result.

Schmittle and Holderried (1959) reported results from the use of methyl bromide in field type experiments. They used a poultry house of concrete block construction with composition roof and concrete floor. The house in an unclean condition with litter not removed nor with any other cleaning, was fumigated. In these experiments the gas concentration in various parts of the building was determined along

TABLE 1

MINIMAL QUANTITY OF METHYL BROMIDE IN LB/1000 FT^3
REQUIRED TO KILL POULTRY PATHOGENS
UNDER DIFFERENT CONDITIONS*

	Time of Sterilization					
	24 hours			36 hours		
	Temp. of Sterilization			Temp. of Sterilization		
	4 C	20 C	30 C	4 C	20 C	30 C
Newcastle Disease Virus	8 lb	2 lb	1 lb			
Salmonella pullorum	9	4.75	4.5	5 lb	3 lb	3 lb
Eimeria tenella	2	1	0.25			

* From Manns and Schmittle (1956)

with the effect of methyl bromide upon agar plate cultures of Aspergillus fumigatus and S. pullorum. The agar plates were placed on top of and beneath the litter and at ceiling level at various locations within the building.

The results of Schmittle and Holderried showed that a building of such a construction, even with all the windows and vents sealed with gas proof plastic, would not maintain a sufficient concentration of methyl bromide to sanitize. Completely enveloping the building with gas proof plastic rectified this problem. A 5 lb dose per 1000 ft^3 did not give adequate control in these experiments but a 7-1/2 lb dose was adequate. This was mainly due to leakage of the gas. The following were found to be important factors which influence the effectiveness of methyl bromide in any test: (1) outside wind velocity as it affects gas leakage, (2) temperature within the building, (3) air movement within the building as it affects layering of the gas, (4) the evaporation rate of methyl bromide after introduction into the building. In calculating the expense of fumigation a cost of 80 cents per lb of methyl bromide was used. The dosage rate was 7-1/2 lb/1000 ft^3. Using these figures, a building with an 8 ft ceiling could be disinfected for 4.8 cents per ft^2 and sealed in plastic for approximately 2.7 cents additional per ft^2, including labor and materials. The total calculated cost of disinfection was approximately 7.5 cents per ft^2 of floor area.

Technical Data on Methyl Bromide

Commercial products

Methyl bromide is available as a liquid packaged under pressure in steel cylinders of capacities of 10, 40, 50, 100, 150, and 400 lb or in small 1 lb "tin" cans. The steel cylinders have air added to bring the total pressure within the cylinder up to 60 lb/in^2 at 40 F. This added pressure assists in forcing the methyl bromide from the cylinder. The cylinders dispense methyl bromide as a liquid by means of a tube from the valve to the bottom of the cylinder. This method preserves the air charge used to force the methyl bromide from the cylinder.

Commercially, methyl bromide is available as a 99 percent pure product or with 2 percent chloropicrin (tear-gas) added to serve as a warning agent of a contaminated atmosphere. Several preparations are also manufactured consisting of methyl bromide dissolved in various solvents which lower its vaporization pressure.

Chemical and Physical Characteristics

The general properties of methyl bromide are listed in Table 2. Methyl bromide is a gas which becomes a liquid at 3.6 C and a solid at -93.7 C. This gas is heavier than air, nonflammable, colorless, practically odorless, and is highly diffusible and penetrating. Methyl bromide is generally non-reactive with most inanimate objects. Upon exposure of gaseous methyl bromide to a source of intense heat such as a flame or a glowing metal surface, decomposition of the gas takes

TABLE 2

TECHNICAL DATA - METHYL BROMIDE

Synonyms	Bromomethane, Monobromomethane
Formula	CH_3Br
Molecular weight	94.95
Density, liquid	1.732 at 0 C and 0 pressure
vapor	3.974 g/l at 20 C and 760 mm mercury pressure
Melting point	-93.7 C
Boiling point	3.56 C
Specific heat, solid at -96.6 C	0.165 cal/gm
liquid at -13.0 C	0.197 cal/gm
vapor at 25 C	0.107 cal/gm
Heat of fusion at -93.7 C	15.05 cal/gm
Heat of vaporization at 3.6 C	60.20 cal/gm
Heat of transition at -99.3 C	1.20 cal/gm
Vapor pressure	1420 mm Hg at 20 C 2 atmospheres at 23.3 C 5 atmospheres at 54.8 C 10 atmospheres at 84.0 C
Solubility	water - at 20 C and with a total pressure of 748 mm Hg = 1.75 gm per 100 gm of solution soluble in all portions in alcohol, ether and carbon tetrachloride
Flash point	nonflammable over wide range of concentration in air with intense source of ignition.
Description	Colorless, transparent, volatile liquid or gas with a burning taste and slight chloroform-like odor
Uses	Fumigant, fire extinguisher, reagent in manufacture of dyes, and the pharmaceutical industry

Data from: Handbook of Chemistry and Physics, Michigan Chemical Company, and Encyclopedia of Chemical Technology

place. The products evolved are hydrogen bromide, bromide, carbon oxybromide, carbon dioxide and carbon monoxide. These decomposition products are corrosive and highly irritating.

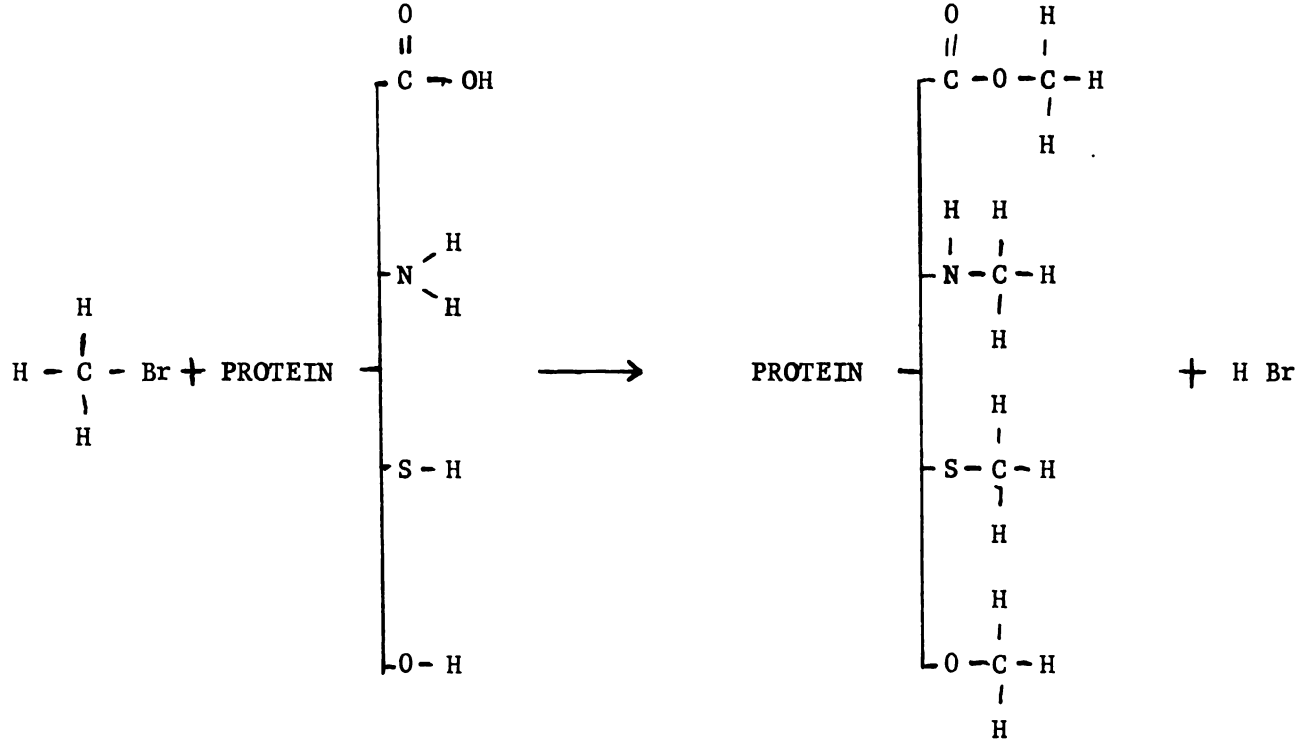
Methyl bromide is relatively insoluble in water but readily soluble in most organic solvents. Liquid methyl bromide is a vesicant, if skin is in prolonged contact with it. The gas is toxic to almost all forms of life. Primarily, the commercial use of methyl bromide is fumigation for rodents and insects within grain mills, warehouses or food plants. Methyl bromide also has extensive usage as a soil fumigant in the control of insects, weeds and microorganisms for tobacco seedbeds, nursery plots, and greenhouse soils. The gas is also used in fire extinguishers, and in organic synthesis by the dye and pharmaceutical industries.

Chemistry of its Bactericidal Activity

The lethal action of methyl bromide as proposed by Phillips (1952 and 1958) is a result of its action as an alkylating agent. Alkylation consists of the replacement of an active hydrogen atom in an organic compound with an alkyl group. The lethal alkylation reaction takes place with the reactive groups which are parts of essential complex enzymes or proteins of the cell. Phillips presents alkylation as the means of action of formaldehyde, ethylene oxide, beta-propiolactone and methyl bromide. Figure 1 presents diagramatically the chemical action involved with methyl bromide acting upon a protein containing free carboxyl, amino, sulfhydryl, and hydroxyl groups. These data were presented by Phillips (1952). The methyl

FIGURE 1

ALKYLATING REACTION OF METHYL BROMIDE WITH A PROTEIN CONTAINING LABILE CARBOXYL, AMINO, SULFHDRYL, AND HYDROXYL GROUPS. PROPOSED BY PHILLIPS (1952)



group of methyl bromide acts as the alkyl group with the bromine combining with the labile hydrogen atom forming hydrobromic acid. Lewis (1948) presented data that methyl bromide is capable of combining with sulfhydrylic groups and produced a progressive and irreversible inhibition of the enzymes urease, succinic dehydrogenase, papain, and yeast respiration. In this reaction Lewis found that hydrobromic acid was formed which then formed bromides. The alkylation theory appears to be supported by the findings of Kolb, Schneiter, Floyd, and Byers (1952) in that neither hydrobromic acid nor methanol killed anthrax spores in the presence of moisture in such concentrations as were effective in the case of methyl bromide.

A review was presented by von Oettinger (1955) of the action and toxicity of methyl bromide upon higher forms of life, especially man and laboratory animals. Dixon and Needham (1946) studied the vesicant effect of methyl bromide. They found that hexokinase was inhibited by methyl bromide. Church, Halvorson, Ramsey, and Hartman (1956) demonstrated heterogeneity in the resistance of spores of Bacillus sp. to ethylene oxide. The percent of resistant organisms could not be increased by selection of resistant spores. These workers also demonstrated that susceptibility to ethylene oxide varies with the fat content of the organism. Removal of the fat with a solvent decreases the resistance.

Phillips (1952) presented some interesting data on the activity of the alkylating agents and other disinfectants in killing

spore and non-spore forming bacteria. Table 3 presents data as given by Phillips. This table lists the relative ratio of resistance of a spore forming bacterium Bacillus globigii as compared to non-spore formers Micrococcus pyogenes and Escherichia coli for various disinfectants. The spore forming bacteria are from 0.5 to 15 times as resistant to the alkylating agents as are the non-spore forming bacteria. Comparing these results with those of the other common disinfectants it is noted that the spore forming bacteria are exceedingly resistant to the lethal action of those disinfectants. Spore forming bacteria are ten thousand to a hundred thousand times more resistant than are the non-spore forming bacteria to the action of the common disinfectants.

Phillips explains the increased susceptibility of the spore state to the alkylating agent in that the reaction can take place with any labile hydrogen. The other disinfectants, however, primarily react only with the sulfhydryl groups. The sulfhydryl group is protected through some means in the process of spore formation. The action of the common disinfectants is thus blocked with the spore protection of the sulfhydryl group but the alkylating agents have many other groups which are still in a reactive stage.

TABLE 3

RELATIVE ACTIVITY OF CERTAIN DISINFECTANTS AGAINST BACTERIAL SPORES AND AGAINST VEGETATIVE CELLS

Disinfectant	Ratio of Resistance
Alkylating agents	
Ethylene oxide	Between 2 and 6
Ethylene imine	Between 0.5 and 10
Methyl bromide	Between 2 and 5
Formaldehyde	Between 2 and 15
Chlorine	
Sodium hypochlorite	About 10^4
Silver	
Movidyn	About 10^4
Phenol	
Trichlorophenol	About 10^3
Quaternaries	
4-Cetyl-4-methyl-morpholinium methyl sulfate	About 10^4

MATERIALS AND METHODS

Methyl Bromide

The methyl bromide used in this study was provided by the Michigan Chemical Corporation, St. Louis, Michigan. The gas was the commercially available product marketed under the trade name "Pestmaster". This product was the commercially pure product, 99 percent CH_3Br. It was packaged in one pound metal containers in which the methyl bromide was maintained as a liquid under pressure.

A small, clean, steel cylinder of the size and design commonly called a lecture bottle was used as the container for methyl bromide. This cylinder was equipped with a micro valve (Hoke valve) in order to dispense the gas at finely controlled flow rates.

The air was removed as completely as possible from this cylinder with a vacuum pump. The cylinder and a 1 lb container of methyl bromide were chilled overnight in a walk-in freezer. Working within the freezer the metal can of methyl bromide was then opened (methyl bromide is a liquid at atmospheric pressure at this temperature) and transferred into the cylinder by suction utilizing the negative pressure within the cylinder. Care was taken not to admit any air into the cylinder. Upon returning the cylinder to room temperature the methyl bromide vaporized giving a pure, easily controlled supply of gaseous methyl bromide.

Gas Measuring Apparatus

Figure 2 illustrates the gas measuring apparatus. The following description describes the apparatus in this figure.

The methyl bromide-filled, lecture cylinder 'A' together with micro-valve 'B' was attached thru means of pyrex tubing to three-way stopcock 'C'. Ball and socket joint 'F' served as a means of disconnecting the cylinder for replenishing purposes. Gas burette 'D' graduated in 0.1 ml with 100 ml capacity was connected with the three-way stopcock 'C' at one end and leveling bulb 'E' at the other end by means of flexible 'Tygon' tubing. Flexible tubings of rubber or plastic were unsatisfactory in that they were easily penetrated by gaseous methyl bromide. Additional pyrex tubing connected the three-way stopcock 'C' with the ball part of ball and socket joint 'F'. In order to maintain the rigidity necessary for this dispensing apparatus it was mounted securely on an upright piece of plywood. All stopcocks and ball and socket joints were sealed by Dow-Corning silicone stopcock grease.

Fumigation Chambers

The fumigation chambers consisted of 250 mm size vacuum desiccators of pyrex glass. These are similar to those described under catalog number 8-631 in the Fisher Scientific Company catalog number 59. These desiccators had a total volume of 11,075 ml. Attached to the desiccator was a three-way stopcock 'H' and the pyrex ball and

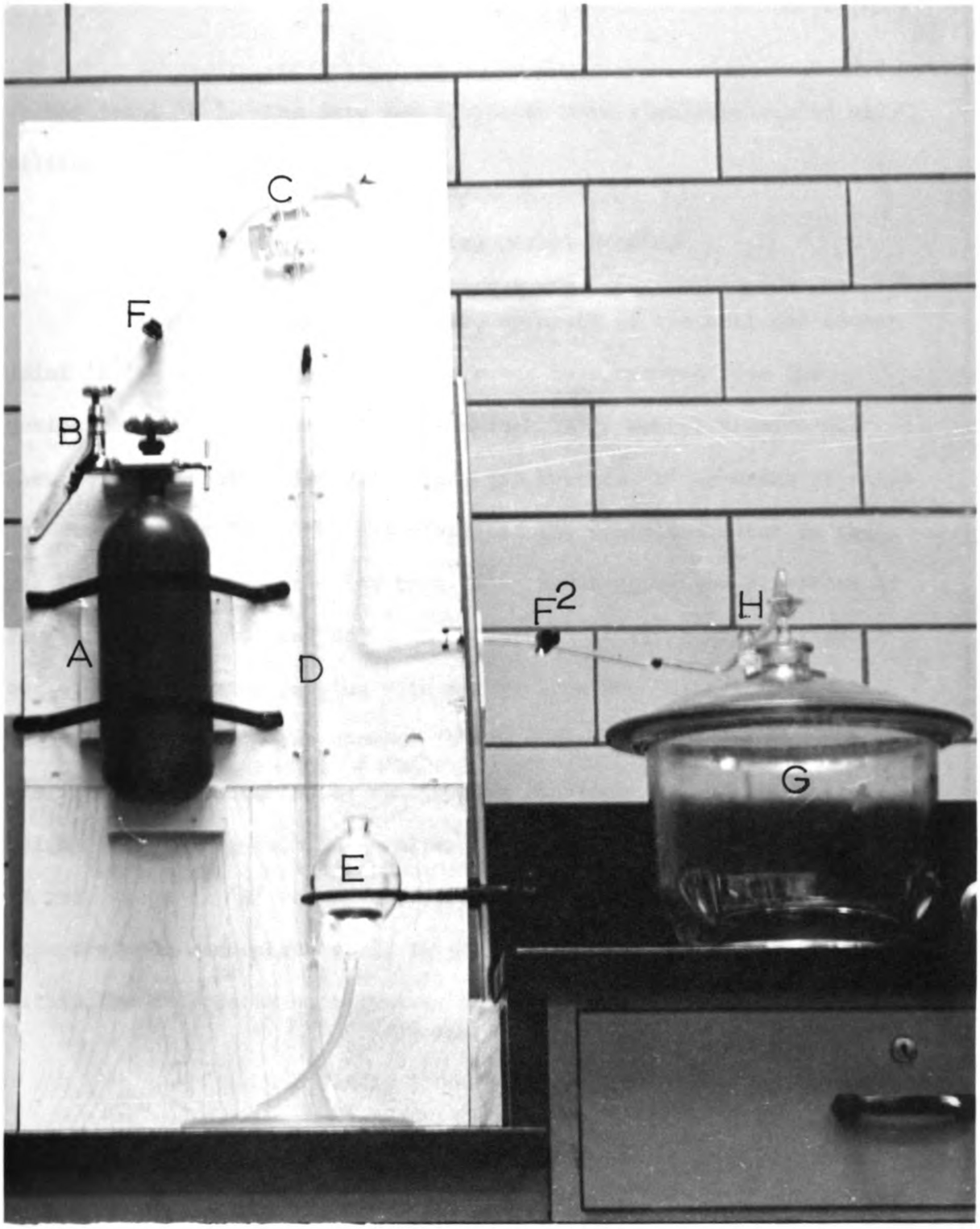


FIGURE 2

THE FUMIGATION APPARATUS

socket joint 'F^2'. The jars and stopcocks were similarly sealed with silicone stopcock grease.

Method of Administering Methyl Bromide

The desiccator was attached by means of the ball and socket joint 'F^2'. A slight quantity of air was then removed from the desiccator through the three-way stopcock 'H'. Methyl bromide was then admitted from cylinder 'A' into gas burette 'D' by means of valve 'B' and stopcock 'C'. The gas displaced the distilled water in the gas burette into the leveling bulb 'E'. By changing the direction of flow at stopcock 'C' and adjusting stopcock 'H' air could be flushed out of the system by purging with methyl bromide. Methyl bromide could be admitted to the chamber 'G' in a measured amount of pure gas from the burette 'D' by readjusting stopcock 'H' and changing the height of leveling bulb 'E'. After admission of the desired amount of gas, stopcock 'H' was then adjusted in such a position that air from the room atmosphere could be admitted to equilibrate pressure within the desiccator with that of the external atmosphere.

Dosage Calculation

Referring to Table 1 the density of methyl bromide vapor is listed as 3.974 g/l at 20 C and at an atmospheric pressure of 760 mm of mercury. From the Handbook of Chemistry and Physics, 35th edition the following information was obtained: 1 lb Avoirdupois = 453.5924 g and 1 ft^3 = 28316 ml. Using appropriate mathematical calculations,

0.0040309 ml of gaseous methyl bromide will give a concentration in 1 ml of fumigated volume equal to 1 lb of methyl bromide per 1000 ft^3. Dosage was calculated for all this work using this formula:

Dosage in pounds per 1000 ft^3 X volume in ml of fumigation chamber X 0.0040309 = ml of gaseous methyl bromide

If dosage was expressed as oz/1000 ft^3, it would be analogous to mg/l.

Salmonella pullorum

The culture of Salmonella pullorum which had just recently been isolated was obtained in the Michigan State University poultry diagnostic laboratory.

After receiving the culture, several nutrient agar (No. B1 Difco*) slants were inoculated and after 24 hours of incubation these were refrigerated and used as stock cultures.

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*Difco Manual, 9th eidtion: Difco Laboratories, Inc., Detroit 1, Michigan, 1953.

EXPERIMENTAL PROCEDURES AND RESULTS

Effects of Methyl Bromide on Bacteriological Media - Experiment I

Sterile nutrient agar and MacConkey agar (No. B 75 Difco) were placed in 20 ml amounts into sterile pyrex Petri dishes 100 x 15 mm. Also prepared were the same media but dispensed without autoclaving into unsterile Petri dishes. One plate of each medium and of each preparation (sterile and non-sterile) was then fumigated. Replicate unfumigated plates were used as controls.

The fumigated plates were exposed for 48 hours at a concentration equal to 11 lb of methyl bromide per 1000 ft^3 of fumigated area. The control plates were held under identical conditions minus the exposure to methyl bromide. After fumigation the plates were aired in room atmosphere for four hours. At this time the sterile, fumigated and control plates were streaked with S. pullorum obtained from the stock culture using an inoculating loop. The inoculation was heavy and was done so as to give good coverage of the plate. Figure 6 illustrates the general procedure of plate streaking. All the plates were then incubated for 48 hours at 37 C and the results recorded.

After fumigation, the autoclaved and non-autoclaved agar plates were identical in appearance to the unfumigated, autoclaved plates. The non-fumigated non-autoclaved agar plates showed considerable growth of many different bacteria and fungi. Figure 3

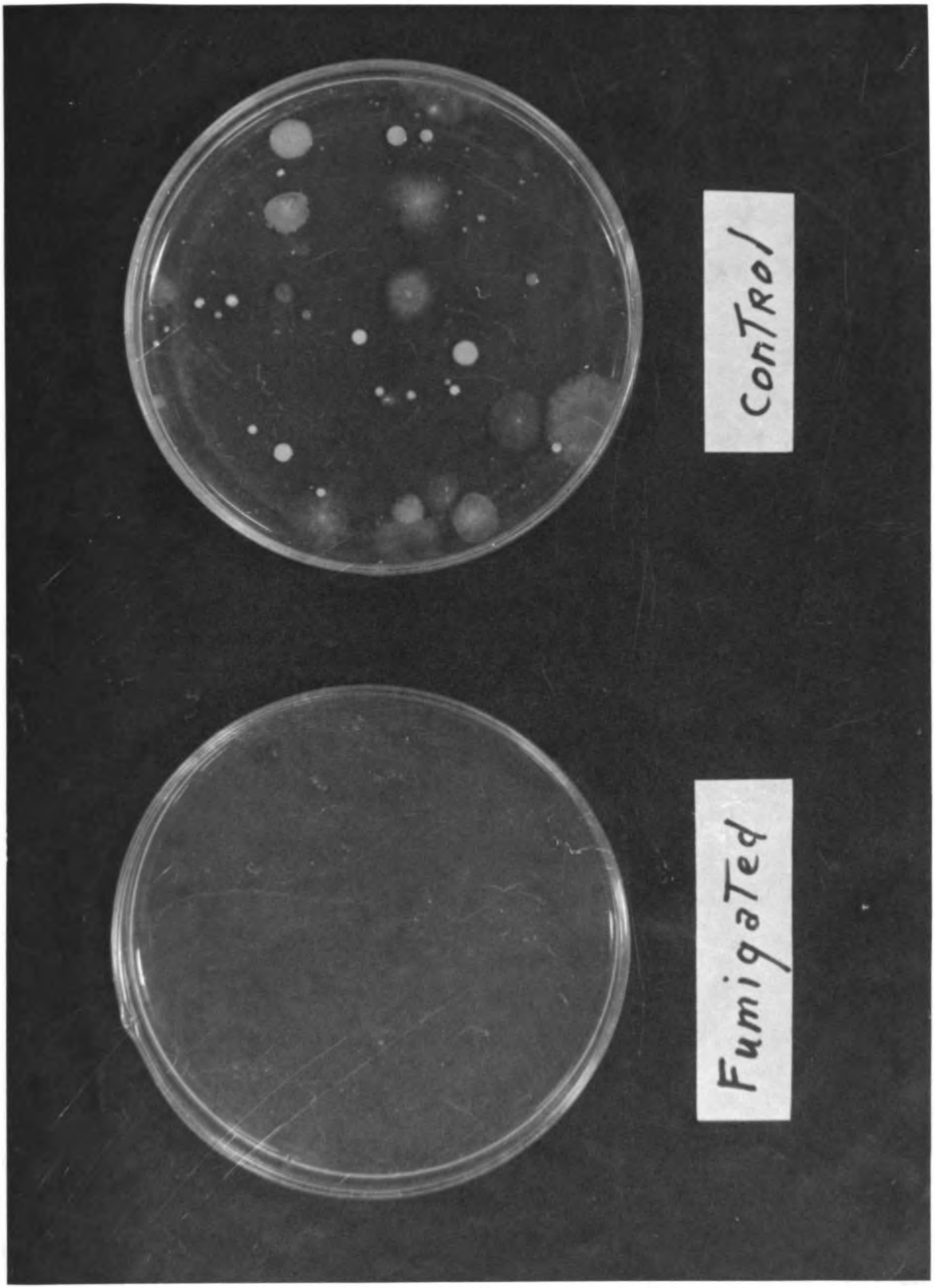


FIGURE 3

NON-AUTOCLAVED NUTRIENT AGAR PLATES FUMIGATED AND NOT FUMIGATED WITH METHYL BROMIDE AT 11 LB/1000 FT^3 FOR 48 HOURS

illustrates the growth present on the non-autoclaved, non-fumigated plate and the absence of growth on the fumigated, non-autoclaved plate.

The growths of S. pullorum on the fumigated and on the autoclaved non-fumigated plates after incubation were essentially identical in amount and appearance.

Adequacy of Counting Techniques - Experiment II

An experimental procedure was developed whereby a known number of organisms could be exposed to methyl bromide for a period of time. After exposure the number of survivals of this population could be determined and the percent of survival calculated. For this work bacteria absorbed onto filter paper strips of a standard grade and size were used. This method offered uniform exposure of the organisms to the gas and provided means of experimentally varying the moisture present in the surroundings of the organisms.

The filter paper strips were sterilized by autoclaving within Petri dishes. After autoclaving the dishes and strips were dried overnight in a 120 C oven. A culture tube of a 24 hour nutrient broth (No. B 3 Difco) culture of S. pullorum was aseptically poured into the Petri dish over the filter paper strips. After allowing five minutes for adsorption, each strip was removed aseptically with sterile forceps to a sterile Petri dish.

To obtain the experimental variation present, the organisms on twelve strips were counted. Each strip was aseptically removed from its Petri dish and placed into a water dilution blank containing

100 ml of saline (0.85 percent NaCl). The bacteria were washed into suspension by vigorous shaking of the bottle and plated.

Table 4 gives the counts obtained from each filter paper strip. The highest count obtained was 8.4 million and the lowest, 3.1 million. The mean was 5.9 million with the variation in count being 44 percent.

Dosage and Time - Experiment III

Using streaked nutrient agar plates, a series of trials was carried out to obtain the time and dosage relationship necessary for complete kill of S. pullorum. The procedure was essentially the same as outlined in Experiment I. Sterile nutrient agar plates were heavily streaked with S. pullorum from the stock culture. The streaking was done with an inoculating loop and in such a manner as to assure heavy growth over the total plate. Duplicate plates were made with one being an unexposed control and one being fumigated.

The plate for fumigation was placed into the desiccator jar which had 100 ml of distilled water in the bottom to maintain proper humidity. The covers of the Petri dishes were not removed. The gas was then introduced according to prior stated procedures. The fumigation was carried out at room temperature which averaged 24 C. The control plates were held under identical conditions in another desiccator jar except that no methyl bromide was added. After fumigation the plates were removed from the jars and incubated for 48 hours at 37 C and the results recorded.

Table 5 and figure 4 constitute a synopsis of the results of 42 trials. The concentration of methyl bromide in lb per 1000 ft^3 is

TABLE 4

VARIATIONS IN NUMBERS OF *S. PULLORUM* UPON STANDARDIZED FILTER PAPER STRIPS

5,500,000	3,100,000
5,400,000	4,900,000
4,500,000	8,400,000
6,100,000	3,800,000
6,200,000	7,700,000
7,800,000	5,800,000

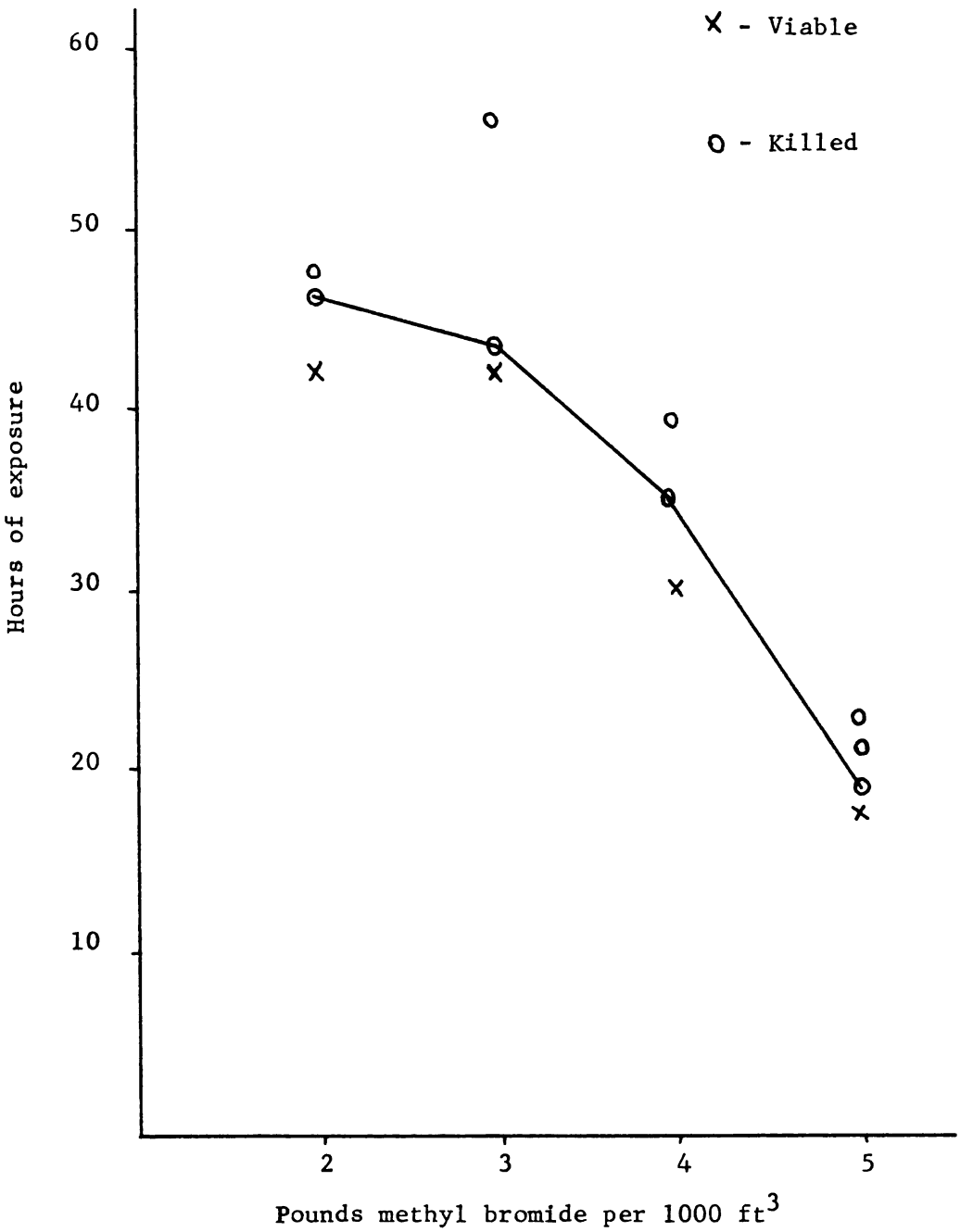
TABLE 5

HOURS REQUIRED TO KILL S. PULLORUM WITH METHYL BROMIDE AT 24 C AND 100 PERCENT RELATIVE HUMIDITY

Dosage Methyl Bromide per 1000 ft^3	Exposure for Complete Kill
2 lb	42 to 47 hours
3	42 to 44
4	30 to 35
5	18 to 23

FIGURE 4

DOSAGE OF METHYL BROMIDE AND EXPOSURE TIME REQUIRED TO KILL S. PULLORUM AT ROOM TEMPERATURE (24 C)



listed in the first column. In the right-hand column are listed the shortest and longest exposure periods in hours found necessary to kill S. pullorum at the respective dosages in a series of several trials.

With 2 lb of methyl bromide per 1000 ft^3 it required from 42 to 47 hours to completely kill S. pullorum. At 3 lb/1000 ft^3, 42 to 44 hours were necessary, whereas at a 4 lb dose 30 to 35 hours proved sufficient. Eighteen to 23 hours of exposure were required to kill S. pullorum at a concentration of methyl bromide equal to 5 lb/1000 ft^3.

Temperature Effects - Experiment IV

Using the same methods as outlined in experiment III the effect of temperature was studied on the activity of methyl bromide in killing S. pullorum. To vary the temperature of fumigation the jars were placed in a walk-in refrigerator having an average temperature of 5 C and in an incubator with an average temperature of 32 C. In all cases the fumigation chambers were allowed to reach temperature equilibrium prior to administering the gas. The plates were removed from the desiccator jars at the end of the exposure period and incubated for 48 hours and the results read. The control plates were maintained under similar conditions except for the exposure to methyl bromide.

Temperature caused a major change in the rate of killing of S. pullorum by methyl bromide. Table 6 is a listing of the results

TABLE 6

EFFECTS OF VARYING TEMPERATURE ON THE DEATH OF *S. PULLORUM* AT 5 LB/1000 FT^3

Temperature	Exposure for Complete Kill
5 C	50 to 111 hours
24	18 to 23
32	10 to 11

of this experiment compiled from data obtained in eight trials using 5 lb of methyl bromide per 1000 ft^3. The variation listed in hours was the shortest and longest period of time needed for killing of S. pullorum in the various trials at that temperature. At 5 C the lethal end point varied from 50 to 111 hours. Ten to eleven hours were necessary for killing at 32 C. Room temperature (24 C) data were obtained from experiment III. At 24 C the time required varied from 18 to 23 hours.

Inactivation Rate - Experiment V

Using filter paper strips impregnated with a broth culture of S. pullorum the death rate was studied. This method using strips of filter paper was essentially the same as outlined in experiment II. The Petri dishes containing the inoculated strips were fumigated for varying time intervals. Bacterial counts were then made as was done in experiment II. These fumigations were carried out at room temperature with each desiccator jar containing 100 ml of distilled water. Methyl bromide was administered at the rate of 5 lb/1000 ft^3. The results are reported as percent kill with unexposed strips serving as controls. Thirty-four trials were carried out at the following time intervals: 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 15, 18, and 21 hours of exposure to methyl bromide.

The average percent survival of S. pullorum after exposure to methyl bromide at 5 lb/1000 ft^3 for varying time intervals is listed in Table 7. The percent survival figures used are the average

TABLE 7

PERCENT SURVIVAL OF *SALMONELLA PULLORUM* AFTER EXPOSURE TO METHYL BROMIDE FOR VARYING PERIODS OF TIME IN THE PRESENCE OF A WATER SATURATURED ATMOSPHERE

Exposure period to 5 lb/1000 ft^3	Average survival
1 hour	74.2%
2	49.7
3	45.0
4	42.3
5	33.3
6	41.8
7	1.40
8	8.92
9	.165
12	.003
15	.002
18	.001
21	.000
24	.000

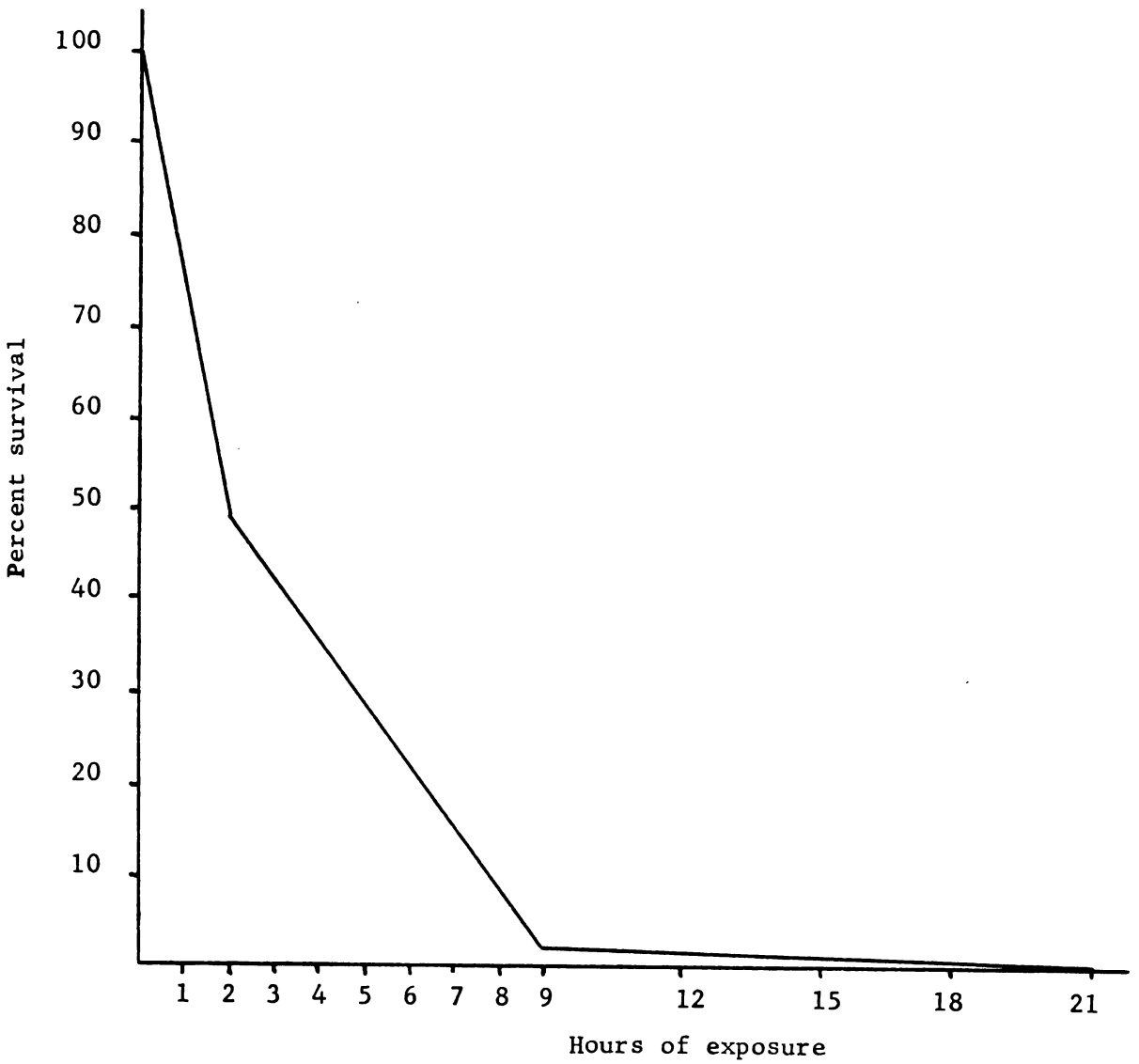
of all the data collected. Figure 5 is a graphic representation of these results.

At one hour of exposure approximately 25 percent kill occurred with an additional hour of exposure only about 50 percent of the organisms were viable. At the 3, 4, 5, and 6 hour intervals the percent survival did not decrease appreciably. During the next three hours of exposure, S. pullorum was killed at a rapid rate. By the end of the ninth hour of exposure less than one percent survived.

Twelve additional hours of exposure were necessary however to kill the remaining few viable organisms. Figure 6 illustrates results obtained when MacConkey agar plates streaked with S. pullorum were fumigated with a concentration of methyl bromide equal to 3 lb/1000 ft^3 for varying time intervals. These plates were fumigated as a part of experiment III but are presented here to illustrate the rate of death as seen visually be examination of the agar plates. The unfumigated control plate in the center illustrates the heavy streaking of each plate with S. pullorum. After 24 hours of exposure to methyl bromide a clearly distinguishable reduction in numbers of the viable bacteria occurred. At thirty hours a very small percentage of organisms remained viable compared with the hundreds present in the original inoculum. Only one organism survived the exposure to methyl bromide for 36 hours. The plate showed no growth being present after 42 hours of fumigation.

FIGURE 5

PERCENT SURVIVAL OF S. PULLORUM AFTER EXPOSURE TO METHYL BROMIDE AT 5 LB/1000 FT^3 AT 24 C



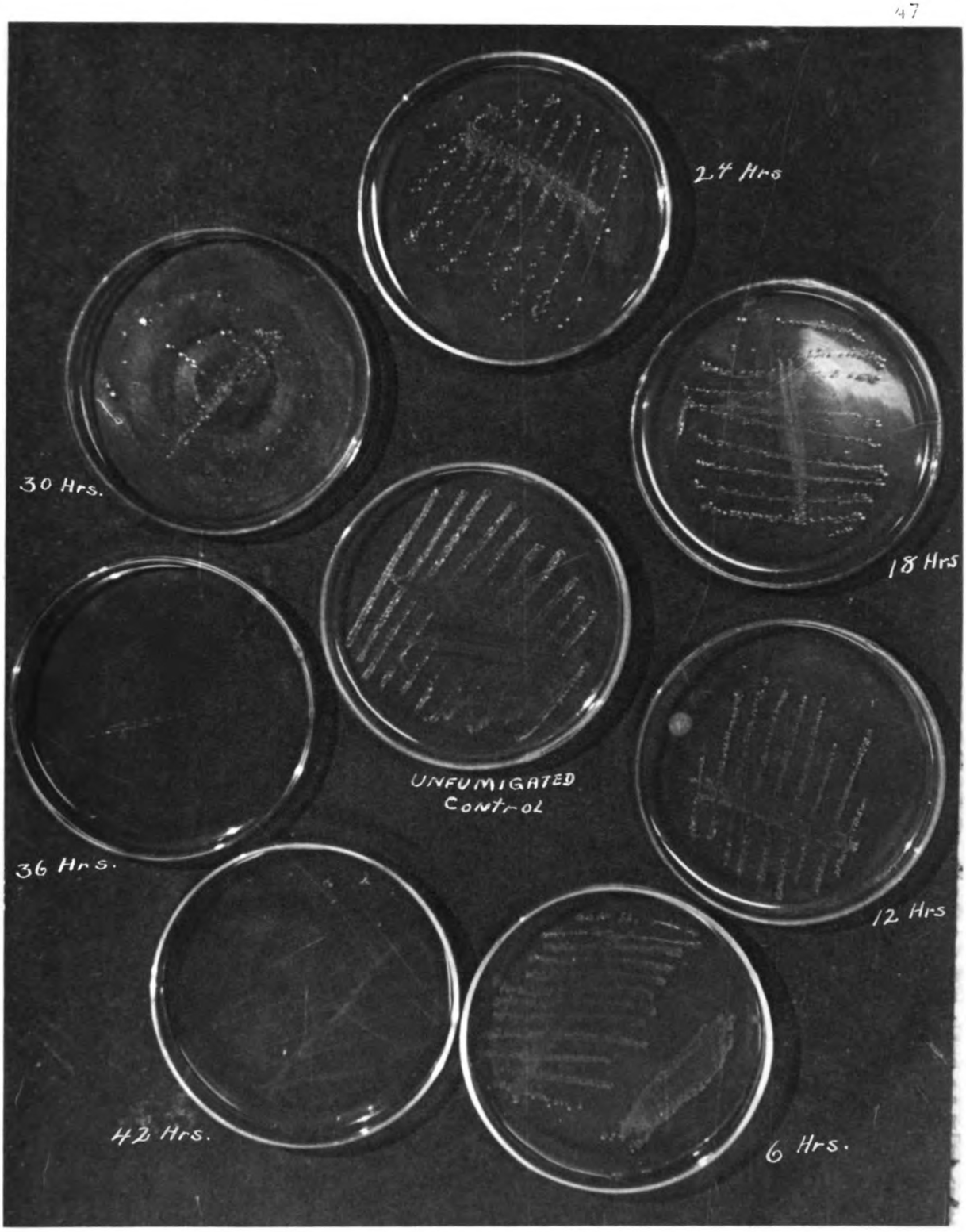


FIGURE 6

MAC CONKEY AGAR PLATES STREAKED WITH S. PULLORUM FUMIGATED WITH 3 LB/1000 FT^3 OF METHYL BROMIDE FOR VARYING TIME INTERVALS

Humidity Effects - Experiment VI

Again using the filter paper strip method, the effect of atmospheric moisture was studied as to its influence on the power of methyl bromide to kill S. pullorum. Difficulties were encountered in maintaining viable controls at low humidities with S. pullorum. Therefore Staphylococcus aureus was used in conjunction with S. pullorum in this study as it was more resistant to desiccation. Trials were made in duplicate using a dose of 5 lb of methyl bromide per 1000 ft^3 for a 24 hour exposure period. Humidity was controlled by using distilled water and calcium chloride. For a dry atmosphere pure calcium chloride was used in the bottom of the desiccator jar. A saturated atmosphere was obtained using 100 ml of distilled water with the 50 percent relative humidity level obtained by a mixture of 35 percent calcium chloride and 65 percent water on a weight basis. Results are recorded as the number of organisms present upon a nutrient agar plate inoculated with 1 ml of the saline used to wash the filter paper strip.

Table 8 is a summary of 24 trials in which S. pullorum and Staph. aureus were exposed to a concentration of methyl bromide of 5 lb/1000 ft^3 for 24 hours at 0, 50, and 100 percent relative humidity. At 100 percent relative humidity the results were the same as those in previous experiments. All the fumigated bacteria were killed. The unfumigated controls at fifty percent relative humidity and in a dry atmosphere had difficulty surviving. No fumigated organisms survived the exposure regardless of the atmospheric moisture.

TABLE 8

EFFECTS OF DIFFERENT HUMIDITY LEVELS ON THE ABILITY OF METHYL BROMIDE AT 5 LB/1000 FT^3 TO INACTIVATE *S. PULLORUM* AND *STAPH. AUREUS*

Surviving Organisms

Relative Humidity	*S. pullorum*		*Staph. aureus*	
	Fumigated	Control	Fumigated	Control
100%	0	T.N.T.C.*	0	10,000
50	0	10	0	1,000
0	0	3	0	1

* Too numerous to count

Incubator Studies - Experiment VII

To learn of the possible use of methyl bromide to control S. pullorum contamination in chick incubators, embryonated eggs were fumigated. Twenty eggs each of 5, 9, 13, 17 and 18 day-old embryos were fumigated in the desiccator fumigation chambers. Table 9 gives the dosage and time of fumigation used. Controls were placed in desiccators for the same length of time but without methyl bromide.

All fumigated embryos were killed and the control, unfumigated embryos involving a long period of exposure to the limited atmosphere of a desiccator jar also died. The control embryos kept in the jars for six hours or less remained viable.

TABLE 9

FUMIGATION OF EMBRYONATED EGGS WITH METHYL BROMIDE IN LARGE DESICCATOR JARS

Age of Embryos	Fumigation		Survival	
	Period	Dose	Fumigated	Controls
Days	Hours	Lb/1000 ft^3	Percent	Percent
5	45	3	0	0
9	45	3	0	0
13	17	3	0	0
17	6	5	0	95
18	3	5	0	100

DISCUSSION

The results obtained in experiment I show that under the experimental conditions methyl bromide was bactericidal for microorganisms contaminating nutrient agar and MacConkey agar media poured into unsterile plates. As evidenced by growth of S. pullorum, no loss of essential nutrients resulted nor did a residual toxicity remain on the fumigated plates. These results agree with the work mentioned in the description of the experiments in which ethylene oxide was used to sterilize bacteriological and mycological media. The results do not adequately evaluate methyl bromide as an agent to sterilize bacteriological media. This evaluation should be made using an organism of fastidious nutritional requirements and different media. Evidence to support this need is found in the work of Sykes (1958) who found that ethylene oxide fumigated serum was inferior to unfumigated serum in the culture of certain fastidious bacteria.

Experiments III and IV demonstrated that methyl bromide did not adversely affect the capacity of the media to support growth of S. pullorum. Agar plates inoculated with S. pullorum, when exposed to sublethal dosages of methyl bromide, showed no visible growth at the end of the fumigation period. The control plates, however, showed good growth during the fumigation period prior to the actual incubation of the plate. This demonstrates that methyl bromide is bacteriostatic in sublethal dosages. Two pounds of methyl bromide per 1000 ft^3 was the lowest dosage used and it proved bacteriostatic.

The data presented by Trickel (1952), Mac Lachlen *et al.* (1953), Phillips (1957 and 1959) and McKeen (1954) on the minimal lethal dose of methyl bromide for microbial life agrees closely with the results reported here. Their experiments had been carefully controlled with adequate precautions taken to prevent the methyl bromide from escaping from the fumigation chamber. These workers also used lengthy exposure periods. Other workers, who have reported that methyl bromide is only effective in extremely high dosages, have either failed to maintain adequate methyl bromide concentration for a period of several hours or the temperature was sub-optimal.

Experiment II showed that the paper strip method had the disadvantage of considerable variance in the counts. This method was used, however, in lieu of any other because of its advantage in that the bacteria were exposed to the varying conditions of the atmosphere. In this humidity experiment, two lethal factors were involved, methyl bromide and environment, probably desiccation.

Results of experiment VI in which atmospheric moisture has little or no effect on the activity of methyl bromide to inactivate *S. pullorum* is in agreement with the work of several other workers. They found that in soil fumigation excess moisture interfered with the activity of methyl bromide to penetrate the soil. None of these workers reported that moisture was necessary for the effectiveness of methyl bromide. The interference with the diffusability of methyl bromide by water is probably a result of its slight solubility in

water. The moisture present in the agar that was sterilized by methyl bromide did not seem to interfere appreciably. Trickel (1952) did not find that moisture was necessary for the inactivation of micro-organisms. Kolb and Schneiter (1950) also presented substantiating evidence in that they found that methyl bromide killed excessively dehydrated spores of Bacillus anthracis. However, in another trial, unless moisture was added to the fumigation chamber the organism was able to survive. Saiki (1952) was able to inactivate members of the Enterobacteriaceae on wet or dry gauze. Edgar and King (1955), as well as Schmittle (1955), did not find that supplementary moisture was necessary.

Kaye and Phillips using ethylene oxide found that it was not as active in excessively high concentrations of moisture nor on organisms that had been lyophilized. Their theory as to the role of moisture in the alkylation reaction is elaborated upon in the review of literature. If these theories can be applied to the action of methyl bromide then the effect of ethylene oxide being readily soluble in water should be considered. Methyl bromide is only slightly soluble in water, therefore, excessive moisture might be detrimental to its action. The difference in the solubilities of methyl bromide and ethylene oxide may be the factor that renders ethylene oxide a more rapid acting bactericidal agent. Because of the lower concentration of methyl bromide ions in a solution, a longer time interval would be necessary for these ions to seek out and react with the labile groups of the organisms.

Methyl bromide fumigation of chicken embryos is not to be recommended, the embryos being more susceptible to this gas than is S. pullorum.

In experiment V the reaction rate of methyl bromide and S. pullorum as evidenced by bacterial mortality, is comparable to that ascribed to most bactericidal chemicals. Of significance here is that 99 percent of S. pullorum are killed in less than 9 hours, however, the remaining one percent of the bacterial population requires an additional 12 exposure hours to enact complete kill. The practical application of this fact is that field disinfection would be considered effective in disease prevention with a 99 percent kill of contaminate pathogens. Combining this fact with the adverse environmental factors acting upon S. pullorum, such as desiccation, adequate field disinfection using methyl bromide should be easily accomplished.

In all conditions where microorganisms or other forms of life were subjected to methyl bromide, death occurred if the gas concentration and exposure period was adequate. Relative to its use by the poultry industry, methyl bromide destroys all parasites even the most resistant, the intermediate hosts of parasites, bacteria, viruses, insects and rodents. The combination of this wide killing spectrum of methyl bromide with its high activity in the presence of organic matter and penetrability make it an ideal disinfectant for use by the poultry industry.

Methyl bromide has the disadvantage of requiring a tight almost hermetically sealed space to contain the gas during fumigation. The gas is less active at lower temperature so that fumigation of exposed areas in the northern states during winter will be limited. The gas is rather costly for large scale fumigations if used at 5 lb/1000 ft^3, the cost being approximately 85 cents to one dollar per pound. Methyl bromide is toxic to man and animals but its popularity and widespread use by other agricultural industries demonstrates that if a few basic precautions are followed this factor is not a serious disadvantage. The toxicity for humans is actually of a much lower order than many other common insecticidal fumigants and sprays in use.

The general non-reactiveness of methyl bromide is a big advantage. This gives a great deal of safety to the fumigation process, thus accidents due to explosion are eliminated. Also methyl bromide does not harm most articles ordinarly fumigated.

The explosiveness of ethylene oxide necessitates mixing it with carbon dioxide which results in increased expense. This mixture then approximates methyl bromide in activity, methyl bromide being 1/10 as active as ethylene oxide.

Ethylene oxide has a slightly higher boiling point, therefore, it is not as active at low temperatures as is methyl bromide. The penetrative activity and general effectiveness of ethylene oxide seems to equal that of methyl bromide. Although ethylene oxide has been shown to affect the nutrition of certain animals fed an ethylene oxide fumigated diet, this fact has not been stated as being true of methyl bromide

(see appendix on toxicity).

Beta-propiolactone has the same basic disadvantage as formaldehyde. It has little penetrating power and needs heat and moisture. Both products require a special procedure for vaporization of the liquid. Beta-propiolactone is also toxic to humans but less than methyl bromide. The advantage beta-propiolactone offers over formaldehyde is its rapid action with resulting easy removal of the gas after fumigation. Neither formaldehyde nor beta-propiolactone are effective disinfectants because they are active only on organisms that are directly exposed. In application on poultry houses, chick boxes, litter, poultry yards, and poultry equipment disinfection by beta-propiolactone and formaldehyde seem unlikely.

SUMMARY AND CONCLUSIONS

Eleven pounds of methyl bromide per 1000 ft^3 sterilized nutrient agar and MacConkey agar plates as evidenced by absence of growth of microbes after 48 hours of incubation. Methyl bromide fumigation did not impart to the media any residual toxicity which inhibited the growth of Salmonella pullorum.

Salmonella pullorum upon agar plates, at 24 C and 100 percent relative humidity, was killed by methyl bromide at 2, 3, 4, and 5 lb/1000 ft^3 with 47, 44, 35, and 23 hours of exposure respectively. A relative humidity of 0, 50, or 100 percent within the fumigation chambers did not alter the activity of methyl bromide at 5 lb/1000 ft^3 in killing Salmonella pullorum and Staphylococcus aureus.

The inactivation rate of Salmonella pullorum by methyl bromide, when percent survival was plotted against time, gave a curve essentially of an exponential character. Exposure of Salmonella pullorum to 5 lb/1000 ft^3 of methyl bromide at 5, 24, and 32 C necessitated fumigation periods of 111, 23, and 11 hours respectively for complete kill.

Methyl bromide at 5 lb/1000 ft^3 was lethal for 17 and 18 day-old chick embryos upon exposure for six or three hours respectively.

REFERENCES

Andrews, J. S., Taylor, A. L. and Swanson, L. E. 1943 Fumigation of soil with methyl bromide as a means of destroying infective stages and intermediate hosts of some internal parasites of mammals. Proc. Helm. Soc. Wash., 10, 4-6.

Anonymous 1939 N. J. Agr. Exp. Sta., Nursery Disease Notes, 12(2), 5-8.

Anonymous 1940 Methyl bromide and methyl alcohol used to control grubs of Asiatic beetle in azalea beds. Flor. Exch. and Hort. Trade World, 94(5), 20.

Baerwald, F. K. 1945 Food Preservation Method. U. S. Pat. No. 2,370,768.

Bakerman, H., Romine, M., Schricker, J. A., Takahashi, S. M. and Mickelsen, O. 1956 Stability of certain B vitamins exposed to ethylene oxide in the presence of choline chloride. J. Agr. Food Chem., 4, 956-959.

Barlow, J. S. and House, H. L. 1956 Ethylene oxide for sterilizing diets. Sci. 123, 229-231.

Boney, W. A. 1948 The efficacy of methyl bromide as a fumigant for the prevention of cecal coccidiosis in chickens. Am. J. Vet. Res., 9, 210-214.

Chapham, P. A. 1950 On sterilizing land against poultry parasites. J. of Helm., 24, 137-144.

Christie, J. R. 1947 Preliminary tests to determine the nematocidal and fungicidal properties of certain compounds when used as soil fumigants. Proc. Helm. Soc. Wash., 14, 23-28.

Church, B. D., Halvorson, H., Ramsey, D. S. and Hartman, R. S. 1956 Population heterogeneity in the resistance of aerobic spores to ethylene oxide. J. Bact., 72, 242-244.

Cotton, R. T. and Roark, R. C. 1928 Ethylene oxide as a fumigant. Ind. Eng. Chem., 20, 805-807.

Cram, E. B. and Hicks, D. O. 1944 The effects of sludge digestion drying, and supplemental treatment on eggs of Ascaris lumbricoides. Proc. Helm. Soc. Wash., 11, 1-9.

Dixon, M. and Needham, D. M. 1946 Biochemical research on chemical warfare agents. Nature, 158, 432-438.

Edgar, S. A. and King, D. F. 1955 The effectiveness of methyl bromide in sterilizing poultry litter. Poul. Sci., 34, 595-597.

Godfrey, G. H. and Young, P. A. 1943 Soil fumigation for plant disease control. Tex. Agr. Exp. Sta. Bul. 628.

Griffith, C. L. and Hall, L. A. 1938 Sterilizing foodstuffs. U. S. Pat. No. 2,107,697.

Griffith, C. L. and Hall, L. A. 1940a Sterilization process. U. S. Pat. No. 2,189,947.

Griffith, C. L. and Hall, L. A. 1940b Sterilization of pancreatin. U. S. Pat. No. 2,189,948.

Griffith, C. L. and Hall, L. A. 1940c Sterilizing colloid materials. U. S. Pat. No. 2,189,948.

Griffith, C. L. and Hall, L. A. 1943 Sterilization of organic materials and spices with ethylene oxide. U. S. Pat. No. 22,284.

Hall, L. A. 1938 Sterilized spices - new factor in food quality control. Food Ind., 10, 424-426 and 464.

Hansen, H. N. and Snyder, W. C. 1947 Gaseous sterilization of biological materials for use as culture media. Phytopath., 37, 369-371.

Harry, E. G. 1956 The application of aerosols to atmospheric and surface disinfection in the poultry industry, II. Atmospheric disinfection and its value as a means of controlling cross infection. Vet. Res., 68, 334-339.

Harry, E. G. 1957 The use of aerosols in poultry husbandry. Agr. Rev. London, 11(8), 30-36.

Hawk, E. A. and Mickelsen, O. 1955 Nutritional changes in diets exposed to ethylene oxide. Sci., 121, 442-444.

Hawkins, L. A. 1939 The use of methyl bromide for the treatment of quarantined plant products. Bur. Ent. Plant Quar., Cir. E-484.

Hoffman, R. K. and Warshowsky, B. 1958 Beta-propiolactone vapor as a disinfectant. App. Micro., 6, 358.

Johnson, F. J. 1945 The effect of chemical and soil treatments on development of wheat mosaic. Ohio J. Sci., 45, 125-128.

Judge, L. F., Jr. and Pelczar, M. 1955 The sterilization of carbohydrates with liquid ethylene oxide for microbiological fermentation tests. App. Micro., 3, 292-295.

Kaye, S. 1949 The sterilizing action of gaseous ethylene oxide, III. The effect of ethylene oxide and related compounds upon bacterial aerosols. Am. J. Hyg., 50, 289-295.

Kaye, S. and Phillips, C. R. 1949 The sterilizing action of gaseous ethylene oxide, IV. The effect of moisture. Am. J. Hyg., 50, 296-306.

Kirby, G. W., Atkins, L. and Frey, C. N. 1936 Recent progress in "Rope" and mold control. Food Ind., 8, 450-488.

Kolb, R. W. and Schneiter, R. 1950 The germicidal and sporicidal efficacy of methyl bromide for Bacillus anthracis. J. Bact., 59, 401-412.

Kolb, R. W., Schneiter, R., Floyd, S. P. and Byers, D. H. 1952 Disinfective action of methyl bromide, methyl alcohol and hydrogen bromide on anthrax spores. Arch. Int. Hyg. Occ. Med., 5, 354-364.

Latta, R., Richardson, H. H. and Kindler, J. B. 1946 Methyl bromide as a delousing agent. U.S.D.A.Bul. 745, 40 pages.

Lear, B. and Mai, W. F. 1950 Use of methyl bromide for killing golden nematode cysts on used bags and in soil samples. Phytopath., 40, 17.

Lear, B. 1951 Use of methyl bromide and other volatile chemicals for soil fumigation. Cornell U. Agr. Exp. Sta. Memoir 303.

Lear, B. 1952 Disinfecting burlap bags and machinery with methyl bromide to prevent spread of the golden nematode of potatoes. Cornell U. Ext. Bul. 866, 4 pages.

Le Goupils, M. 1932 Les proprites insecticides du bromure de methyle. Revue Path. Veg. et Ent. Ag., 19, 169-172.

Lewis, S. E. 1948 Inhibition of sulfhydryl enzymes by methyl bromide. Nature, 161, 692-693.

Livingstone, E. M. 1940 Fumigation of bulk soil with methyl bromide for the white fringed beetle in New Orleans. Bur. Ent. Plant. Quar., Div. of Control Investigations Multigraphed Cir.

Mac Lachlan, D. S., Monro, H. A. U., Radicot, H. N. and King, J. E. 1953 Fumigation of used bags with toxic gases for control of bacterial ring rot of potatoes. Can. J. Agr. Sci., 33, 132-140.

McKeen, C. D. 1954 Methyl bromide as a soil fumigant for controlling soil-borne pathogens and other organisms in vegetable seedbeds. Can. J. Bot. 32, 101-115.

Mackie, D. B. and Carter, W. B. 1937 Methyl bromide as a fumigant, a preliminary report. Bul. Dept. Agr. Calif., 26, 153-162.

Manns, M. M. and Schmittle, S. C. 1956 Method for sterilizing used textile feed bags. U. S. patent applied for Serial No. 627,557.

Mrak, E. M. 1941 The deterioration of dates. 18th Annual Date Growers Institute Report, Calif., pages 3-4.

Munnecke, D. E. and Ferguson, J. 1953 Methyl bromide for nursery soil fumigation. Phytopath., 43, 375-377.

Munnecke, D. E. and Lindgren, D. L. 1954 Chemical measurement of methyl bromide concentration in relation to kill of fungi and nematodes in nursery soil. Phytopath., 44, 605-606.

Newhall, A. G. 1946 Soil fumigants for plant disease control. Soil Sci., 61, 67-81.

Newhall, A. G. and Lear, B. 1948 Soil fumigation for fungous control with methyl bromide. Phytopath., 38, 38-43.

Newhall, A. G. 1955 Disinfestation of soil by heat, flooding and fumigation. Bot. Rev., 21, 189-250.

Nordgren, G. 1939 Sterilization efficiency of gaseous ethylene oxide. ACTA Path. Micro. Skand. Suppl. 40.

Phillips, C. R. and Kaye, S. 1949 Sterilizing action of gaseous ethylene oxide, I. A review. Am. J. Hyg., 50, 270-279.

Phillips, C. R. 1949 The sterilizing action of gaseous ethylene oxide, II. Sterilization of contaminated objects with ethylene oxide and related compounds. Am. J. Hyg., 50, 280-288.

Phillips, C. R. 1952 Relative resistance of bacterial spores and vegetative bacteria to disinfectants (an abstract). Bact. Rev., 16, 135.

Phillips, C. R. 1957 Gaseous sterilization, Chapter 30. In Antiseptics, Disinfectants, Fungicides, and Sterilization, 2nd edition. Edited by G. F. Reddish. Lea and Febiger, Philadelphia, Pa.

Phillips, C. R. 1958 Gaseous sterilization. Becton, Dickenson Lectures on Sterilization, Seton Hall College of Medicine and Dentistry, Jersey City, N. J.

Phillips, C. R. and Warshowsky, B. 1958 Chemical disinfectants. Ann. Rev. Micro., 12, 525-550.

Phillips, C. R. 1959 Personal communication.

Phillips, G. L. and Easter, S. S. 1943 Methyl bromide fumigation, a review. Bur. Ent. Plant. Quar. Cir. 601.

Phillips, G. L. 1955a Grain fumigation I. Agr. Chem., 10(1), 55-56, 117-121.

Phillips, G. L. 1955b Grain fumigation II. Agr. Chem., 10(2), 41-43, 133, 135.

Richardson, H. H. and Johnson, A. C. 1935 Studies of methyl bromide in greenhouse and vault fumigation. U.S.D.A. Tech. Bul. 853, 1-20.

Saiki, E. 1952 Disinfecting effect of chloropicrin, methyl bromide, hydrocyanic acid, and formalin on bacteria. Nagoya J. Med. Sci., 15, 270-276. From Br. Abs. Med. Sci., 1, 482.

Salle, A. J. and Korzenovsky, M. 1942 Effect of a vacuum on destruction of bacteria by germicides. Proc. Soc. Exp. Biol. Med., 50, 12-16.

Schmittle, S. C. 1955 Studies on Methyl bromide, I. The efficacy of methyl bromide fumigation on Newcastle disease virus. Poul. Sci., 34, 1219.

Schmittle, S. C. 1959 Personal communication.

Schmittle, S. C. and Holderried, J. A. 1959 Personal communication.

Schrader, H. and Bossert, E. 1936 Alkylene oxides for killing pests and germs. U. S. Patent No. 2,037,439.

Stark, F. L. and Lear, B. 1947 Control of nematodes by gaseous sterilization. Phytopath., 37, 698-711.

Stover, R. H. and Koch, L. W. 1952 Methyl bromide as a soil fumigant for controlling disease and weeds in tobacco seed-beds. Sci. Agr., 32, 411-420.

Swank, G., Jr. 1951 A preliminary report on a new compound, trans 1,4-dibromobutene-2 for the control of damping off. Plant Dis. Reptr., 35, 492-493.

Swanson, L. E. and Taylor, A. L. 1943 Control of cattle parasitic and free living nematodes by soil fumigation with methyl bromide. Proc. Helm. Soc. Wash., 10, 1-3.

Sykes, G. 1958 Disinfection and Sterilization. D. Van Nostrand Company, Inc., Princeton, N. J.

Taylor, A. L. and McBeth, C. W. 1940 Preliminary tests of methyl bromide as a nematocide. Proc. Helm. Soc. Wash., 7, 94-96.

Taylor, A. L. and McBeth, C. W. 1941 A practical method of using methyl bromide as a nematocide in the field. Proc. Helm. Soc. Wash., 8, 26-28.

Trickel, R. J. 1952 Mold inhibitors for food products. Ph.D. thesis, Univ. of Wisconsin.

von Oettinger, W. F. 1955 The halogenated aliphatic, olefinic, cylic, aromatic, and aliphatic-aromatic hydrocarbons including the halogenated insecticides, their toxicity and potential dangers. Public Health Serv. Publ. No. 414.

Wensley, R. N. 1953 Microbiological studies on the action of some selected soil fumigants. Can. J. Bot., 31, 277-308.

Whelton, R., Phaff, H. J., Mrak, E. M. and Fisher, C. D. 1946 Control of microbiological food spoilage by fumigation with epoxides. Food Ind., 18, 23-25 and 318-320.

Wilson, A. T. and Bruno, P. 1950 The sterilization of bacteriological media and other fluids with ethylene oxide. J. Exp. Med., 91, 449-458.

Windmueller, H. G., Ackerman, C. J., and Engel, R. W. 1956 Alterations in the nutrition value of casein by exposure to ethylene oxide. J. Nutr., 60, 527-537.

Windmueller, H. G. 1956 Ethylene oxide and alteration of nutritive value of casein. M. S. Thesis, V. P. I., Blacksburg, Va.

Windmueller, H. G. and Engel, R. W. 1956 A preliminary report on ethylene oxide and effects on casein. Fed. Proc., Fed. Am. Soc. Exp. Biol., 15, 386.

Yesair, J. and Cameron, E. J. 1938 Present status of sterilization of spices. The Canner (convention number), 86 (12 p. 2), 108-110.

APPENDIX

The following references are listed as being beyong the main scope of this dissertation. They are however directly pertaining to the topic of gaseous disinfection with methyl bromide. Their inclusion here is so that this thesis could serve as a complete reference.

Toxicity

Brooks, V. J. and Jacobs, M. B. 1958 Poisons, Properties, Chemical Identification, Symptoms and Emergency Treatment. 2nd Ed. D. Van Nostrand Co., Inc., New York.

Dudley, H. C. 1939 Bromide content of fruits and vegetables following fumigation with methyl bromide. Ind. Eng. Chem., Anal. Ed., 11, 259-261.

Dudley, H. C. et al. 1940 Studies on foodstuffs fumigated with methyl bromide. U. S. Public Health Reports, 55, 2251-2275.

Irish, D. D. et al. 1941 Chemical changes of methyl bromide in the animal body in relation to its physiological effects. J. Ind. Hyg., 23, 408-411.

Lewis, S. E. and Eccleston, K. 1946 Residues in wheat flour fumigated with methyl bromide. J. Soc. Chem. Ind., 65, 149-152.

Miller, C. D. 1945 Thiamine content of cereals before and after treatment with carbon bisulfide and methyl bromide to destroy insects. J. Am. Diet. Assoc., 21, 516-517.

Reohm, L. S., Shrader, S. A. and Stenger, V. A. 1942 Bromide residues in cereals fumigated with methyl bromide. Cer. Chem., 19, 235-242.

Reohm, L. S., Stenger, V. A. and Shrader S. A. 1943 The fumigation of dairy products with methyl bromide. J. Dry. Sci., 26, 205-211.

Spencer, H. C. et al. 1944 Response of rats maintained on diets fumigated with methyl bromide. Food Res., 9, 11-18.

von Oettinger, W. F. 1958 Poisoning, A Guide to Clinical Diagnosis and Treatment. 2nd Ed. W. B. Saunders Co., Philadelphia.

Wintsringham, F. P., Hellyer, G. C. and McKay, M. A. 1958 Effects of methyl bromide on phosphorus metabolism in the adult housefly, Musca domestica L. Biochem. J. 69, 640.

Application methods

Anonymous 1939 Methods of use of methyl bromide on plants. Bur. Ent. Plant Quar. Bul. 499.

Anonymous 1953 Methyl bromide bulletin, Michigan Chemical Co., St. Louis, Mich.

Anonymous 1955 Methyl bromide dooms Khapra beetles. Pest Control, 23(4), 22-26.

Hassler, Karl 1956 Some facts about fumigation tarps. Pest Control, 24(2), 25-26.

Latta, R. 1941 Methyl bromide fumigation of imported perishable food products. Bur. Ent. Plant Quar., Cir. E-521.

Parker, M. E. 1948 Food-Plant Sanitation. McGraw-Hill Book Co., Inc. New York.

Phillips, G. L. 1955 Grain fumigation I. Agr. Chem. 10(1), 55-56, 117-121.

Phillips, G. L. 1955 Grain fumigation II. Agr. Chem. 10(2), 41-43, 133, 135.

Steinweden, J. B. 1945 Methyl bromide fumigation. Calif. State Dept. Agr. Bul., 34, 4-14.

Young, T. R., Jr. 1955 Inexpensive heat exchanger for methyl bromide dry wood termite fumigations. Pest Control, 23(3), 45-46.

ANALYSIS

Anonymous 1952 Directions for methyl bromide analysis. Michigan Chemical Co., St. Louis, Mich.

Anonymous 1953 Instruction manual for Gow-Mac gas master. Gow-Mac Instrument Co., 100 Kings Road, Madison, N. J.

Frear, D. E. H. 1942 Chemistry of Insecticides and Fungicides. D. Van Nostrand Co., Inc., New York.

Monro, H. A. W., Buck Land, C. T., and King, J. E. 1953 Preliminary observations on the use of the thermal conductivity method for the measurement of methyl bromide concentrations in ship fumigation. Ann. Rept. Ent. Soc. Ontario, 84, 71-76.

Phillips, G. L. and Bulger, J. W. 1953 Thermal conductivity method for measuring methyl bromide. Bur. Ent. Plant Quar., E-851.

Shrader, S. A., Beshgetoor, A. W. and Stenger, V. A. 1942 Determination of total and inorganic bromide in foods fumigated with methyl bromide. Ind. Eng. Chem., Anal. Ed., 14, 1-4.

Stenger, V. A., Shrader, S. A., and Beshgetoor, A. W. 1939 Analytical methods for methyl bromide. Ind. Eng. Chem., Anal. Ed., 11, 121-124.

General

Allen, M. W. and Raski, D. J. 1950 The effect of soil type on the dispension of soil fumigants. Phytopath., 40, 1043-1053.

Anonymous 1951 Encyclopedia of Chemical Technology. Interscience Encyclopedia, Inc., New York.

Bailey, S. F. and Smith, L. M. 1951 Handbook of Agricultural Pest Control. Industry Publication, Inc., New York.

De Ong, E. R. 1948 Chemistry and Uses Insecticides. Reinhold Publishing Corp., New York.

Metcalf, R. L. 1957 Advances in Pest Control Research. Vol. 1. Interscience Publishers, Inc., New York.

Sampson, R. E. and Ludwig, R. A. 1956 Laboratory studies on the evaluation and activity of anti-fungal fumigants. Can. J. Bot., 34, 37-43.