# Malathion and Fenitrothion in Stored Wheat: Low Temperature Degradation, Distribution, and Quantification of Metabolites

by

© Mohamed Hafez K. Abdel-Kader

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MOHAMED HAFEZ K. ABDEL-KADER

A thesis submitted to the Faculty of Graduate Studies of
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## ABSTRACT

The effect of Canadian prairie temperatures on residue degradation and residue translocation of malathion and fenitrothion in stored wheat has been studied. Methods for the simultaneous FPD/GLC analysis of each insecticide and its metabolites have been developed and were employed to quantify major metabolites found in treated wheat samples.

Malathion and fenitrothion (EC) were applied as water-based emulsions at 0.8 mL/kg to provide a deposit of 8 ppm (AI) on hard red spring wheat. The treated grain was stored in the dark at -35, -20, -5, 5, 10, 20, and 27 C. Very little (< 3%) breakdown of both insecticides occurred on wheat that had been stored at -35 and -20 C for 72 weeks. Insecticide residues decreased generally with increase of temperature and duration of storage. At the end of the storage period, 74, 59, 26, 5, and 4% of the initial deposit of malathion remained on wheat stored at -5, 5, 10, 20, and 27 C, respectively. Corresponding values for fenitrothion were: 82, 65, 44, 10, and 4%. Fenitrothion appeared to be somewhat more persistent on wheat than malathion particularly at low temperatures.

In a second study, water-based emulsions of malathion and fenitrothion were applied at 8 and 12 ppm to wheat of 12.5% moisture content to compare residue distribution in fractions milled from wheat samples stored at -5, 10 and 20 C over a period of 12 months. High levels of insecticide residues were contained in the bran and middlings. Less malathion than fenitrothion was found in the bran after 12 months of storage

of wheat at the three temperatures tested. Comparatively smaller amounts of insecticide residues were found in the flour than in the bran or middlings. Less fenitrothion than malathion was present in the flour. Insecticide residues recovered from milled fractions decreased generally, with increase of storage temperature and the duration of storage period. On whole grain, fenitrothion degraded at a relatively slower rate than malathion particularly at lower storage temperatures. Residues recovered from fractions of wheat stored at 20 C were comparatively lower than residues found at 10 or -5 C.

Simple and rapid methods for the simultaneous quantitative analysis of malathion and fenitrothion and their major metabolites in stored wheat were developed. Analysis of the extract was conducted using FPD/GLC (526 nm) after derivatization with diazomethane or diazoethane. Recoveries of both compounds and their metabolites from fortified wheat were greater than 90%. These developed methods were used to quantitatively determine major metabolites found in wheat treated with malathion or fenitrothion and stored at 20 C for 12 months. Dimethyl phosphorodithioic acid, malathion monoacid, and malathion diacid were the major metabolites of malathion . Major metabolites of fenitrothion were demethyl fenitrothion, 3-methyl-4-nitrophenol, and dimethyl phosphorothioic acid. Confirmation of these metabolites was carried out by chemical derivatization plus FPD/GLC and by thin-layer chromatography.

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For My Wife

DEBBIE

For Her Patience And Encouragement

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## Chapter I

## INTRODUCTION

Millions of tons of cereal are wasted each year through spoilage of various sorts. Yet hunger is widespread, and populations increase ever more rapidly. Protection of food supplies through sound practices is thus a matter of the most vital importance.

Insect pests have demonstrated amazing abilities to overcome man's best effort to control them; storage of grain and grain products has always involved risk of infestation. It is therefore clear that continued research on the control of insect pests encountered during storage of grain and grain products is essential to ensure abundant supplies of high quality food for the expanding population of the world.

Among the numerous insect pests which cause losses and deterioration to stored food grains and cereal products are the rusty grain beetle, Cryptolestes ferrugineus (Stephens) and the red flour beetle, Tribolium castaneum (Herbst). These two species are well known in the Canadian grain industry as major biotic factors responsible for grain heating, spoilage, and grade losses (Sinha, 1971). These pest species can, if left unchecked, cause a substantial loss of food grains during storage.

Although thorough sanitation practices are essential in maintaining quality and abundance of stored grain and grain products, prestorage treatment of grain and granaries with contact insecticides is generally the most effective management method available to reduce insect popula-

tion to tolerable levels. Malathion is the major contact insecticide used on grain and in grain storage facilities because of its high toxicity to several stored-product insects and its low mammalian toxicity (oral $LD_{50}$ rat= 2800 mg/kg body weight) (Martin and Worthing, 1977).

The continued and repeated use of this compound has been accompanied in many areas by the rise in populations of resistant strains of T. castaneum ,e.g., in Nigeria (Hayward, 1962; Parkin et al., 1969), the U.S.A. (Spiers et al., 1967; Zettler, 1974, 1975), Australia (Champ and Campbell-Brown, 1970), and the United Kingdom (Dyte and Blackman, 1970). Resistant stains are now being further spread through international trade (Dyte and Blackman, 1970). There is, thus, a need for alternative insecticides that will control a variety of insects that attack stored grain and grain products. Fenitrothion may be used as a grain protectant and it is a possible alternative to malathion.

## Scope and Objectives of Study

Treatment of stored grain with contact insecticides, in many instances, has been used effectively to confer long-term protection against insect infestation. Persistent residues of contact insecticides on the grain may be useful from the standpoint of insect pest control although increasing consumer demands have necessitated the setting of tolerances for pesticide residues and their metabolites in stored products. Therefore tolerance levels were established by international committees (FAO/WHO, 1967, 1968 a,b, 1969 a,b, 1973 a,b,) and accepted by national regulatory agencies, e.g., Agriculture Canada (Anon., 1977).

Thus, the need for detailed checks on persistence of insecticides and the bioaccumulation of their toxic metabolites in stored products after

treatment and, particularly, prior to consumption cannot be overstressed.

In temperate regions such as the prairie provinces of Canada, infested grain may be treated with an insecticide to control insects prior to being offered for sale. Treated grain may pass into commercial marketing channels or it may be stored under a wide range of environmental conditions. The fact that increased grain moisture in stored grain in general shortens persistence of insecticides residues is well known (Watters, 1959; Strong and Sbur, 1960). However, the influence of widely varying temperatures, such as those experienced in Western Canada, on residue degradation and distribution of malathion and fenitrothion has not been studied.

Following application of malathion or fenitrothion to stored grain, residues may be degraded into various metabolites. Many of these metabolites pose a toxicological hazard to mammals (Kovacicova et al. 1973; Bender and Westman, 1976; Rainsford, 1978; Talcott et al. 1979). It is therefore important to quantify the major metabolites that may be found in stored treated grain. Rapid, sensitive, and reliable methods are therefore required to enable analysis of both insecticides and their major breakdown products in studies of their persistence and fate in stored grain. Since most laboratories rely on gas-liquid chromatography (GLC) for routine pesticide analysis, it is desirable to develop sensitive GLC methods for malathion and fenitrothion and their metabolites.

The objectives of the studies reported herein may be divided into the following four main areas:

1. To study the influence of Canadian prairie temperatures on the rate of malathion and fenitrothion degradation in stored wheat.
2. To study the translocation and residue distribution of both insecticides in grain stored at various temperatures.
3. To develop suitable analytical methodology for the accurate and precise determination of malathion and fenitrothion and their metabolites in wheat.
4. To quantify residue levels of metabolites for both compounds in stored grain.

# Chapter II

# LITERATURE REVIEW

## 2.1 ROLE OF CHEMICALS IN REDUCING STORAGE LOSSES

The use of chemicals to protect stored grain from the attack of insects has increased steadily since about 1890 (Deong, 1948). There was occasional mention of chemical control prior to that date; through the eighteenth century, such early attempts were sporadic. An example of early use of insecticide was the burning of sulfur to check insect infestation of stored products, and as a disinfectant (Deong, 1948).

An important means of minimizing grain losses during storage is the control of insect pests by application of pesticides to the storage premises or fumigation of the grain bulk. Techniques for the protection of stored grain have been developed to a high degree. Grain protectants consist of formulations of chemicals having toxic and repellent action, or both, for grain damaging insects. Generally, they are applied to prevent infestation, but they may be applied to destroy existing infestation (Donald, 1958). Since grain protectants are applied directly to the grain, they must have low toxicity to man and higher animals. The insecticides approved for use on stored grain are limited because of possible toxic effects on vertebrates. The mode of action for many of these compounds such as the organophosphates, and carbamates is the disruption of neural transmission by inhibiting the enzyme (acetylcholinesterase) that returns the synapse between nerve cells to normal polar-

ity after a stimulus has passed through (O'Brien, 1976). Unfortunately, the nervous systems of other organisms function in much the same way making it difficult to find selective insecticides (O'Brien, 1976).

Malathion, because of its effectiveness against stored grain insects and its low toxicity to mammals, has been approved for use on grain. Canada, U.S.A., Brazil, France, Italy, and the U.K. are among the countries which allow residues of malathion in grain up to a limit of 8 ppm (FAO/WHO, 1969 a,b). Kenya has a tolerance level of 12.5 ppm. The Joint FAO/WHO Food standard program, Codex Alimentarius Commission, has recommended an international tolerance of 8 ppm malathion in stored grain (FAO/WHO, 1973 a,b).

## 2.2 RESISTANCE OF STORED-PRODUCT INSECTS TO MALATHION

Although malathion appears to be well suited for use as a grain protectant in most situations, there is evidence that some strains of insect pests are developing resistance to it. The problem of malathion resistance in stored product pests is complicated by the fact that it is being spread from country to country in ships through international trade. Even countries which have not yet taken to large scale and extensive use of insecticides have to contend with malathion resistant strains of insect (Pasalu et al. 1974). Malathion resistant strains of red flour beetle, T. castaneum, have been reported in numerous locations around the world (Spiers and Zettler, 1969; Champ and Campbell, 1970 a,b; Dyte and Blackman, 1970, 1972; Champ and Girish, 1974, Pietere and Schuten, 1974; Zettler, 1975; Champ and Dyte, 1976). Dyte and Blackman (1965) found a strain of T. castaneum from Nigeria with resistance as

high as 200-fold to malathion after selection pressure. Parkin and Forster (1962) reported T. castaneum, collected from stored peanuts in Nigeria had resistance as high as 52-fold to malathion. Bhatia et al. (1971) compared two strains of T. castaneum for resistance to malathion. One strain had been maintained in the laboratory without exposure to insecticides. The other was collected from rice bags in a storage warehouse that had received repeated surface treatments with malathion. Results indicated a definite resistance to malathion of the beetles collected from the warehouse. Champ and Dyte (1976) reported that strains resistant to insecticides are now known in 1 mite, 5 moths, and 11 beetles which attack stored products. At least 10 species have developed resistance to malathion and 5 to pyrethroids. They also indicated that malathion resistance has been encountered in T. castaneum in at least 70 countries and the confused flour beetle, Tribolium confusum (du Val), and the lesser grain borer, Rhyzopertha dominica (F.), in 25 countries.

## 2.3 PROTECTANTS FOR STORED PRODUCTS

The malathion resistance in T. castaneum and other stored product insects is becoming widespread and severe and may lead to control failure if the use of malathion is continued (Dyte, 1970; Zettler, 1975). Consequently, new insecticides as grain protectants are needed. Fenitrothion, O,O-dimethyl-O-(3-methyl-4-nitrophenyl)phosphorothioate, a contact insecticide and stomach poison which exhibits practically no vapor action, is considered a relatively safe, broad-spectrum insecticide. Half-life in an alkaline solution (pH 12) is 272 min at 30 C and 123 min

at 40 C (Nishizawa, 1976), and emulsifiable concentrates are stable under normal storage conditions.

Fenitrothion has been shown to be more effective than malathion against some stored-product insects. Lemon (1967) found that fenitrothion was more effective than bromophos or malathion when applied topically to ten species of stored-product beetles. He also found that fenitrothion was the most effective of the three compounds as a protectant on wheat against the granary weevil, Sitophilus granarius (L.). In laboratory experiments fenitrothion was more toxic than malathion to T. castaneum on wheat (Strong and Sbur, 1965). Under practical conditions, 2 ppm fenitrothion on wheat or barley provided protection against the saw-toothed grain beetle, Oryzaephilus surinamensis (L.) equal to that of 10 ppm malathion (Green and Tyler, 1966; Tyler and Green, 1968).

Among a group of organophosphorus compounds about equal to or safer than malathion, Strong and Sbur (1968) found fenitrothion had potential for use as a commodity protectant, as a direct spray application, and as a residual surface treatment in tests with adults of stored insect pests. Champ et al. (1969) and Kashi (1972) reported higher effectiveness of fenitrothion over diazinon and malathion against the rice weevil, Sitophilus oryzae (L.), and the lesser grain borer, Rhyzopertha dominica (F.). Dyte and Blackman (1972) found that a malathion-resistant strain of T. castaneum with a 260-fold level of resistance to malathion was cross-resistant to malaoxon (X 19), and fenthoate (X 25) but not to mevinphos nor to five of the organophosphorus insecticides including fenitrothion. They concluded that T. castaneum does not develop cross-resistance to insecticides which lack carboxyesters in the leaving

group. Weaving (1975) reported that a dose of 8 ppm fenitrothion on corn was much more effective than an equal dose of malathion in controlling the maize weevil. LaHue and Dicke (1976, 1977 a,b) reported that fenitrothion was more effective than malathion, and that a dose of 8 ppm fenitrothion gave good protection to wheat, shelled corn, and sorghum for 12 months against rice weevils, lesser grain borers, and confused and red flour beetles. Ardley and Sticka (1977) showed that fenitrothion at one half the application rate of malathion appeared to give equivalent protection against the deterioration of stored wheat caused by attack from stored product insect pests.

In laboratory studies, in which doses of 2.5, 5.0, 7.5, and 10 ppm of fenitrothion and malathion were applied to wheat, Hyari et al. (1977) indicated that after the grain had been stored for 12 months, fenitrothion was consistantly more effective than malathion against S. oruyzae, R. dominica, T. castaneum, and T. confusum. Recently, LaHue and Kadoum (1979) applied fenitrothion and malathion to plywood surfaces to determine their toxicity against the above mentioned insect pests. They found that fenitrothion was more effective than malathion and concluded that fenitrothion is a promising grain protectant.

## 2.4 DEGRADATION OF INSECTICIDES IN STORED GRAIN

Because stored grain may be used at any time for human consumption, the level of pesticide residues must be kept at a minimum for the safety of the consumer. The insecticide is applied either as a dust or as a liquid spray formulation and its subsequent absorption and metabolism are affected by the time and mode of application. The rate of degrada-

tion is influenced by physical and enzymic factors, and by the length of time the residues on the grain are exposed to these conditions.

### 2.4.1 Temperature

Temperature has marked effects on the velocity of enzyme-catalyzed reactions, and, if enzymic activity is plotted as a function of temperature, a curve is obtained (Figure 1) wherein the stimulating and denaturing effect of increased temperature are offset. Since denaturation has a distinctly high temperature coefficient, a rise in temperature results in a progressively smaller increase of activity until the apex of the curve is reached at the optimum temperature. Denaturation predominates beyond this point (Devlin, 1969).

Thus it can be seen that high temperatures in stored grain can have profound effects on the rate of metabolism of insecticides applied directly to the grain or absorbed from treated sacks and storage structures.

Strong and Sbur (1960) showed by bioassay that a decrease in residual effectiveness of malathion applied at 10 ppm in wheat at 10% moisture content (mc) occurred with increase of storage temperature from 10 to 50 C and that temperatures in the region of 50 C so limited the effective life of this insecticide in wheat that the moisture content of the grain could be discounted, although increases in the moisture content also increased the breakdown rate. They showed similar effects, in varying degrees, with Dibrom, DDVP, ronnel, Guthion, and diazinon (Strong and Sbur, 1964). Koivistionen (1961) also noted that the temperature of storage had a marked effect on malathion disappearance, with greater losses occuring at about 20 C than at about 4 C when apples and beans

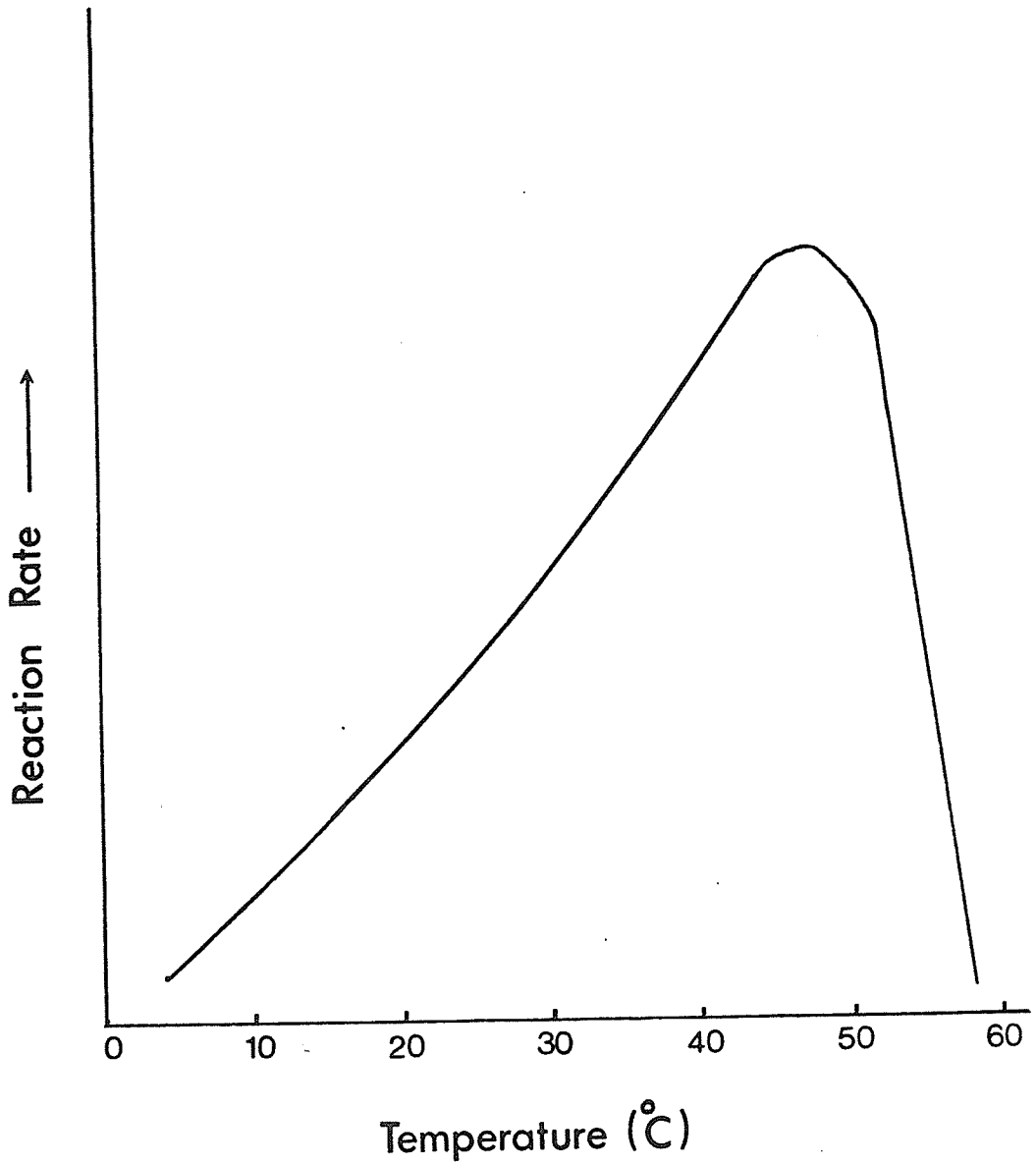



Figure 1: Typical effect of temperature on an enzyme-catalyzed reaction (Devlin, 1969)

were treated with malathion after harvest. Godavari Bai (1964), in his studies on the persistence of malathion on treated food grains under different conditions of storage and processing, found that high temperature results in loss of malathion on the grains and milled materials during storage.

Using data from chemical analysis, Minett et al. (1968) demonstrated temperature and moisture dependence of malathion breakdown on wheat. At increasing storage temperature, degradation of malathion was found to increase, particularly at high moisture levels.

Eichler and Knoll (1974), in studing the degradation of bromophos in stored wheat, observed that the type of formulation and the degree of moisture content of the stored grain have no significant influence on the degradation of the compound when the treated wheat was stored at 15 C. However, by increasing the storage temperature to 26 C, the rate of decomposition was significantly increased.

In laboratory studies, Wilkin et al. (1973) compared the periods of effectiveness of malathion and iodofenphos at 10 ppm on English wheat stored at 17, 30, or 35 C. The results of the experiment over a 20 week period showed that iodofenphos has a longer effective life than malathion against O. surinamensis , even on warm grain. The authors explained that the longer persistence of iodofenphos is associated with much slower rate of breakdown of the compound.

Watters and Mensah (1979) studied the stability of malathion applied to wheat stored for 9 months at 10, 20, and 30 C. Residue analysis showed a gradually increasing rate of breakdown of the insecticide from 10 - 30 C.

### 2.4.2 Moisture content

Metabolic activity in the grain increases rapidly at moisture contents above 14 to 15% which Kretovitch (1945) terms the "critical" level.

The wheat grain when absorbing water, holds part of it in the interstices by capillary force while the rest is more firmly bound as a thin film on all the surfaces. Such absorbed water has low vapor pressure, slightly higher density, and a different dielectric constant from pure water; moreover, it is not free to flow and cannot serve as a solvent and medium in which biochemical changes can occur in the living cell (Rowlands, 1967). The increase in metabolic activity above the critical level is explained by the appearance of free water in the cells, a necessary prerequisite for enzyme activity. Kretovitch (1945) showed that the essential enzymic activity catalyzing oxidation-reduction and hydrolytic reactions in stored wheat increased slowly with increase of moisture content from 11 to 14% and then sharply above 15% and up to 20%.

Watters (1959) found that malathion in wheat lost its insecticidal properties more quickly when the grain moisture content exceeded 13.5%. Malathion applied at 2 ppm to wheat of 13.5% moisture content was more effective than malathion applied at 16 ppm to wheat of 15.5% moisture content against the rusty grain beetle, Cryptolestes ferrugineus . Malathion was ineffective when applied at 16 ppm to wheat at 18% moisture content.

Strong and Sbur (1960) applied malathion to wheat with moisture contents ranging from 10 to 20%. They found a reduction in mortalities of granary weevil, rice weevil, and red flour beetle with an increase in

moisture content. They found that malathion remained effective for a considerable time on wheat with a 12% moisture content, but that the toxicity decreased very rapidly at moisture levels above 14%, which they considered to be the critical level of moisture in stored wheat.

Coulon (1966) carried out tests to study the effect of grain humidity on the control of granary weevils afforded by malathion applied to stored wheat with moisture contents of 13.5, 14, 15.6, 17.1%. Results revealed that the rapidity with which malathion lost its effectiveness during a 13-week period was in direct relation to the moisture content of the grain, and that artificial drying of damp grain improved the persistence of the compound.

Kadoum and LaHue (1969) reported that increase in moisture content caused a rapid breakdown of malathion residues in grain sorghum. Green et al. (1970) also assessed the influence of moisture content of the grain on the persistence of bromophos and malathion by comparing their effectiveness when applied at 10 and 20 ppm to warm moist grain against saw-toothed grain beetles. Applied to wheat of 18.5% moisture content stored at 30 C, bromophos lost effectiveness after six weeks to the same extent as malathion. The high dosage rate did not give longer protection with either compound.

Kadoum and LaHue (1975) determined that malathion degraded rapidly the first 28 days after treatment of wheat at 15% moisture content, and that 37% of the applied malathion (10 ppm) remained at that time.

### 2.4.3 Viability of the grain

Kadoum and LaHue (1972), in a study of the effect of viable and sterilized sorghum grain on malathion degradation during 9 months storage, found that higher residues were retained by sterilized than by live sorghum grain. Rowlands (1964) found higher malathion degradation rates on live wheat than on autoclaved wheat. Tyler et al. (1966) reported that malathion degradation was higher on freshly harvested wheat and barley than that on grain which had been stored for some time after harvest.

Locations and types of enzymes concerned in metabolism of insecticides in stored wheat will be discussed in Section 2.6.1.

### 2.4.4 Baking and processing

Allessandrini (1965) reported 84 to 92% loss of malathion residues when flour is made into bread. He also found that 89 to 90% loss of the insecticide when flour was converted to pasta, and that cooking pasta resulted in degradation of the insecticide beyond the point of detectability.

Acton and Hertel (1966) observed that no malathion residues occurred above the detection limit of 0.3 ppm in bread baked from flour milled from wheat treated with malathion at 26 ppm, but found over 1 ppm malathion in bread baked from whole wheat flour that had been milled from whole wheat treated wiith 8 ppm.

Bengston et al. (1974) determined 95 to 97% loss of fenitrothion residues when flour, milled from wheat treated at 4 ppm, was made into bread. Lockwood et al. (1974) applied fenitrothion at 2 to 16 ppm, and

malathion or tetrachlorvinphos (each at 10 to 80 ppm) to paddy (rough) rice, wheat, and sorghum. Preparation involving boiling or steaming (wet cooking) resulted in complete degradation of residues of all three protectants so far as detectable with methods sensitive to less than 0.1 ppm. Dry cooking methods, used in preparing chapatties from wheat and sorghum, resulted in average losses of 43 to 75% of residues.

## 2.5 PENETRATION OF INSECTICIDES INTO STORED GRAIN

The rate at which insecticides penetrate into stored grain affects their intermediate if not ultimate metabolic fate, and also the persistence of their residues.

Penetration is affected by many factors other than the nature of the insecticide: the moisture content, age, variety, and viability of the grain itself, whether the insecticide is applied as a dust or spray, and whether the spray is a suspension, emulsion, or solution.

### 2.5.1 Measuring penetration

The only reliable methods for determining internal and external distribution of insecticides applied to grain are those dependent on mechanical separation of the grain components before extracting the insecticide, i.e., industrial or lab-scale milling, or alternatively, dissection of the dry grain by hand (Rowlands, 1967).

### 2.5.2 Effect of site of application on penetration

The region of the grain surface that actually receive the insecticidal dose can affect the rate of penetration. When uniform, undamaged grains were treated by topical application of bromophos to the attachment region, brush area, center of the crease (ventral side), or center of the dorsal side (Figure 2), dissection and analysis of the intact bromophos showed that more rapid penetration occured via the attachment region than from the crease area; absorption from back or brush areas was relatively slow (Rowlands, 1966 a).

### 2.5.3 Nature of the insecticide

The electronic properties of the insecticide molecule, particularly as they determine its water-solubility, affect the penetration of insecticide into grain above the critical moisture level. The more water soluble the compound, e.g., dimethoate (3-4%), the more readily it penetrates into the grain (Rowlands,1971). In grain below the critical moisture content, however, the less water soluble compounds, e.g., bromophos (water solubility is 40 ppm) and malathion (145 ppm) still penetrate more slowly than dimethoate, but their respective rates are the reverse of those in damp grain; e.g., bromophos penetrates more rapidly into dry grain than malathion (Rowlands, 1971).

As mentioned previously, the moisture content of the grain affects the persistence of applied insecticide. Generally speaking, the higher the internal moisture content, the slower the penetration of water-insoluble compounds. This is connected with the appearance of free water in grain above the "critical" level (Rowlands, 1967). Penetration rates for

the individual compounds do not appear to vary much inside the two ranges 10 to 14 and 15 to 20% moisture content.

### 2.5.4 Type of formulation

Contact insecticides are usually applied as aqueous suspensions of water dispersible powders, as aqueous sprays of emulsion concentrates, or as dry dusts, either the powdered insecticides or the insecticide diluted with an inert carrier.

Rowlands and Horler (1967) attempted to measure the uptake by grain, at two moisture levels, of malathion applied either as an emulsion spray or as a dry dust. Results indicated more rapid penetration of malathion applied as a spray than when it was applied in dust formulations into the dry grain. This could be due either to the greater affinity of dry grain for the aqueous emulsion or to the fact that non-hydrophilic substances, e.g., the oil deposited from the emusion and, perhaps, the pesticide, penetrate more easily into dry grain (Rowlands and Horler, 1967).

Uptake of the less water-soluble compounds from dust or water-dispersible suspensions is achieved chiefly by the unprotected germ, via the attachment region.

### 2.5.5 Location of residues within the grain kernels

Schesser et al. (1958) milled samples of wheat of unknown temperature and moisture content that had been treated with malathion, methoxychlor, or lindane residues. They reported that the highest residues were in the bran and shorts, with very little insecticide contained in the flour.

Strong et al. (1961) sprayed wheat of 10 and 13% moisture content at 5 ppm with malathion formulated as an emulsion, suspension, or solution, and stored the treated grain for three months at 32 C. Very little residual malathion was detected in the flour: 0.2 ppm from emulsion treatment, 0.1 ppm from the suspension, and 0.01 ppm from the solution.

Roan and Srivastava (1965) stored wheat in open containers after spraying with diazinon emulsion. A stable level of penetration was reached within 45 days. Milling of samples taken after 24 hours revealed 57% of the residue in the bran, 26% in the shorts, and 17% in the flour. Rowlands and Horler (1967) concluded from experiments in which malathion was applied topically in hexane to indiviual grains that, even in grain of moisture content as low as 7%, there is an effective transport system moving the insecticide from one tissue to another. McGaughey (1971, 1972) investigated the amounts of insecticide present in the milled fractions of three varieties of malathion treated rice. He found that treating levels between 10 and 20 ppm caused excessively high residues in the bran, and that these high residues persisted through 7 to 11 months of storage.

Kadoum and LaHue (1972) made a useful study of the penetration of malathion emulsion into sorghum when applied at 10 ppm to both viable and sterilized grains. One day after treatment, 13.8% of the dose was inside (i.e. in the endosperm) the live kernels and 21.4% in the sterile; residues on the outside (i.e. in the bran fraction) of the grain accounted for 86.2% and 78.6% of the dose, respectively. After one month the corresponding values were 20.7% of initial dose in the live and 34.1% in the sterile grains, with recoveries of 51.4 and 48.3% from the outer surfaces.

Kadoum and LaHue (1974) measured the amounts of malathion that penetrated the kernels of corn, sorghum , and wheat stored at 26 C. They found that all three grains showed more residues inside the kernels one month after treatment than they did at 24 hours, 3, and 6 months. They concluded that the type of grain did not affect the movement of malathion residues into the kernel.

Rowlands and Bramhall (1977) studied the insecticide behavior within the grain by a series of experiments at room temperature using $^{14}C$-malathion. They showed that wheat grains absorbed malathion, giving high concentration in the aleurone layer of the seed coat and in the scutellum of the germ (Figure 2). Only very small amounts were found in the starchy endosperm. Similar findings were reported for malathion (Kadoum and LaHue, 1977) and methyl phoxim (Alnaji and Kadoum, 1979) when the treated wheat stored at 26 C for 12 months.

Mensah et al. (1979) determined residue levels of malathion, bromophos, iodofenphos, and primiphos-methyl in fractions from treated wheat stored at 23 C for 6 months. They showed that high levels of residues were present in the bran and middlings. Less malathion than bromophos, iodofenphos, or primiphos-methyl, was found in the bran and middlings after 6 months of storage. Comparatively smaller amounts of insecticide residues were found in the flour (0.3 - 2.2 ppm) than in the bran or middlings (6.9 - 21.3 or 5.9 - 17.0 ppm, respectively).

## 2.6 INSECTICIDE METABOLISM IN STORED GRAIN

Once on or in the grain, organic insecticides are subject to metabolism by a variety of enzymes. Depending on their chemical structures, the compounds may be oxidized, hydrolyzed, decarboxylated, dehalogenated, or dealkylated.

### 2.6.1 Locations of enzymes involved in metabolism

Figure 2 suggests how enzymic reactions in the main area of metabolic activity in the wheat grain may attack insecticide molecules and it seems that compounds which are slow to penetrate the seed coat and reach the germ and endosperm will have greater opportunity to be oxidized than those penetrating rapidly (Rowlands, 1967).

#### 2.6.1.1 Oxidation

The phenoloxidase type enzymes are located in the seed coat of freshly harvested wheat and have been shown to oxidize phosphorothionates to phosphorothiolate and phosphate (P=S to P=O), and substituted (chlorinated) cresols to di- and trihydroxy compounds *in vivo* (Rowlands, 1966 b, 1968, 1970 a). Rowlands (1966) showed also that the oxidative activity in the seed coats was apparent for three to four weeks in grain treated immediately after harvest but that the activity had practically ceased in grain treated more than two to eight weeks after harvest.

Rowlands (1966 b) observed slight phosphorothioate oxidation in the germ tissues of freshly harvested wheat grains. He found that the fat-peroxide systems, using either a wheat germ lipoxidase or commercial catalase or perioxidase, would concurrently oxidize P=S to P=O.

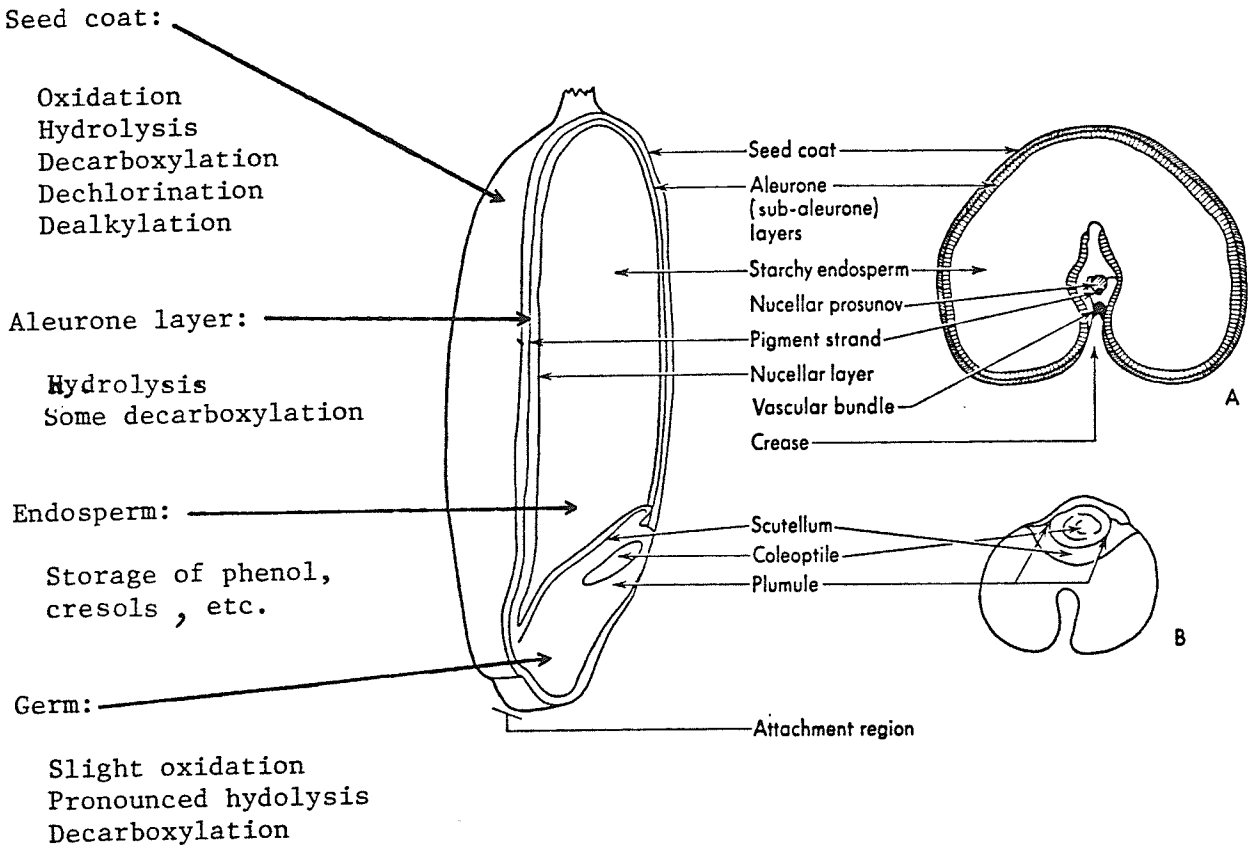



Figure 2: The wheat grain: Sections through grain showing regions of insecticide metabolism A = above crease, and B = at germ (Rowlands, 1971)

### 2.6.1.2 Hydrolysis

Enzymes in stored grain that catalyze the hydrolysis of ester substrates (i.e., esterases) and which may attack insecticidal ester substrates are of two types, the lipases, which catalyze the hydrolysis of simple derivatives of organic carboxylic acids, and the phosphatases, which split phosphoric and thiophosphoric esters. These enzymes have been reported to occur in wheat, in the aleurone layers of the endosperm and in the scutellum of the germ (Engel,1947, 1948, Singer 1948, Rowlands 1966 b)

The age of the wheat grain after harvest had no effect on hydrolytic activity towards differing substrates, both insecticidal and non-insecticidal, and the lower levels of hydrolase activity showed no variation in grain of moisture content ranging from 10 to 14%; activity increased at moisture levels above the "critical level" (Rowlands, 1971).

From the data available on the location of hydrolytic activity, insecticides would be liable to hydrolytic attack regardless of where they penetrate to inside the grain as they will be in contact with the enzymes.

### 2.6.1.3 Decarboxylation

Decarboxylation normally plays a role in the later degradation of insecticide metabolites produced in and on grain, rather than in initial detoxification. Thus, dimethoate carboxylic acid, malathion mono- and dicarboxylic acids could be further degraded to assimilable or non-toxic products. Most of the decarboxylase activity determined in the grain has been associated with the germ (Harary et al., 1953; Cheng et al., 1958) and in the aleurone layer of the endosperm (Rowlands, 1966 b).

### 2.6.1.4 Dehalogenation

Rowlands (1968) found very slight dehydrochlorination of DDT to DDE in stored wheat under aerobic conditions but once the intergranular air was consumed and anaerobic conditions prevailed, reductive dechlorination to DDD took place. These reactions were localized in the aleurone layer of the endosperm.

### 2.6.1.5 Dealkylation

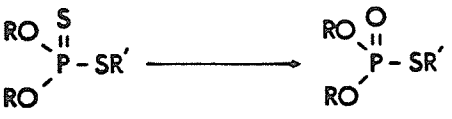
O-Dealkylation has frequently been identified as a degradation pathway of insecticides in mammals, insects, plants, and microorganisms (Aharoni and O'Brien, 1968). There is sometimes clear specificity of attack limited to methyl, rather than ethyl phosphate esters. Dealkylation has been shown to take place in the aleurone layer and in the germ of the wheat grain (Rowlands, 1971).

It has been reported that S-dealkylation, which might be a possible degradation pathway of certain minor insecticide products such as S-alkyl isomers, is much less likely to occur (Rowlands, 1971).

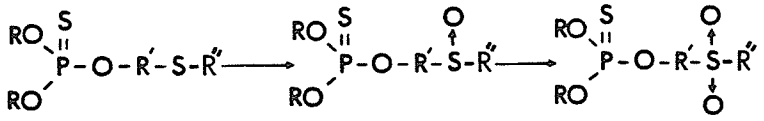
## 2.6.2 Metabolism of organophosphorus insecticides in stored grain

Organophosphorus insecticides are metabolized more rapidly by grain than most other insecticides. Phosphorothioates or dithioates of low toxicity may be oxidized to more insecticidally active compounds, or their phosphate and phosphorothiolate analogs; thioethers may be converted to sulfoxide or sulfone derivatives as follows:


Phosphorothioate Phosphate



Phosphorodithioate Phosphorothiolate

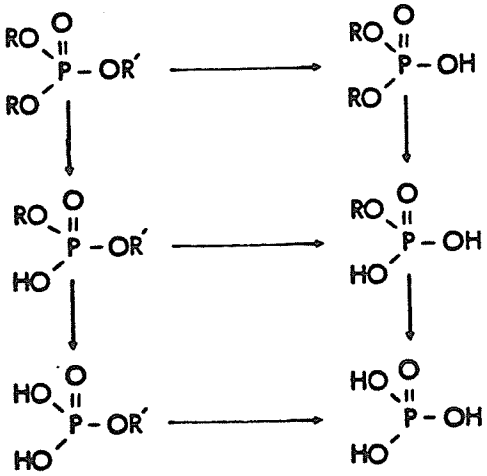


Thioether Sulfoxide Sulfone

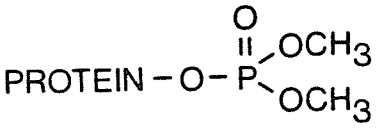
Compared to the organochlorine compounds, organophosphorus insecticides are generally of relatively short persistence and are easily hydrolyzed to innocuous products that can be absorbed into the normal metabolic system of organisms involved.

### 2.6.2.1 Phosphates

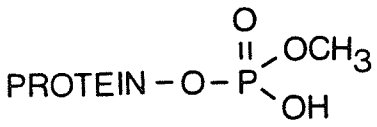
Consideration of the enzymology of grain suggests hydrolytic attack as the main metabolic pathway of phosphates in stored grain (Rowlands, 1971). Triphenyl phosphate was degraded to di- and monophenyl phosphates by stored wheat (Rowlands, 1965 b) and dimethyl dichlorobromophenyl phosphate, the phosphate analogue of bromophos, was similarly converted to dimethyl phosphate and dichlorobromophenol (Rowlands, 1966 b); presumably, ultimate degradation to inorganic phosphate occurred. The likely degradative pathways for phosphates are the following:



Rowlands (1970 b) has shown that DDVP breaks down rapidly on wheat grain to give mainly dimethyl phosphate phosphorylated protein derivatives. Lesser amounts of demethyl-DDVP, monomethyl phosphate, and phosphoric acid were found. Investigation of these phosphorylated proteins, after Sephadex gel fractionations, suggested that there was little molecular weight difference between the extractives of DDVP treated and untreated wheat, and that DDVP probably dimethyl-phosphorylated avaliable hydroxy groups of the protein molecule, e.g.,




This complex underwent rapid demethylation during storage to give a more stable form:




Digests of complexes extracted from treated wheat were shown to yield mono- and dimethyl phosphoryl derivatives of amino acids which was slowly attacked by acid phosphatase to yield mono- and dimethyl phosphates. The overall degradation pathway is shown in Figure 3.

### 2.6.2.2 Phosphorothioates and phosphorodithioates

The phosphorothioates undergo two major metabolic reactions in grain: oxidation and hydrolysis. Although compounds with a side chain that can be hydrolyzed to give carboxylic acids, e.g., malathion and dimethoate (Rowlands, 1967), may be further degraded by decarboxylation, the ulti-

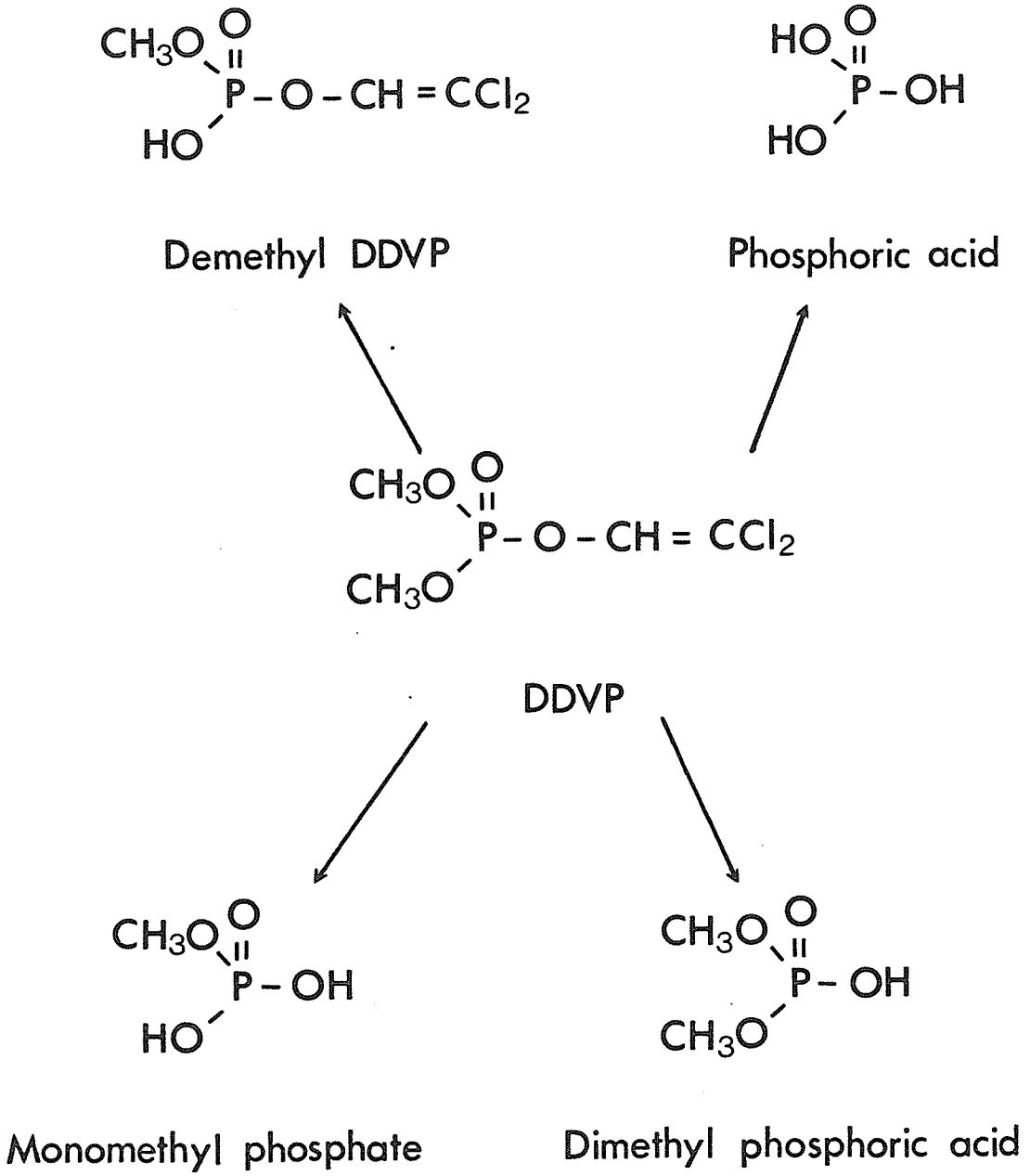



Figure 3: Metabolism of DDVP in wheat grain (Rowlands, 1970 b).

mate product of metabolism is inorganic phosphate or thiophosphate (Figure 4).

1. Compounds with aryloxy-(or arylthio-) phosphate side chains:

   In the few cases where the metabolism of such compounds in stored grain has been determined, e.g., bromophos and fenitrothion, the results, based on hydrolytic rather than oxidative degradation, have shown that demethyl derivatives are the main products. Rowlands (1966 a) found that bromophos was metabolized by wheat at 12% moisture content and stored at 20 C to yield demethyl bromophos, traces of dimethyl phosphorothioate and dichlorobromophenol, and small amounts of the phosphate analog of bromophos (Figure 5). Ultimately the demethyl bromophos was degraded to monomethyl phosphorothioate, thiophosphoric acid, and dibromochlorophenol.

2. Compounds with alkoxy- (or alkylthio-) phosphate side chains:

   The relevant compounds in this catagory are, for instance, malathion and dimethoate. These have the possibility of esterase or amidase catalyzed hydrolysis in addition to phosphatase degradation and this alternative site of attack seems to account for their pronounced selective toxicity between insects and mammals (O'Brien, 1961). Thus the thiolo-side chain may be degraded to carboxlyic derivatives (Figures 6,7).

   Rowlands (1964) studied the metabolism of malathion in stored maize and wheat. Products identified qualitatively by thin-layer chromatography were dimethyl phosphorodithioic acid and malathion diacid after 6 months of storage. No malaoxon was found, but in

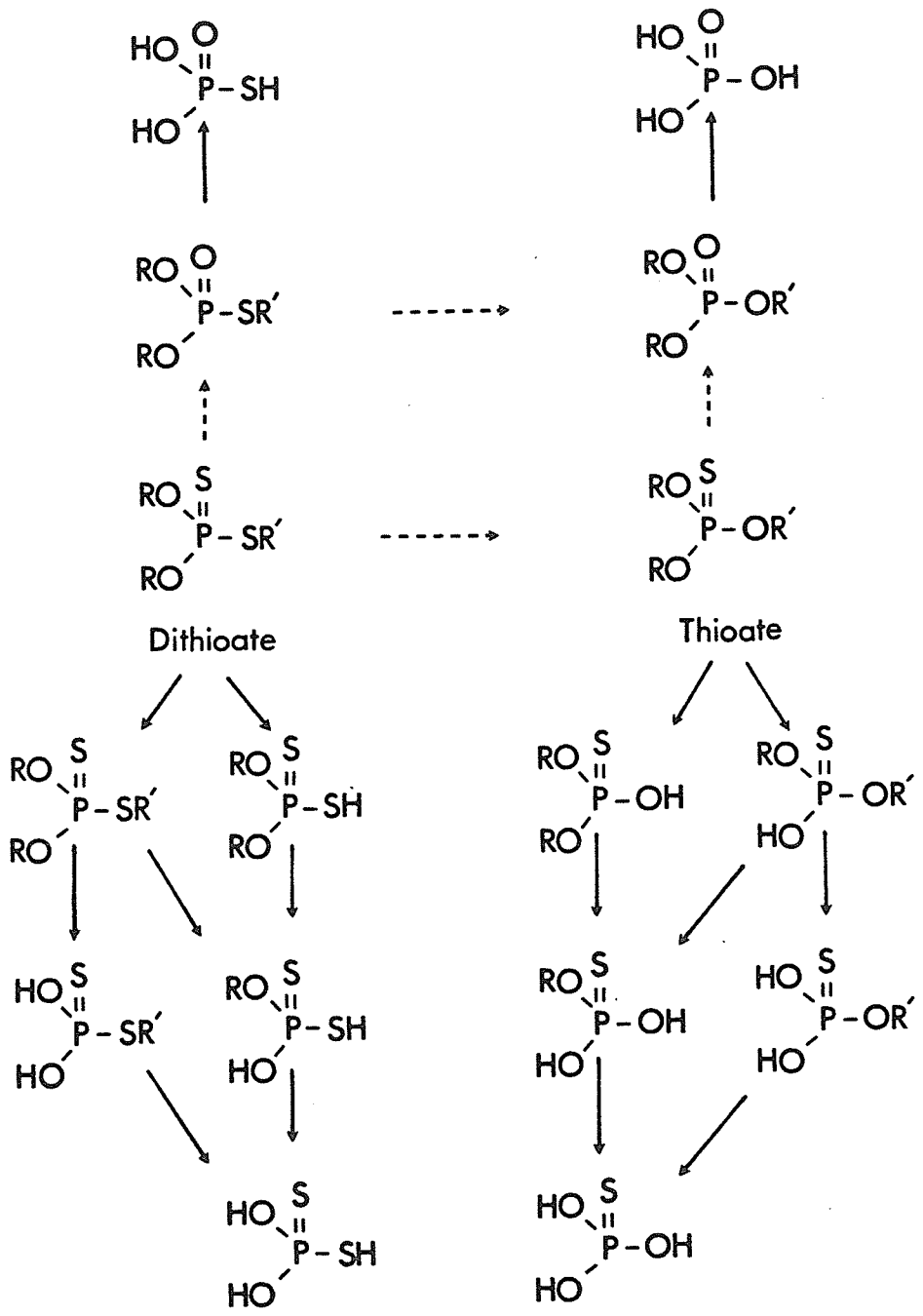



Figure 4: Degradation pathways of dithioates (left) and thioates (right) : ----→ (oxidation), ⟶ (hydrolysis) . R and R´ can be alkyl or aryl groups.

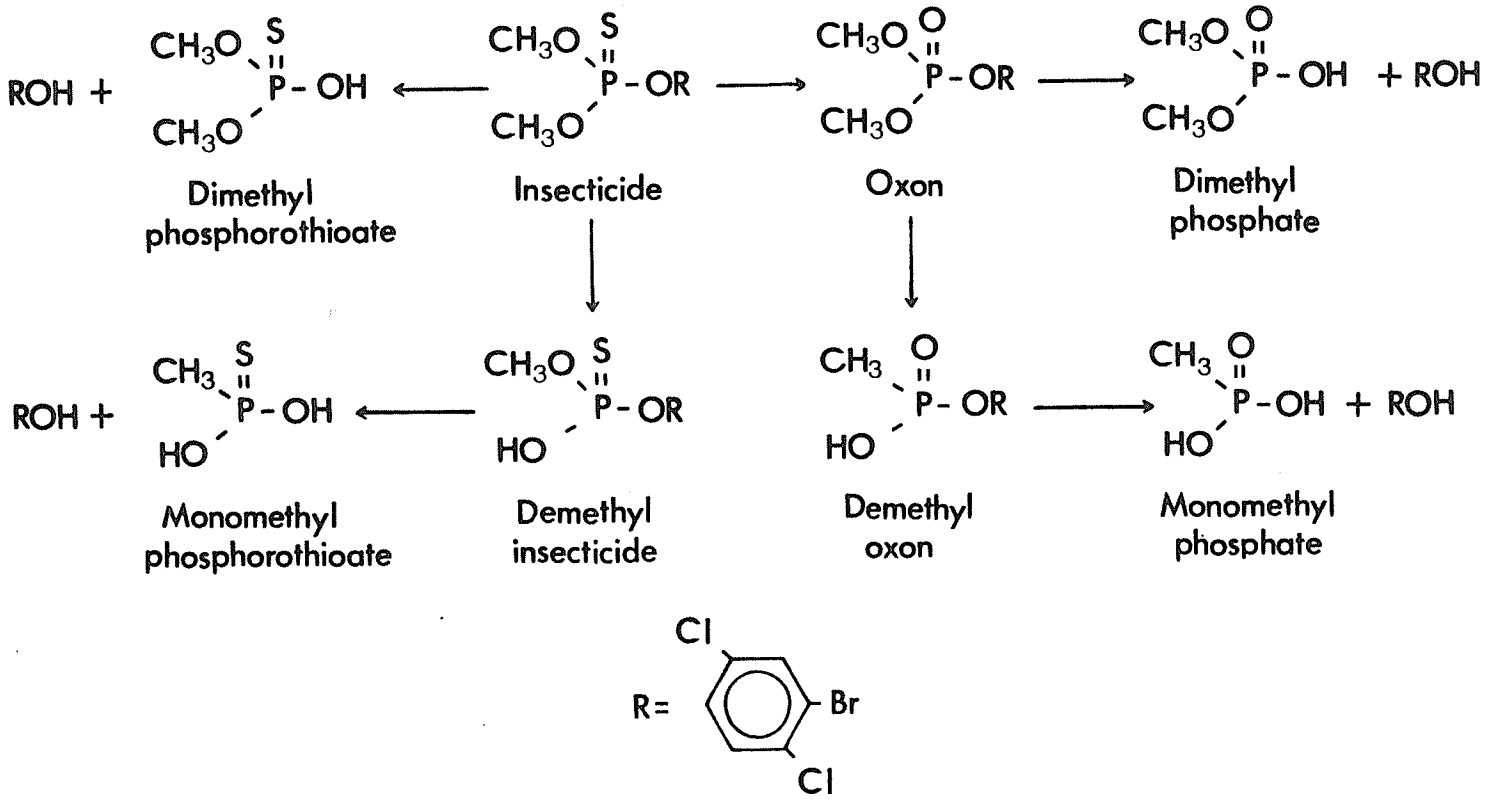



Figure 5: Metabolism of bromophos in grain (Rowlands, 1966 a).

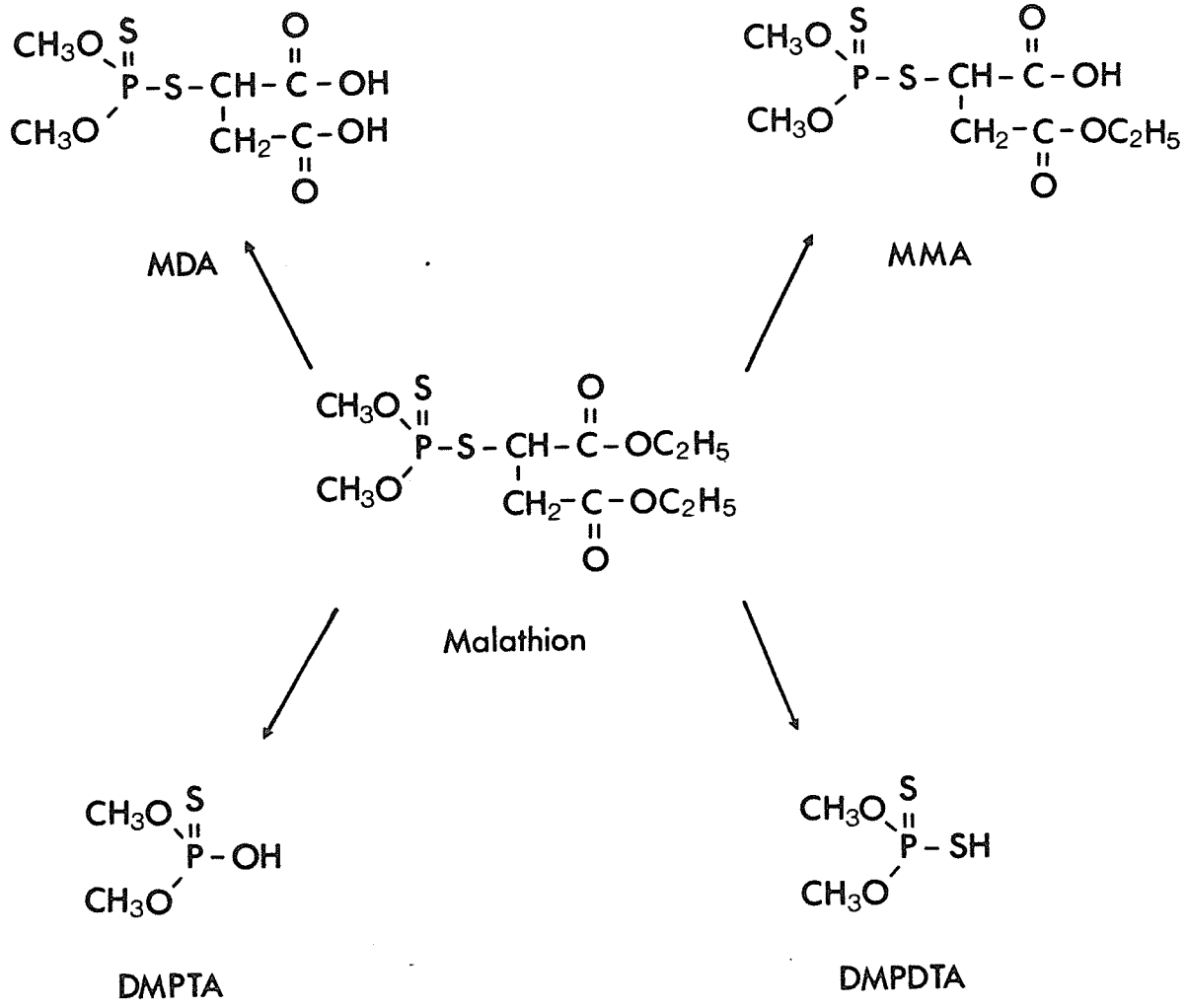



Figure 6: Metabolism of malathion in wheat (Rowlands, 1964).

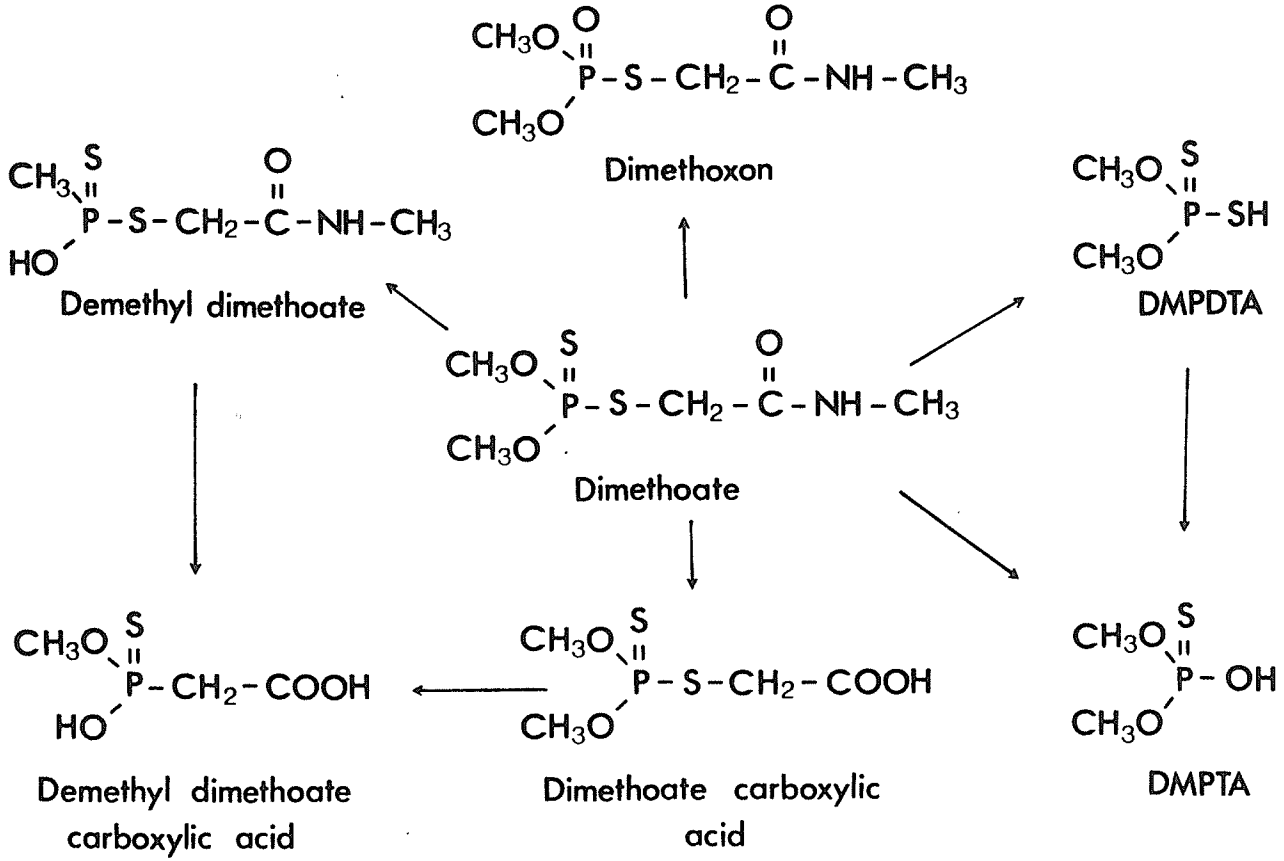



Figure 7: Pathways of dimethoate degradation in stored grain (Rowlands, 1966 c)

later work, Rowlands (1965 a,b) showed that when freshly harvested wheat was treated with malathion, production of malaoxon was initially rapid and continued for three to four weeks. A sample from the same wheat bulk treated after two to eight weeks from harvest showed almost no oxidative activity.

Dimethoate was found to degrade rapidly in stored grain and sorghum (Rowlands, 1966 c). The rapid degradation was in part attributable to the speed with which dimethoate enters the grains, possible due to its relatively higher water solubility. Only slight oxidation was detected during the first seven days of storage in both wheat and sorghum. On the other hand, hydrolysis of dimethoate was very marked and demethyl dimethoate, the demethyl derivative of dimethoate carboxylic acid, and dimethyl phosphorodithioic acid were found in both commodities (Figure 7). Later Rowlands (1966 c) showed that dimethoate carboxylic acid was slowly degraded to trimethyl phosphorodithioate, i.e., decarboxylated, by enzymes of the germ and aleurone layer of wheat.

## 2.7 ANALYTICAL METHODOLOGY FOR MALATHION AND FENITROTHION AND METABOLITES IN STORED GRAIN

### 2.7.1 Analysis of parent compounds

Several methods for the determination of malathion and fenitrothion in stored grain have been reported in the literature. Levi and Nowiciki (1974) have modified a method for the screening of organochlorine pesticides in wheat to permit rapid, simultaneous screening for malathion, fenitrothion, and other common organophosphorus residues. The sample is extracted in a ball-mill with ethyl ether and hexane and the extract is

analyzed by gas-liquid chromatography (GLC) using an alkali flame ionization detector (AFID).

Varca et al. (1975) reported a method for the analysis of malathion and dichlorvos in stored grain. The method involves extraction by shaking with benzene, Florisil column cleanup, and GLC determination using electron capture detection (ECD) or AFID. Desmarchelier et al. (1977) described a method for the analysis of fenitrothion in stored wheat. Using methanol or acetone as the extraction solvent, samples were either macerated or Soxhlet extracted for 36 or 6 hours, respectively. The extract was then analyzed by AFID-GLC.

Kadoum and LaHue (1977 and 1979) extracted malathion from wheat and corn by shaking with acetonitrile saturated with hexane. The extract was cleaned up on a Kontes Chromflex column and analyzed by EC-GLC.

### 2.7.2 Analysis of metabolites

No method has been reported to date for the extraction and determination of fenitrothion and its metabolites which may be found in stored wheat. The only published method for the analysis of malathion carboxylic acids in fortified grain was reported by Kadoum (1969). It involves a somewhat lengthy extraction process, methylation with $BF_3$-methanol, column cleanup, and GLC determination of the derivatized metabolites by ECD or phosphorus thermionic detector.

Shafik et al. (1969, 1971, 1973) and Lores and Bradway (1977) have reported methods for the simultaneous GLC analysis of six dialkyl metabolites, common to many organophosphorus pesticides, in human blood and urine using the flame photometric detector (FPD) and the derivatization

procedure described by Stanley (1966). Two of these compounds, namely, dimethyl phosphorodithioate and dimethyl phosphorothioate, have been reported as metabolites of malathion (both compounds) or fenitrothion (the latter compound).

Greenhalgh (1974) developed a method for the simultaneous analysis of fenitrothion (F) and its degradation products, amino fenitrothion (AF), fenitrooxon (FO), and S-methyl fenitrothion (SMF) from water. The analysis of the extract was conducted using AFID-GLC. Greenhalgh et al. (1980) described a procedure for F, AF, SMF, and de-S-methyl fenitrothion (DSMF) in natural aquatic systems. Samples were extracted with ethyl acetate prior to and after acidification to pH 1. The first extract contained F and AF, and the second, DMF and DSMF. Analyses of the extracts were performed by AFID-GLC.

Recently, Volpe and Mallet (1980) developed a method for the simultaneous analysis of fenitrothion and five of its metabolites, AF, SMF, formyl fenitrothion (FF), and hydroxymethyl fenitrothion (HMF) from water. Samples were passed through either XAD-4 or 7 resins and the compounds were eluted with an organic solvent such as ethyl acetate or methylene chloride. Extracts were then analyzed by FPD-GLC.

The use of high pressure liquid chromatography (HPLC) for the analysis of fenitrothion and metabolites has been reported. Abe et al. (1979) developed a procedure for the simultaneous analysis of F, FO, DMF, and 3-methyl-4-nitrophenol (MNP) using HPLC coupled to an ultraviolet detector (UV) at 260 nm. Cochrane et al. (1979) reported an analytical method for the quantitation of contaminants in technical grade fenitrothion using $UV_{254}$-HPLC. The chromatographic system used, gave good separation of F, FO, SMF, MNP and carboxy fenitrothion.

## Chapter III

## EXPERIMENTAL

### 3.1 EFFECT OF STORAGE TEMPERATURE ON THE RATE OF DEGRADATION OF MALATHION AND FENITROTHION IN STORED WHEAT

#### 3.1.1 Reagents

1. Solvents: all were pesticide grade- Caledon Co., Ontario.
2. Insecticide analytical standards: Malathion (O,O-dimethyl-S-1,2-di( ethoxycarbonyl)ethyl phosphorodithioate) was supplied by American Cyanamid, U.S.A.; Fenitrothion (O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate) was provided by Stauffer Chemical Co. U.S.A.
3. Insecticide formulations: Malathion 83.6% (w/v) emulsifiable concentrate (EC) was supplied by Cyanamid of Canada Ltd., Montreal; Fenitrothion 95.8% (w/v) EC was provided by Stauffer Chemical Co., U.S.A.
4. Chromosorb W HP, 80/100 mesh and OV-101: (Chromatographic Specialties,Ontario.
5. Grain: Hard red spring wheat, Neepawa variety, was used. The moisture content of the grain was tempered either by aeration or by addition of calculated volumes of distilled water and tumbled on a Norton rotary mixer for one hour and then left for seven days to equilibrate to the desired moisture content (12.5%). The moisture content was determined at room temperature with a Mois-

ture Master 101 A (CAE Industries Ltd., Canada) with a precision of $\pm$ 0.5%.

### 3.1.2 Apparatus

1. Rotary grinder: GS Iona high speed coffee grinder- Model CG8 (General Signal Appliances Ltd., Canada).
2. Extraction apparatus: 50 mL round bottom stainless steel centrifuge tubes (International Equipment Co., No. 613), stainless steel caps fitted with Teflon "O" ring gaskets, stainless steel balls of approximately 1.75 cm diameter.
3. Paasche Type H airbrush sprayer fitted with a No. 5 nozzle (Paasche Airbrush Co., U.S.A.).
4. Gas liquid chromatograph: Tracor Micro Tek 220 equipped with Melpar flame photometric detector (Tracor Inc., U.S.A.).

### 3.1.3 Methodology

#### 3.1.3.1 Adjusting moisture content

Hard red spring wheat, Neepawa variety, stored in a temporary wooden bin for three months was collected into 27.2 kg capacity jute bags. The grain was thoroughly cleaned of weed seeds and other foreign materials with a Clipper M-2B (A.T. Ferrel and Co., U.S.A.) to give uniform grain kernels, and divided into 10 kg lot samples. These cleaned wheat samples were tempered to 12.5 percent moisture content by adding a calculated volume of distilled water and mechanically tumbling for one hour. The tempered wheat was kept in tied polyethylene bags for seven days to equilibrate before the application of insecticides. Prior to the insec-

ticide treatments, the moisture content of each sample lot (12.5% ± 0.1%) was determined with a Moisture Master 101 A and the grain was divided further into 2 kg lots.

### 3.1.3.2 Insecticide treatment

Emulsifiable concentrates containing malathion 83.6% active ingredient (AI) and fenitrothion 95.8% AI were diluted with distilled water to obtain desired concentrations that when applied at 0.8 mL/kg wheat would provide a deposit of 8 ppm AI. The moisture content of the wheat was increased by less than 0.1% during insecticide application. The insecticide emulsion (1.6 mL) was applied to the wheat (2 kg lots) spread thinly in a galvanized iron tray 81 X 41 cm, with sides 4 cm high. The insecticide was sprayed evenly over the grain surface with a Paasche airbrush sprayer at a constant pressure of 0.52 $kg/cm^2$, the nozzle being held about 20 cm above the grain surface during application. Each lot of treated wheat was mixed for five minutes with a small metal shovel. The treated lots were thoroughly mixed together to ensure initial uniformity of residue levels, and samples were taken for insecticide determination. The remainder was transferred to screw cap jars (240 mL) and stored in the dark at -35, -20, -5, 5, 10, 20, and 27 C. Control wheat samples were transferred into the jars without being treated and were stored under similar conditions.

### 3.1.3.3 Sampling for residue analysis

Four jars of treated wheat and an untreated control were selected randomly at 1, 3, 6, 12, 24, 36, 48, and 72 weeks after insecticide application from each storage temperature for residue determination.

### 3.1.3.4 Extraction procedure

1. Extraction efficiency studies:

Two extraction procedures using different solvents were investigated to extract malathion and fenitrothion residues from fortified wheat.

a) The ball-mill extraction method:

Fifty-gram samples of uncontaminated hard red spring wheat of 12.5% moisture content were placed in flasks and fortified at concentrations of 0.05, 1.0, and 5.0 ppm (w/w) with acetone solutions of malathion and fenitrothion (1 mL). The flasks were stoppered, agitated by hand, and stored in a deepfreeze (-35 C) for 24 hours after which the samples were ground for 30 seconds in a coffee grinder.

Ten-gram aliquots of fortified wheat were transferred into extraction tubes and one stainless steel ball added to each. Thirty mL of hexane, ethyl ether-hexane (3:97, v/v) or methanol were added, the caps were sealed in place and the extraction tubes mounted horizontally on a wrist-action shaker and agitated for one hour. The tubes were centrifuged for five minutes and aliquots of the extract were transferred into small vials.

b) Soxhlet extraction method:

Ten-gram samples of wheat fortified at 0.05, 1.0, or 5.0 ppm were weighed into Soxhlet thimbles and topped with small plugs of glass wool. The extraction thimbles were then inserted into the Soxhlet apparatus and the wheat exhaustively

extracted with 200 mL of hexane, ethyl ether-hexane (3:97, v/v), or methanol for 6 hours. The extracts were concentrated in a Bucchi Rotovapor evaporator to about 20 mL and final volume adjusted to 25 mL with each solvent.

Triplicate samples of fortified wheat were prepared for each solvent used and for each extraction method investigated. Parallel tests of extraction of unfortified samples were run with the extraction efficiency tests.

2. Extraction of residues from stored grain:

Results from the above investigation, to be discussed later, showed that the use of methanol and the ball-milling extraction technique was efficient, rapid and economical, and was the method of choice to extract residues of malathion or fenitrothion from wheat treated at 8 ppm and stored at various temperatures for up to 72 weeks.

The contents of each jar was well mixed and a 50 g subsample was ground for 30 seconds in a coffee grinder. After thorough mixing of the ground subsample, a 10 g aliquot was weighed into an extraction tube and extracted with methanol (30 mL) as described above. Aliquots (1-5 uL) of the methanolic extract were injected into the gas liquid chromatograph without further clean-up.

### 3.1.3.5 Gas-liquid chromatographic analysis

A Tracor Micro-Tek 220 gas chromatograph equipped with a Melpar flame photometric detector, operated in the phosphorus mode at 526 nm, was

used to analyze the insecticide residues. The operating conditions were as follows: 0.6 m X 4 mm id silanized Pyrex glass column packed with 5% OV-101 on Chromosorb W HP, 80/100 mesh; column temperature (isothermal) 190 C, detector temperature 220 C, inlet temperature 210 C; gas flow rates, nitrogen (carrier gas) 70 mL/min; hydrogen 200 mL/min; air 90 mL/min; oxygen 20 mL/min; chart speed 3 mm/min. A 10 cm long glass sleeve liner containing about 2.5 cm silane treated glass wool was inserted in the injection port of the column. This was performed in order to trap any coextractives present in the samples onto the glass wool, thus avoiding any alteration of the column performance and consequently prolonging its efficiency and reproducibilty. The glass sleeve liner was changed periodically, usually after 25 - 30 injections of the sample extract. Before reuse, it was thoroughly rinsed with acetone followed by hexane and then dried under a stream of nitrogen.

Sample injections were alternated with injections of standards. The external standard method of quantitation, using linear regression equations, was performed to calculate amounts of malathion and fenitrothion residues in stored grain.

### 3.1.3.6 Wheat germination test

Samples of hard red spring wheat were taken before and 1, 6, and 12 months after treatment with malathion and fenitrothion for germination test according to Seitz et al. (1975). Two hundred kernels were placed between moist paper towels in an aluminum foil folder and held at room temperature (25 C) for 6 days. Germinated seeds were then counted.

## 3.2 TRANSLOCATION OF MALATHION AND FENITROTHION IN KERNELS OF WHEAT STORED AT VARIOUS TEMPERATURES

### 3.2.1 Reagents

1. Solvents: all were pesticide grade- Caledon Co., Ontario.
2. Insecticide analytical standards and formulations: Same as described in Section 3.1.1.
3. Grain: Hard red spring wheat, Neepawa variety.

### 3.2.2 Apparatus

1. Ottawa Micro mill: Agriculture Canada, Engineering Research Services, Ontario.
2. Paasche Type H airbrush sprayer fitted with a No.5 nozzle (Paasche Airbrush Co., U.S.A.).
3. Gas-liquid Chromatography: Tracor Micro Tek 220 equipped with Melpar flame photometric detector.

### 3.2.3 Methodology

#### 3.2.3.1 Insecticide treatment

Hard red spring wheat (12.5% mc) was treated with emulsifiable concentrates of malathion and fenitrothion at concentrations of 8 and 12 ppm as described in Section 3.1.3.2. Immediately after treatment, samples (100 g) were taken for milling with an Ottawa Micro Mill into bran, middlings, and flour for initial insecticide determination in the milled fractions. Residues in whole ground grain also were determined. The remainder was transferred into screw-capped jars (240 mL) and stored in the dark at -5, 10, and 20 C.

Four jars were taken randomly, at 1, 3, 6, and 12 months after treatment from each storage temperature for residue determination in both milled fractions and whole ground wheat.

### 3.2.3.2 Extraction procedure

The ball-mill extraction method described in Section 3.1.3.3 was followed. Residues of malathion and fenitrothion were extracted from aliquots of wheat (ground, 10 g) and milled fractions (10 g flour and 5 g bran and middlings) with 30 mL methanol. Aliquots (1 uL) of methanolic extracts from ground wheat, bran, and middlings and 5 uL from flour were injected into the gas chromatograph without further cleanup.

### 3.2.3.3 Gas-liquid chromatographic analysis

Residue analysis of malathion and fenitrothion was conducted by injecting aliquots of methanolic extracts, alternated with injections of standards, into the GLC. GLC operating conditions and the method of quantitation of residues were identical to that outlined in Section 3.1.3.4.

### 3.2.3.4 Determination of ash content in wheat flour

To examine whether or not the flour fraction was contaminated with the bran fraction during milling of wheat, the approved method of the American Association of Cereal Chemists (1976) was followed. Five g of flour of a well-mixed sample was weighed into the ashing dish, and placed in a muffle furnace at 150 C ; the temperature was gradually increased to 350 C. The flour was left to incinerate until light gray ash

was obtained or to a constant weight. The dish was cooled in a desiccator and weighed soon after room temperature was attained. Percentage ash content in the flour sample was then determined. This procedure was replicated five times.

## 3.3 ANALYTICAL METHODS DEVELOPMENT AND QUANTITATIVE DETERMINATION OF MALATHION AND FENITROTHION AND THEIR METABOLITES IN STORED WHEAT

### 3.3.1 Reagents

1. Solvents: all were pesticide grade- Caledon Co., Ontario.
2. Analytical standards: See Table 1 for details.
3. Potassium carbonate (3%, w/v): Three g of anhydrous potassium carbonate (Macco Reagent A.C.S.) were dissolved in 100 mL distilled water.
4. Thallium (III) chloride (1%, w/v): One g of $TlCl_3 \cdot 4H_2O$ (Alfa division, U.S.A.) was dissolved in 100 mL distilled water.
5. 2,6-Dibromo-N-chloro-p-benzoquinoneimine (0.5%, w/v): 0.5 g of the spray reagent (Eastman Kodak Co., U.S.A.) was dissolved in 100 mL ethanol.
6. Anhydrous sodium sulfate: Reagent grade- Fisher Scientific.
7. p-N,N-dimethylaminocinnamaldehyde:(Sigma Chem. Co., U.S.A.)-0.5% (w/v) in ethanol-acetic acid (1:1,v/v).
8. Diazomethane and diazoethane: generated from N-methyl-N´-nitro-N-nitrosoquanidine and N-ethyl-N´-nitro-N-nitrosoquanidine, respectively (Aldrich Chem.Co.) (Caution-potential carcinogen, mutagen, irritant) according to the method of Stanley (1966).
9. Silica gel: 60-200 mesh Grade 950, Fisher Scientific Co.
10. Hydrochloric acid: 4 N saturated with sodium chloride.

11. Glass wool: Silanized (Applied Science Laboratories Inc.).

12. Chromosorb W HP, 80/100 mesh and OV-101, OV-225 and Carbowax 20 M: (Chromatographic Specialties, Ontario).

TABLE 1

Details for analytical standards of malathion and fenitrothion and metabolites used

| Compound | Chemical name |
|---|---|
| Malathion* (M) | O,O-dimethyl S-(1,2-dicarbethoxy) ethyl phosphorodithioate |
| Malaoxon (MO) | O,O-dimethyl S-(1,2-dicarbethoxy) ethyl phosphorothiolate |
| Malathion monoacid (MMA) | O,O-dimethyl S-(1-ethoxycarbonyl-2-carboxy) ethyl phosphorodithioate |
| Malathion diacid (MDA) | O,O-dimethyl S-(1,2-dicarboxy) ethyl phosphorodithioate |
| DMPDTA | O,O-dimethyl phosphorodithioic acid |
| DMPTA | O,O-dimethyl phosphorothioic acid |
| Fenitrothion** (F) | O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate |
| Fenitooxon (FO) | O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphate |
| S-Methyl fenitrothion (SMF) | O,S-dimethyl O-(3-methyl-4-nitrophenyl) phosphate |
| O-Demethyl fenitrothion (DMF) | O-methyl O-(3-methyl-4-nitrophenyl) phosphorothioate |
| MNP | 3-methyl-4-nitrophenol |
| O-Demethyl S-methyl fenitrothion (DMSMF) | S-methyl O-(3-methyl-4-nitrophenyl) phosphate |

* Analytical standards of malathion and its metabolites were supplied by American Cynamid, U.S.A.

** Analytical standards of fenitrothion and its metabolites were provided by Stauffer Chemical Co., U.S.A.

### 3.3.2 Apparatus

1. Gas liquid chromatographs: Varian model 2440, equipped with a tritium foil electron capture (EC) detector and a Tracor Micro Tek 220 equipped with Melpar flame photometric detector.
2. Rotary grinder: GS Iona model CG8 (General Signal Appliances Ltd., Canada).
3. Glassware: Treated prior to use with Dri-film (SP 5800, 15% in toluene), a silanizing reagent, to prevent adsorption of residues and standards.
4. TLC silica gel (G) plates: precoated TLC with silica gel (0.25 and 0.5 mm thickness) Brinkman Instruments, U.S.A.

### 3.3.3 Methodology

#### 3.3.3.1 Analytical method development for malathion and its metabolites in wheat

1. Fortification of wheat:

Fifty gram samples of grain (12.5% moisture content) were fortified at concentrations of 5.0, 1.0, 0.5, and 0.1 ppm (w/w) with a mixture of malathion (M), malathion monoacid (MMA), malathion diacid (MDA), dimethyl phosphorodithioic acid (DMPDTA), and dimethyl phosphorothioic acid (DMPTA) in acetone (1 mL). The fortified wheat samples were allowed to equilibrate for 1 hour after which they were ground for 30 sec in a coffee grinder.

2. Extraction of malathion and metabolites:

Two extraction procedures were investigated. The first procedure (Figure 8) utilizes a one step extraction process and may be used for malathion and its metabolites, excluding malaoxon (MO) which has the same GLC retention time as malathion monoacid (MMA). However, the second technique, which involves separation of neutral compounds from acidic metabolites (Figure 9), could be used to analyze malathion and all other metabolites.

a) One step extraction process:

An aliquot (25 g) of fortified wheat was weighed into a centrifuge bottle (250 mL). One hundred mL of acidified acetone (0.5 to 1 mL of 2N HCl) were added, and the bottle was stoppered and shaken on a mechanical shaker for 1 hour. The acetone extract was filtered and transferred quantitatively to a 500 mL round bottom flask and concentrated to 2 - 3 mL under vacuum at 40 C. The extract was then transferred to a 15 mL centrifuge tube to await derivatization for malathion metabolites present in the sample.

b) Extraction plus separation of the acidic metabolites:

Wheat (25 g) fortified with M, MMA, MDA, DMPDTA, DMPTA, and malaoxon (MO) at the concentration of 1.0 ppm was extracted with acidified acetone (100 mL) as described above. The acetone extract was concentrated to 2-3 mL and transferred quantitatively into a 150 mL beaker containing 100 mL water. The aqueous solution was adjusted to pH 8.5 with 3% potassium carbonate and transferred to a 250 mL separatory funnel. Mala-

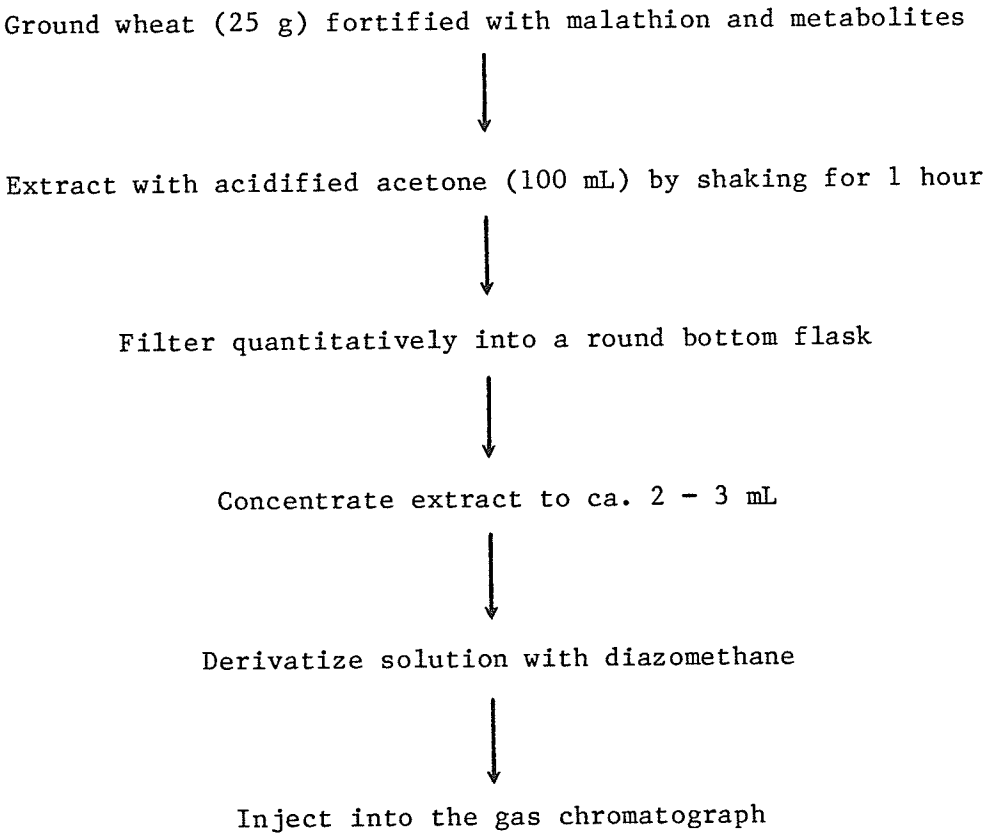



Figure 8: Scheme for the extraction of malathion and metabolites from fortified wheat

thion and malaoxon were removed by extracting the aqueous solution with two 30 mL portions of methylene chloride. The methylene chloride extracts were dried over anhydrous sodium sulfate and the combined extracts concentrated in a water bath (45 C) for determination of malathion and malaoxon using a stream of nitrogen.

The aqueous solution was transferred to a 150 mL beaker and adjusted to pH 2 with 6N HCl saturated with sodium chloride and then returned to the separatory funnel. MMA and MDA were removed by extracting the aqueous solution with ethyl acetate (2 X 50 mL). DMPDTA, and DMPTA, were removed from the aqueous solution by extracting with ether (2 X 30 mL). The ethyl acetate extract was dried over sodium sulfate as before and concentrated to 2-3 mL under vacuum at 40 C. The ether was dried, concentrated under a gentle stream of nitrogen to approximately 1 mL, and then transferred into a 15 mL centrifuge tube.

3. Derivatization of malathion metabolites:

Malathion metabolites present in acetone, ethyl actate, or ether extracts, resulting from procedure (a) and (b), respectively, were methylated with diazomethane (2-3 mL). The tubes were left to stand for 30 min in a well-ventilated hood. The solution was concentrated, under a gentle stream of nitrogen, as required for determination of malathion and metabolites.

4. Cleanup procedure:

The methylated extract may be injected into the gas chromatograph without further cleanup. However, the following cleanup

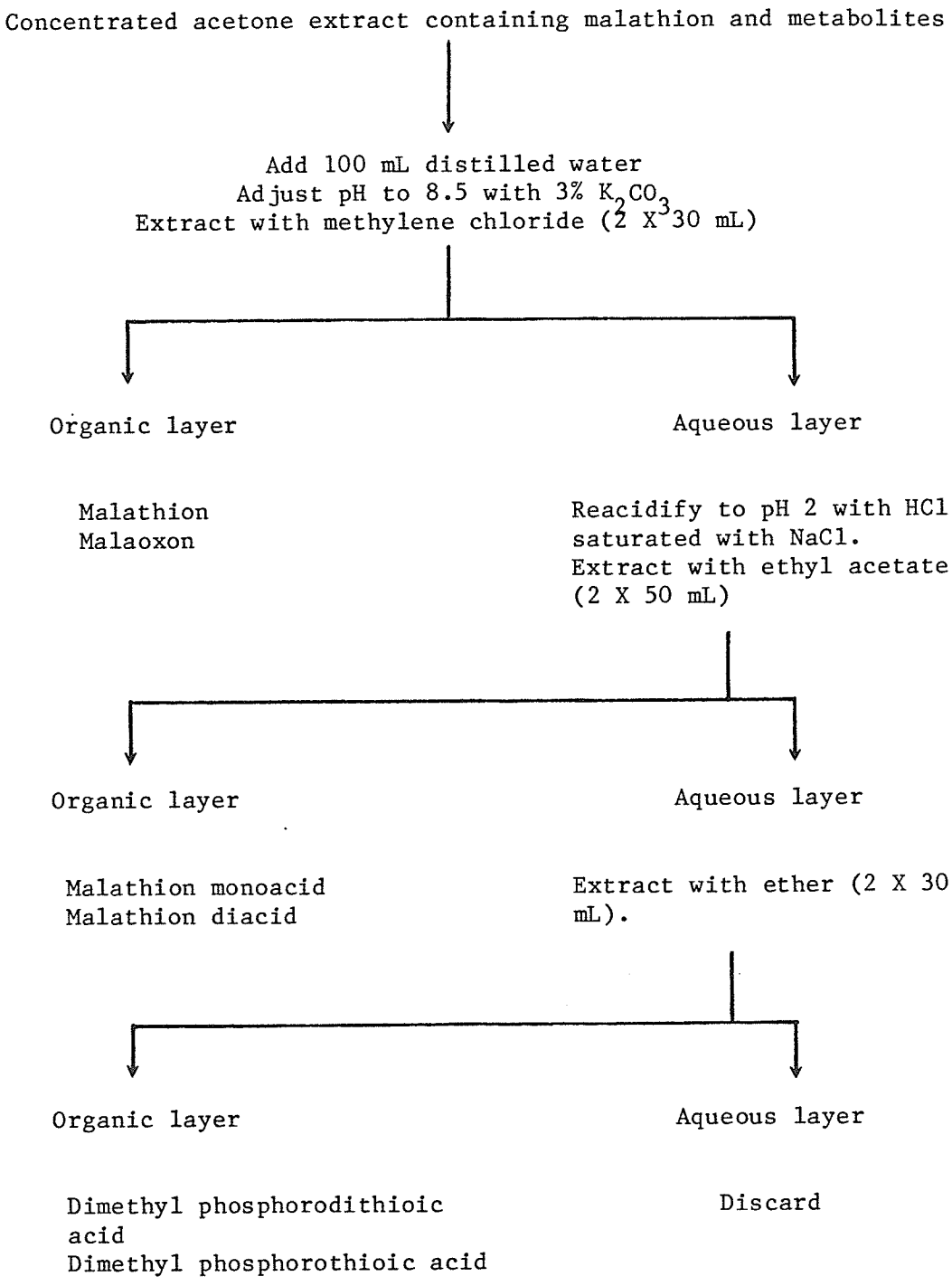



Figure 9: Scheme for the extraction and isolation of malathion and metabolites from fortified wheat

procedure could be used for extracts obtained from extraction procedure (a). This was found necessary when consecutive injections of the concentrated samples, which contain lipids, would alter the performance of the GLC column.

A silica gel chromatographic micro-column was prepared by packing a plug of glass wool loosely into a disposable Pasteur pipet, 23 cm in length, having a tip opening ca. 1 mm, and top opening 5 mm inside diameter. One and half grams of silica gel were added, followed by 1 gram of anhydrous sodium sulfate. The column was tapped to obtain good packing and then washed with 5 mL hexane.

Prior to column chromatography, the methylated extracted was evaporated to ca. 0.5 mL in a water bath maintained at 40 C, using a gentle stream of nitrogen. Distilled water (9 mL), benzene (5 mL), and sodium chloride (5 g) were added and the contents mixed on a Vortex mixer for 1 min and the layers allowed to separate. With the aid of a disposable pipet, the benzene extract was transferred quantitatively to the prewashed chromatography column and the eluate was immediately collected in a 25 mL centrifuge tube. The aqueous layer was extracted with an additional 5 mL benzene, and the benzene extract transferred to the column just as the previous extract reached the sodium sulfate layer. The sides of the columns were rinsed with 3 mL benzene. The elution of the column was continued with 10 mL of 10% (v/v) ethyl acetate in benzene. The eluate was then concentrated to 0.5 - 5 mL in a water bath at 40 C, using a gentle stream of nitrogen.

5. Gas-liquid chromatographic analysis:

A Tracor Micro Tek 220 gas chromatograph equipped with a Melpar flame photometric detector, operated in the phosphorus mode at 526 nm, was employed. The operating conditions were as follows: 1.5 m X 4 mm (id) silanized Pyrex glass columns packed with either 5% OV-101 or 3% OV-225 on Chromosorb W HP, 80/100 mesh; column temperature (isothermal) 80, 120, or 175 C, detector temperature 220 C, inlet temperature 210 C; gas flow rates, nitrogen (carrier) 50 mL/min; hydrogen 180 mL/min; air 100 mL/min; oxygen 20 mL/min; chart speed 3 mm/min

New columns were conditioned by placing a small piece of silanized glass wool in the inlet end of the column and adding approximately 10 cm of 10% Carbowax on Chromosorb W, acid washed 80/100 mesh. The column was heated overnight at 240 C with a nitrogen flow of 20 mL/min. During the conditioning period, the column was not connected to the detector. The Carbowax and the glass wool separator plug were replaced with either 5% OV-101 or 3% OV-225, the carrier flow was adjusted to 50 mL/min, and the column was conditioned an additional hour at 240 C. This conditioning procedure was repeated when sensitivity and efficiency of the column became unacceptable because of peak tailing, lack of peak separation, or the lack of reproducibility.

### 3.3.3.2 Analytical method development for fenitrothion and its metabolites in wheat

1. Fortification of wheat:

An acetone standard solution (1 mL) containing fenitrothion (F), fenitrooxon (FO), S-methyl fenitrothion (SMF), O-demethyl fenitrothion (DMF), O-demethyl S-methyl fenitrothion (DMSMF), 3-methyl-4-nitrophenol (MNP), and dimethyl phosphorothioic acid (DMPTA) was used to fortify wheat samples (50 g, 12.5% mc) at concentrations of 5.0, 1.0, 0.5, and 0.1 ppm. The fortified wheat samples were allowed to equilibrate for 1 hour after which they were ground for 30 sec in a coffee grinder.

2. Extraction of fenitrothion and metabolites:

Two extraction procedures were employed, the first to analyze all compounds when fenitrothion residue level was present in small amounts. If the latter was present in high concentration, this would alter the separation and, consequently, the quantitation of demethyl fenitrothion (to be discussed in detail later). Such a problem could be overcome by isolating neutral from acidic compounds (Figure 10).

a) Shaking with a solvent:

Fenitrothion and metabolites were extracted from wheat (25 g) by shaking with acidified acetone or methanol (100 mL) for 1 hour. The extract was filtered quantitatively into a 250 mL round bottom flask, concentrated to 2 to 3 mL under vacuum at 40 C, and transferred into a 15 mL centrifuge tube to await derivatization of fenitrothion metabolites.

b) Extraction plus separation of the acidic metabolites:

Wheat (25 g) fortified with F, FO, DMF, SMF, DMSMF, MNP, and DMPTA was extracted with acidified acetone which was concentrated to 2 to 3 mL as described above. The acetone extract was transferred into a 150 mL beaker containing 100 mL distilled water. The aqueous layer was adjusted to pH 8.5 with 3% potassium carbonate and F, FO, and SMF were removed with methylene chloride as previously described for malathion and malaoxon [(3.3.3.1) 2.b]. The aqueous layer was readjusted to pH 2 with 6 N HCl saturated with sodium chloride and DMF, DMSMF, MNP, and DMPTA were isolated with ethyl acetate and ether as mentioned earlier for acidic malathion metabolites [(3.3.3.1) 2.b].

3. Derivatization of fenitrothion metabolites:

Diazoethane (2 - 3 mL) was used to ethylate fenitrothion metabolites present in acetone, ethyl acetate, or ether extracts. The centrifuge tube was left to stand for 30 min in a well-ventilated hood. The solution was concentrated, under a gentle stream of nitrogen as required for determination of fenitrothion and metabolites.

The extract was injected into the gas chromatograph without further cleanup, or the cleanup method described earlier was employed, particularly when a large number of samples were to be analyzed. This was found necessary to avoid saturation of the GLC column with lipids that would alter its separation performance.

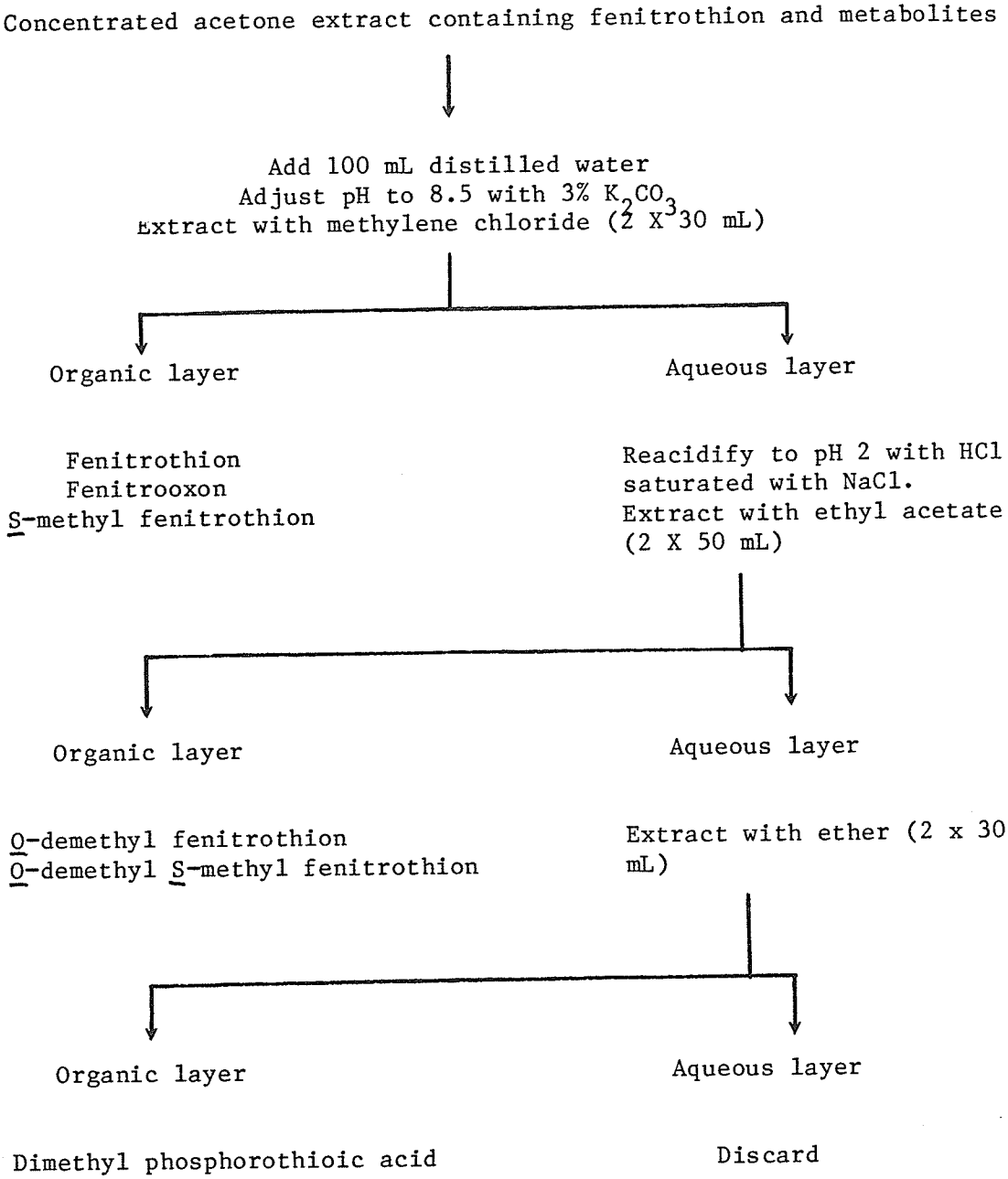



Figure 10: Flow chart for the extraction and isolation of fenitrothion and metabolites from fortified wheat

4. Gas-liquid chromatographic analysis:

A Varian model 2440, equipped with tritium foil electron-capture detector was used to analyze MNP by injecting aliquots of the acetone extract obtained from method (a) The operating conditions were: 1.5 m X 2 mm silanized Pyrex glass column packed with 3% OV-101 on Chromosorb W AW DMCS, 80/100 mesh; column temperature 140 C, injector temperature 180 C; detector temperature 190 C; nitrogen carrier-gas flow rate 40 mL/min, chart speed 3 mm/min

Phosphorus containing compounds were analyzed by FPD/GLC. Operating conditions were similar to those described in 3.3.3.1(5) except that the column temperature used was 160 C instead of 175 C.

### 3.3.3.3 Quantitative determination of malathion and fenitrothion and metabolites in stored wheat

1. Insecticide treatment:

Hard red spring wheat (12.5% mc) was treated with water-based emulsions containing either malathion or fenitrothion at the level of 12 ppm. After thorough mixing of the sprayed wheat, samples were taken for initial insecticide determination. The remainder was transferred into screw-capped jars and stored in the dark at 20 C.

Four jars were taken randomly at 1, 3, 6, and 12 months after treatment for quantitative determination of malathion and fenitrothion and their metabolites.

2. Residue analysis:

Analytical methods developed and described above were used to analyze residue levels of both insecticides and their metabolites. Sample injections were alternated with injections of standard mixtures. The external standard method of quantitation, using linear regression equations, was performed to calculate amounts of malathion and fenitrothion and their metabolites in the stored grain.

### 3.3.3.4 Confirmation tests

Two different techniques were employed to identify and confirm malathion and fenitrothion metabolites found in stored wheat.

1. Chemical derivatization:

Stored wheat samples treated with either malathion or fenitrothion were extracted according to procedure (b). Ethyl acetate fractions, containing malathion monoacid and malathion diacid or demethyl fenitrothion were concentrated to about 2 mL and derivatized with diazoethane or diazomethane, respectively. These reactions convert the metabolites to their parent compounds. Aliquots of the alkylated extracts were injected along with injections of parent compound standards into the FPD/GLC. Retention times for the resulting peaks were then compared.

2. Thin-layer chromatography:

a) Malathion and metabolites:

Stored grain (50 g) was extracted with acidified acetone, which was cleaned up as previously described. The extract was

concentrated to about 0.5 mL, using a gentle stream of nitrogen. Standard solutions of M, MO, MMA, MDA, DMPTA, DMPDTA and their mixtures, wheat extract, and extract from control (untreated wheat) were spotted with micropipets (varying between 5 to 50 ul) on the chromatoplate. The plate was developed twice in benzene:acetic acid (4:1, v/v) or once in benzene: diethyl ether:acetic acid (8:2:1, v/v/v) to the scored line (15 cm) in a rectangular chromatographic chamber lined with filter paper. The plate was allowed to dry at room temperature and sprayed with an ethanolic solution of 2,6-dibromo-N-chloro-p-benzoquinoneimine (0.5% wt/v). The plate was then placed in an oven (110 C) for 5 minutes. The $R_f$ values of the separated components calculated for malathion and metabolites found in stored wheat were compared with those calculated for the standards.

b) Fenitrothion and metabolites:

Stored treated wheat (50 g) was extracted with acidified acetone which was cleaned up as before and then concentrated to 0.5 mL. Standard solutions of F, FO, DMF, MNP, SMF and their mixtures, grain extract, and extract from the control were spotted on the chromatoplate. The plate was developed in ether:isooctane (7:3, v/v) to the scored line (10 cm). The plate was allowed to dry at room temperature and sprayed first with aqueous solution of thallium chloride (1%, wt/v) followed by a 0.5% solution of p-N,N-dimethylaminocinnamaldehyde in ethanol:acetic acid (1:1, v/v). The $R_f$ values of the separated

components found in stored grain were calculated and compared with those obtained for the standards.

# Chapter IV

# RESULTS AND DISCUSSION

## 4.1 EFFECT OF STORAGE TEMPERATURE ON THE RATE OF DEGRADATION OF MALATHION AND FENITROTHION IN STORED WHEAT

### 4.1.1 Extraction efficiency studies

Table 2 shows the averages of three determinations for recovery of malathion and fenitrothion residues from fortified wheat using the ball-milling and Soxhlet techniques. Both techniques were found to be efficient using the three solvent systems tested. The ball-milling extraction has an added advantage of being rapid and thereby enabling a large number of samples to be handled economically, both in terms of solvent (30 mL per sample in comparision to 200 mL for the Soxhlet method) and time (1 hour in comparison to 4 hours for the Soxhlet method). Although all solvents tested were generally suitable for the removal of malathion and fenitrothion residues from fortified wheat, methanol was chosen as the extraction solvent because of its relative cheapness and its reported consistency for the extraction of pesticide residues from foodstuffs (Crisp and Tarrant, 1971; The Malathion Panel, 1973; Desmarchlier, 1977).

TABLE 2

Percent recovery of malathion and fenitrothion by ball-milling and Soxhlet extraction methods using different solvents

| Solvent | Fortification level (ppm) | % Recovery* Malathion | | Fenitrothion | |
|---|---|---|---|---|---|
| | | Ball-milling | Soxhlet | Ball-milling | Soxhlet |
| Hexane | 0.05 | 97.5±2.5 | 97.8±1.5 | 96.7±2.3 | 97.1±2.0 |
| | 1.00 | 98.2±1.1 | 97.7±1.2 | 97.1±2.4 | 97.9±1.9 |
| | 5.00 | 97.8±1.3 | 98.3±1.4 | 98.0±2.5 | 98.1±2.2 |
| ** | 0.05 | 97.7±2.3 | 96.0±2.6 | 96.2±1.4 | 96.3±3.1 |
| | 1.00 | 99.8±1.0 | 97.3±1.0 | 97.4±1.9 | 98.1±1.5 |
| | 5.00 | 98.7±1.9 | 97.4±2.1 | 97.6±2.3 | 98.3±1.5 |
| Methanol | 0.05 | 99.3±0.8 | 98.1±1.7 | 99.6±0.8 | 99.1±1.1 |
| | 1.00 | 99.1±1.5 | 97.3±1.5 | 99.4±0.7 | 98.3±1.4 |
| | 5.00 | 99.3±1.8 | 97.5±2.1 | 99.7±0.7 | 98.6±1.7 |

* Average of 3 determinations

** Ethyl ether: hexane (3:97, v/v)

### 4.1.2 Standard calibration curves

Standard calibration curves for malathion and fenitrothion are shown in Figure 11. These compounds gave linear FPD-GLC responses over the range of 0.2 to 9.5 ng. Freshly prepared standarad solutions were used for quantification of insecticide residues at each sampling period. Table 3 shows the retention time and limit of detectability of malathion and fenitrothion.

TABLE 3

Retention time and limit of detectability of malathion and fenitrothion

| Insecticide | Retention time (min) | Detection limits (ng) |
|---|---|---|
| Malathion | 2.12 | 0.01 |
| Fenitrothion | 2.61 | 0.05 |

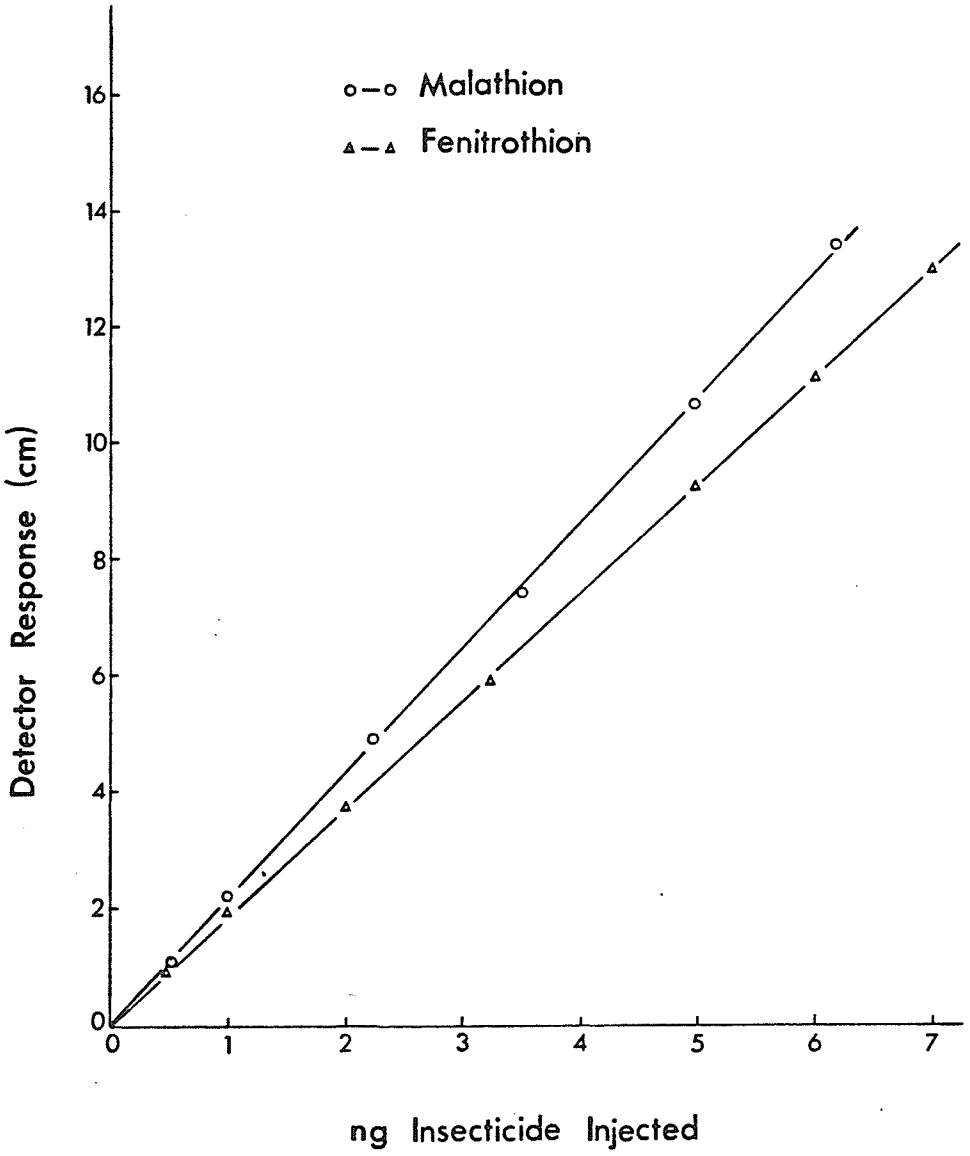



Figure 11: FPD-GLC linearity response to malathion and fenitrothion

### 4.1.3 Analysis of residues in wheat stored at various temperature

Tables 4 and 5 list residues of malathion and fenitrothion, respectively, found on wheat during the 72-week study. Samples were analyzed immediately after sampling except for those taken at 1, 3, and 6 weeks in which case they were analyzed within a week after storage at -35 C. GLC analysis immediately after treatment showed that 7.61 $\pm$ 0.14 ppm malathion and 7.56 $\pm$ 0.12 ppm fenitrothion were initially deposited on wheat. Very little (< 3%) breakdown of both insecticide residues occurred on wheat that had been stored at -35 and -20 C for 72 weeks. Insecticide residues decreased with increase of temperature and duration of storage. After 72 weeks, 74, 59, 26, 5 and 4% of the initial deposit of malathion remained on wheat stored at -5, 5, 10, 20 and 27 C, respectively. Corresponding values for fenitrothion were 82, 65, 44, 10 and 4%. The data show the storage temperature is an important factor influencing the persistence of insecticide residues on grain. In general, the results obtained at 20 and 27 C agreed closely with those obtained by Hyari et al. (1977) and Mensah and Watters (1979) for malathion and Hyari et al. (1977) and LaHue and Dicke (1977) for fenitrothion.

Figure 12 compares percentage residue degradation of both insecticides. There were no apparent differences in the percentage residue levels of both insecticides on wheat stored at -35 or -20 C. As the temperature increased, fenitrothion was more persistent than malathion at each period of assessment, particularly at lower temperatures. At the end of the storage period (504 days), 26, 41, 74% of the initial application of malathion had degraded from wheat stored at -5, 5, and 10 C. Corresponding values for fenitrothion were 18, 35, 56%.

TABLE 4

Malathion residues* ± SD (ppm) on wheat (12.5% mc) after a 7.61±0.14 ppm application of a malathion emulsion spray

| Sampling Period (weeks) | Storage temperature (C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | -35 | -20 | -5 | 5 | 10 | 20 | 27 |
| 1 | 7.66 ±0.12 | 7.61 ±0.09 | 7.59 ±0.11 | 7.53 ±0.13 | 7.50 ±0.11 | 7.08 ±0.16 | 6.62 ±0.12 |
| 3 | 7.58 ±0.01 | 7.58 ±0.10 | 7.45 ±0.11 | 7.24 ±0.15 | 6.75 ±0.09 | 5.53 ±0.14 | 5.19 ±0.26 |
| 6 | 7.53 ±0.16 | 7.45 ±0.10 | 7.37 ±0.10 | 6.90 ±0.21 | 5.60 ±0.19 | 4.92 ±0.22 | 2.74 ±0.18 |
| 12 | 7.51 ±0.10 | 7.34 ±0.21 | 7.15 ±0.08 | 6.39 ±0.23 | 5.21 ±0.12 | 3.11 ±0.12 | 1.88 ±0.11 |
| 24 | 7.54 ±0.06 | 7.49 ±0.09 | 6.95 ±0.12 | 5.74 ±0.18 | 4.05 ±0.22 | 2.13 ±0.16 | 1.20 ±0.25 |
| 36 | 7.25 ±0.13 | 7.50 ±0.13 | 6.83 ±0.10 | 5.26 ±0.20 | 2.86 ±0.13 | 1.31 ±0.20 | 0.75 ±0.15 |
| 48 | 7.55 ±0.11 | 7.45 ±0.12 | 6.63 ±0.14 | 4.99 ±0.15 | 2.14 ±0.25 | 0.96 ±0.13 | 0.53 ±0.14 |
| 72 | 7.53 ±0.10 | 7.48 ±0.07 | 5.66 ±0.24 | 4.48 ±0.26 | 2.01 ±0.11 | 0.41 ±0.11 | 0.32 ±0.10 |

* Average of four replications

TABLE 5

Fenitrothion residues* ± SD (ppm) on wheat (12.5% mc) after a 7.56±0.12 ppm application of fenitrothion emulsion spray

| Sampling Period (weeks) | Storage temperature (C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | -35 | -20 | -5 | 5 | 10 | 20 | 27 |
| 1 | 7.63 ±0.08 | 7.51 ±0.10 | 7.61 ±0.04 | 7.50 ±0.09 | 7.41 ±0.09 | 7.22 ±0.18 | 6.84 ±0.05 |
| 3 | 7.61 ±0.06 | 7.58 ±0.04 | 7.50 ±0.09 | 7.40 ±0.07 | 7.01 ±0.23 | 6.25 ±0.09 | 5.14 ±0.21 |
| 6 | 7.53 ±0.04 | 7.55 ±0.11 | 7.42 ±0.12 | 7.15 ±0.05 | 5.85 ±0.26 | 4.66 ±0.16 | 3.01 ±0.25 |
| 12 | 7.39 ±0.14 | 7.48 ±0.07 | 7.10 ±0.22 | 6.66 ±0.12 | 5.25 ±0.31 | 3.10 ±0.26 | 2.04 ±0.20 |
| 24 | 7.48 ±0.11 | 7.50 ±0.13 | 6.87 ±0.09 | 5.89 ±0.08 | 4.35 ±0.17 | 2.25 ±0.14 | 1.37 ±0.18 |
| 36 | 7.71 ±0.03 | 7.62 ±0.03 | 6.61 ±0.11 | 5.54 ±0.23 | 3.95 ±0.19 | 1.66 ±0.20 | 0.94 ±0.06 |
| 48 | 7.41 ±0.08 | 7.46 ±0.12 | 6.49 ±0.13 | 5.19 ±0.14 | 3.61 ±0.09 | 1.06 ±0.21 | 0.46 ±0.16 |
| 72 | 7.45 ±0.06 | 7.46 ±0.01 | 6.21 ±0.24 | 4.89 ±0.18 | 3.34 ±0.21 | 0.76 ±0.07 | 0.27 ±0.07 |

* Average of four replications

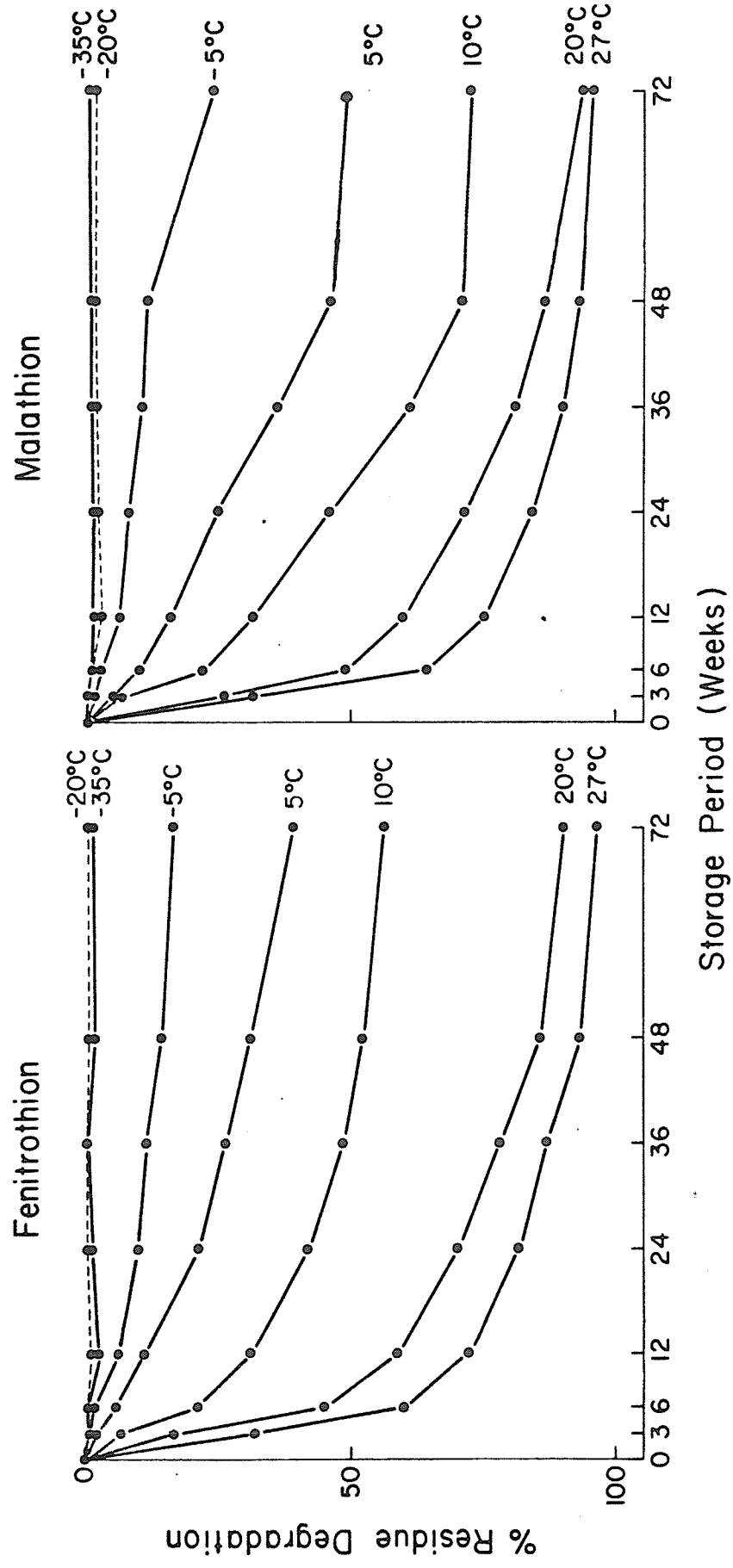



Figure 12: Percentage residue degradation of malathion and fenitrothion in wheat during 72 weeks of storage at various temperatures.

Values in Table 6 (calculated by summation of % insecticide remaining at each sampling period and dividing by total sampling intervals,i.e. 8) compare percent insecticide residues recovered from wheat during the 72 week study. The data confirm the above findings that fenitrothion persisted longer than malathion during the study period.

The presented data can be used as a guide to determine optimium rates of application of malathion and fenitrothion to grain harvested and stored at various temperatures. The data also indicate that fenitrothion may be considered as an alternative to malathion for the protection of stored grain from insect infestation provided its use has been approved for this purpose.

TABLE 6

Mean* percent insecticide residues recovered from wheat (12.5% mc) stored at various temperatures during 72 weeks

| Storage temperature (C) | Insecticide | |
|---|---|---|
| | Malathion | Fenitrothion |
| -35 | 99.2 | 99.6 |
| -20 | 98.3 | 99.3 |
| -5 | 91.3 | 92.2 |
| 5 | 79.6 | 82.9 |
| 10 | 59.4 | 67.4 |
| 20 | 40.1 | 43.7 |
| 27 | 31.5 | 33.2 |

* Mean of four replicates

### 4.1.4 Rate of degradation of insecticides in stored wheat

While present studies were underway, Desmarchelier (1978) reported that the loss of fenitrothion from treated stored wheat held at fixed temperatures and moisture contents is an apparent first-order reaction. The study was conducted under typical tropical storage temperature conditions (i.e., 20 C and higher). It was therefore desirable to examine the data obtained for malathion and fenitrothion from treated wheat stored under typical Canadian prairie storage temperatures (Tables 4 and 5) which are somewhat lower.

In a first-order reaction the rate of decay of insecticide per unit time is proportional to its concentration, i.e.;

$$-dC/dt = kC \qquad (1)$$

$$\ln C/C_o = -kt \qquad (2)$$

where C = insecticide residue at time t

$C_o$ = insecticide residue at t=0

t = time, in weeks, after application

k = rate constant

Rearranging equation (2) gives:

$$\ln C = \ln C_o - kt \qquad (3)$$

Therefore plotting ln C vs. t should give a straight line with a slope equal to - k.

Figures 13 and 14 show the semi-log plots for the degradation of malathion and fenitrothion, respectively, in wheat stored at different temperatures. Straight lines were obtained for both compounds at -35, -5 , 5, and 10 C and two straight intersecting lines at 20 and 27 C.

In the latter cases, the degradation of the insecticides proceeded in two stages, the first rapid and the second slower. The second stages observed for 20 and 27 C were found to be linear indicating that the process at this stage is first order.

Because the curves in Figures 13 and 14 (for 20 and 27 C) appeared to be multi-exponential, they were resolved using a standard graphical resolution method assuming they were composed of two exponentials (simplest case). Thus,

$$C = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \qquad (4)$$

would represent the curves of 20 and 27 C in Figures 13 and 14. In equation (4), $k_1$ , $k_2$ , $A_1$ , and $A_2$ are constants. The values of $k_1$ and $k_2$ are different. If the linear parts of Figures 13 and 14 (i.e., the second slower stages of the 20 and 27 C curves) are assumed to be represented by

$$C = A_2 e^{-k_2 t} \qquad (5)$$

then the values of $A_2$ and $k_2$ can be obtained using semi-log linear curve fitting method. Thus in order to obtain the values of $A_1$ and $k_1$, the calculated values of $A_2 e^{-k_2 t}$ are deducted from the observed values of C during the rapid first stages and the resulting values are plotted as before. According to equation (4) and (5) such a plot should be a straight line:

$$\ln ( C - A_2 e^{-k_2 t} ) = \ln ( A_1 e^{-k_1 t} + A_2 e^{-k_2 t} - A_2 e^{-k_2 t} )$$

$$= \ln ( A_1 e^{-k_1 t} ) = \ln A_1 - k_1 t \qquad (6)$$

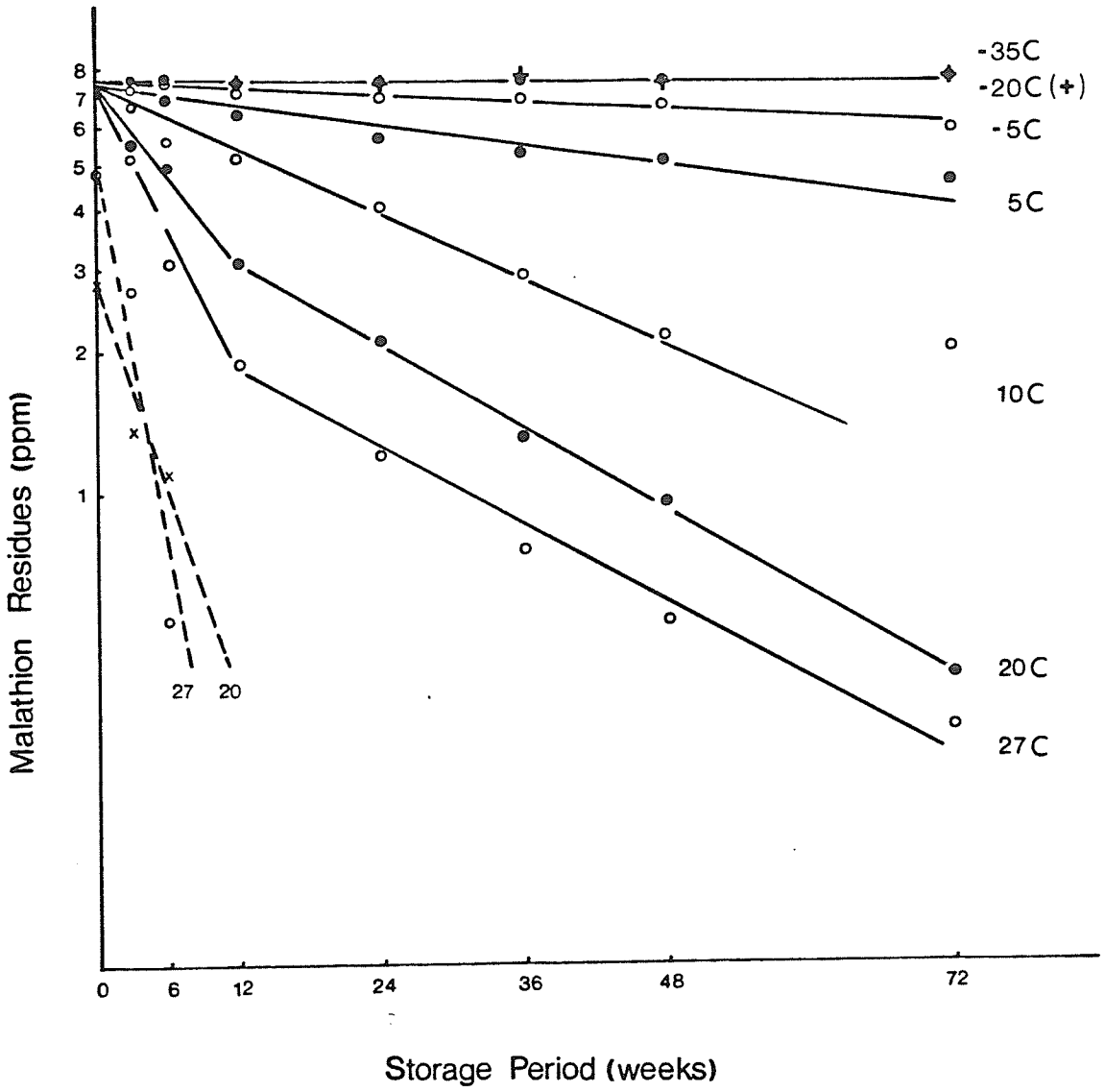



Figure 13: Semilogarithmic plot of malathion concentration vs. time after application (dotted lines show resolved first exponential for 20 and 27 C at 0, 3, and 6 weeks)

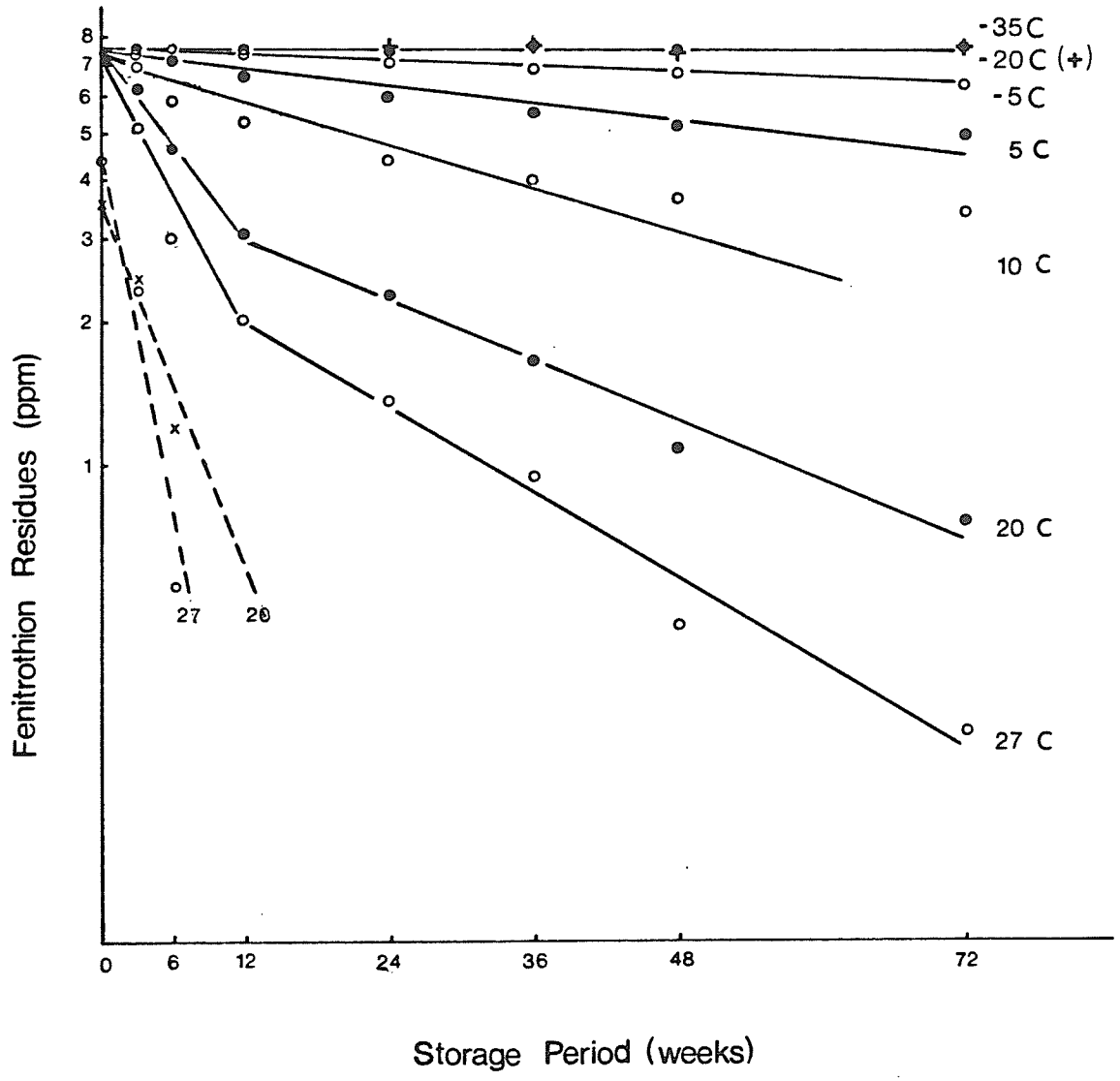



Figure 14: Semilogarithmic plot of fenitrothion concentration vs. time after application (dotted lines show resolved first exponential for 20 and 27 C at 0, 3, and 6 weeks)

The values of $A_1$ and $k_1$ can easily be obtained from such a plot.

This relationship of equation (5) was plotted for both malathion and fenitrothion; the resulting curves appear in Figures 13 and 14 as dotted lines. Tables 7 and 8 show rate constants and correlation coefficients for rate constants k for malathion and fenitrothion, respectively. Correlation coefficients for the data on semi-log plots were better than 0.930 except for degradation at -20 and -35 C.

The two stages of the reaction discussed above may be explained as follows:

(a) In the first stage the insecticide molecules are in contact with grain enzymes which quickly catalyze their metabolism (Rowlands, 1966 a). The duration of this stage is rather short at 20 and 27 C (12 weeks) and increases as the storage temperature decreases.

(b) In the second stage, due to the accumulation of hydrolytic metabolites which inhibit the action of enzymes (for example, phosphatase) the insecticide molecules break down less rapidly. At blocked individual active sites, inhibiting metabolites must themselves break down further to enable these enzyme sites to catalyze the metabolism of parent compound molecules (Rowlands, 1966 a).

Rowlands (1964) attributed the degradation of malathion in stored wheat to chemical and enzymic hydrolysis. He found that the insecticide degraded in both living and autoclaved wheat. In the latter case, it was assumed that no enzymic action could occur. Desmarchelier (1978) suggested that the rate determining step in the break down of fenitrothion is a transport mechanism although the details of the reaction are not fully explained.

TABLE 7

Rate constants and correlation coefficients for loss of malathion on grain

| Temperature (C) | Rate Constant ($week^{-1}$) | Correlation Coefficient |
|---|---|---|
| 27 | 0.3637 | 0.964 |
| 20 | 0.1556 | 0.963 |
| 10 | 0.0256 | 0.993 |
| 5 | 0.0069 | 0.974 |
| -5 | 0.0034 | 0.972 |
| -20 | 0.0007 | 0.355* |
| -35 | 0.0014 | 0.518* |

* The small values are probably due to the fact that the reaction was not long enough and variation between points was higher than variation due to degradation.

TABLE 8

Rate constants and correlation coefficients for loss of fenitrothion on grain

| Temperature (C) | Rate Constant ($week^{-1}$) | Correlation Coefficient |
|---|---|---|
| 27 | 0.3435 | 0.977 |
| 20 | 0.1831 | 0.982 |
| 10 | 0.0155 | 0.958 |
| 5 | 0.0064 | 0.968 |
| -5 | 0.0027 | 0.975 |
| -20 | 0.0001 | 0.213* |
| -35 | 0.0002 | 0.233* |

* The small values are probably due to the fact that the reaction was not long enough and variation between points was higher than variation due to degradation.

Further elucidation of the degradation mechanisms for these insecticides in stored wheat can best be carried out following more detailed examination of the kinetics of these reactions.

### 4.1.5 Effect of temperature on rate of degradation

The effect of temperature on the rate of decay of malathion and fenitrothion follow the Arrhenius equation:

$$k = A \exp (-E_a/RT)$$

Thus, if log k (reaction rate) were plotted vs. the reciprocal of absolute temperature, a straight line would result. Figure 15 shows plots of rate constants vs. the reciprocal of absolute temperatures for malathion and fenitrothion. The plots of k vs. 1/T for both compounds are linear for the temperature region -35 to 27 C. In the plots (Figure 13), $k_1$ values were selected for both 20 and 27 rather than $k_2$ because $k_1$ values provided the best fit straight line with the k values obtained for the lower temperatures. Regression analysis of the slope,

$$\text{slope} = -E_a/(2.303)(1.99)$$

yielded activation energy ($E_a$) equal to 13.37 and 16.18 kcal per mole for malathion and fenitrothion, respectively. The latter value was closely similar to the activation energy value of 13.61 kcal per mole reported by Desmarchelier (1978) for fenitrothion applied to wheat at 10 ppm.

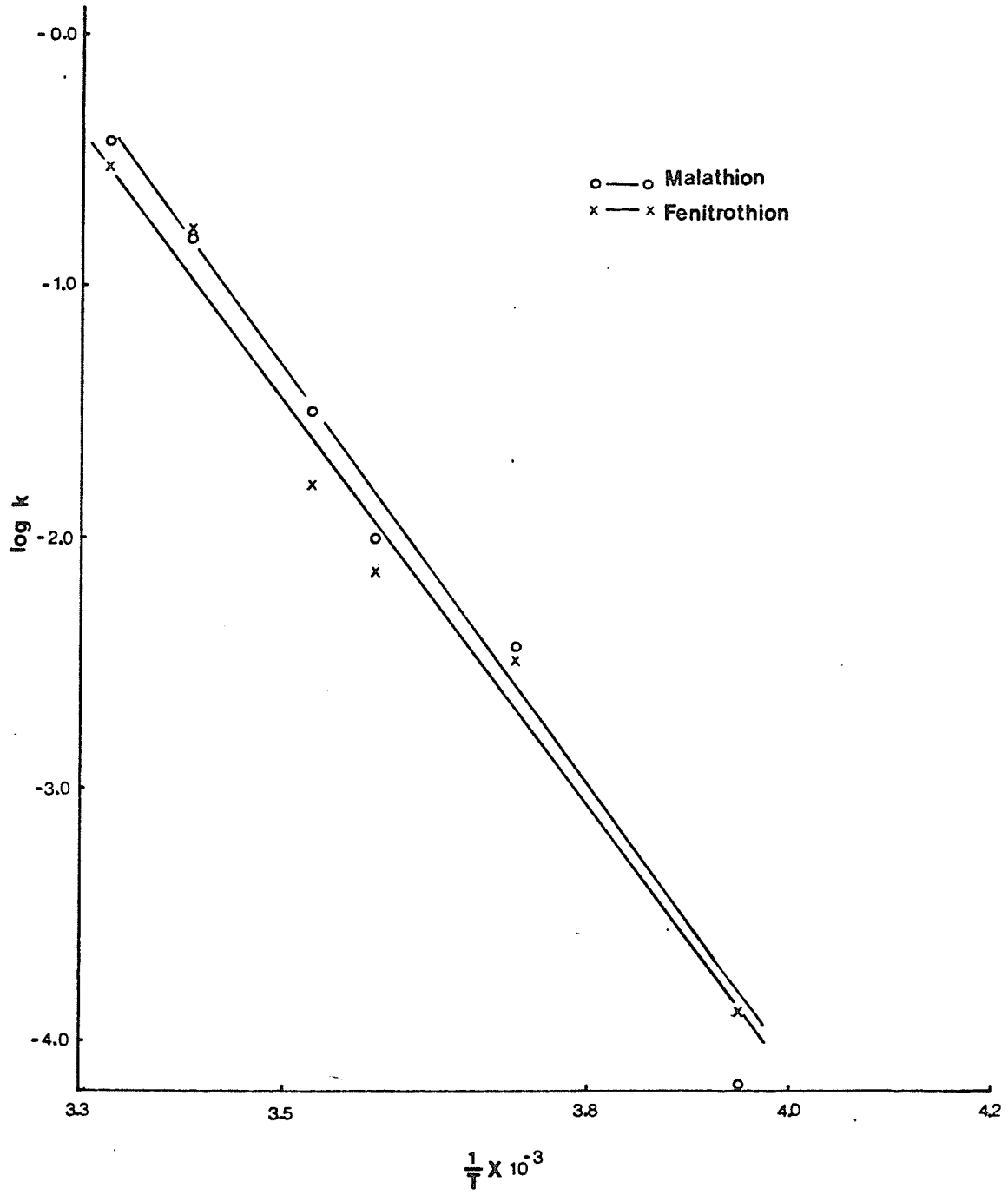



Figure 15: Arrhenius plots of log k vs. reciprocal of absolute storage temperatures for malathion and fenitrothion

### 4.1.6 Wheat germination tests

Table 9 shows there were no measurable differences in germination among the treated samples and the control during the test period. The germination control was only tested prior to insecticide treatment. La-Hue (1976) found that none of the four organophosphorus insecticides tested affected corn germination during his 21-months study.

TABLE 9

Effect of malathion and fenitrothion on wheat germination

| Intervals after treatment (months) | % Germination* | |
|---|---|---|
| | Malathion | Fenitrothion |
| Before treatment | 92.0 | 92.0 |
| 1 | 89.8 | 91.6 |
| 6 | 91.0 | 89.9 |
| 12 | 91.5 | 90.9 |

* Average of four replicates

## 4.2 TRANSLOCATION OF MALATHION AND FENITROTHION WITHIN KERNELS OF WHEAT STORED AT VARIOUS TEMPERATURES

### 4.2.1 Determination of ash content in wheat flour

The ash content of flour samples was determined in order to examine whether or not the flour fraction was contaminated with the bran fraction during milling of the treated wheat. An average value of 0.39 ± 0.01% ash content was obtained. According to Martin and Leonard (1967), the maximium ash content present in uncontaminated flour is 0.42%; a higher value is an indication of contamination with bran. This figure shows that the flour fraction obtained from milled wheat in this study was not contaminated.

### 4.2.2 Residues in milled fractions of wheat stored at different temperatures

Milling of the wheat yielded on average, by weight, 16.5% bran, 12.5% middlings, and 71.0% flour. The bran consists mainly of the outer layers of grain (pericarp, seed coat, and aleurone layer) and middlings are made up of shorts (fine particles of bran), germ and coarse particles of flour.

No insecticide residues were found in the control wheat samples or their milled fractions. Residue analysis of treated wheat immediately after insecticide application showed that most of the intended dosages were recovered; i.e., 98.8 - 98.9% for malathion and 99.1 - 99.3% for fenitrothion. Table 10 shows residue distribution in milled fractions from wheat immediately treated with malathion and fenitrothion at two dosage rates.

TABLE 10

Mean* ± SD malathion and fenitrothion residues (ppm) found on wheat and milled fractions immediately after treatment of wheat at 8 and 12 ppm

| Insecticide | Dosage (ppm) | Whole** | Bran | Middlings" | Flour |
|---|---|---|---|---|---|
| Malathion | 8 | 7.91±0.06 | 26.61±0.63 | 18.81±0.25 | 1.75±0.03 |
| | 12 | 11.87±0.09 | 34.52±0.32 | 28.60±0.28 | 2.81±0.05 |
| Fenitrothion | 8 | 7.95±0.09 | 29.33±0.64 | 15.69±0.26 | 1.45±0.06 |
| | 12 | 11.90±0.13 | 36.18±0.38 | 29.78±0.49 | 2.68±0.11 |

* Mean of four replications

** Ground wheat

" Shorts, wheat germ, and coarse particles of flour

#### 4.2.2.1 Malathion

Table 11 shows residue data of malathion found on whole wheat and in milled fractions. Residues on wheat samples stored at 20 C before and after milling were lower than those on wheat stored at 10 C, which in turn were lower than those at -5 C. After 12 months storage, 6.70, 4.52, and 1.16 ppm malathion were recovered from wheat treated at 8 ppm and stored at -5, 10, and 20 C, respectively. Corresponding residues at the higher application rate were 8.81, 6.75, and 1.91 ppm. Residue data on milled fractions showed higher concentrations of malathion in the bran than in the middlings or flour at each period of assessment and at each storage temperature tested except for malathion applied at 8 ppm on wheat stored at 20 C (3.06 and 2.59 ppm in middlings and bran, respectively). The amount of residues on bran were significantly higher than on the other fractions particularly during the first 3 months of storage and at lower temperatures. Malathion applied at 12 ppm resulted in a residue of 2.81 ppm in the flour fraction immediately after treatment, but this decreased to 2.10, 1.96, and 0.78 ppm after 12 months of storage at -5, 10, and 20 C, respectively.

#### 4.2.2.2 Fenitrothion

Residue degradation of fenitrothion on wheat and in milled fractions (Table 12) was similar to that observed for malathion. In fractions milled from wheat, high residues were found in the bran throughout 12 months of storage except for bran milled from wheat treated at 8 ppm and stored at 20 C. As high as 35.31 ppm fenitrothion was found in the bran in contrast to 28.05 and 2.65 ppm in the middlings and flour, respec-

TABLE 11

TABLE II. Mean* ±SD malathion residues (ppm) found on wheat and milled fractions after 1, 3, 6, and 12 months of storage of wheat treated at 8 or 12 ppm and stored at various temperatures.

| Dosage (ppm) | Storage Period (months) | Storage Temperature (C°) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -5 | | | | 10 | | | | 20 | | | |
| | | Whole** | Bran | Middlings" | Flour | Whole | Bran | Middlings | Flour | Whole | Bran | Middlings | Flour |
| 8 | 1 | 7.84±0.04 | 25.41±0.22 | 17.92±0.15 | 1.66±0.01 | 7.23±0.10 | 22.18±0.44 | 17.01±0.72 | 1.64±0.00 | 6.18±0.12 | 18.27±0.42 | 15.51±0.44 | 1.56±0.03 |
| | 3 | 7.34±0.12 | 23.14±0.13 | 16.83±0.59 | 1.65±0.04 | 6.35±0.07 | 20.16±0.18 | 15.21±0.21 | 1.64±0.02 | 3.55±0.24 | 10.18±0.08 | 9.33±0.96 | 1.10±0.26 |
| | 6 | 6.93±0.16 | 21.72±0.09 | 14.55±0.14 | 1.61±0.03 | 5.81±0.08 | 17.91±0.26 | 12.76±0.16 | 1.56±0.03 | 2.41±0.16 | 6.07±0.11 | 7.01±0.27 | 1.01±0.06 |
| | 12 | 6.70±0.06 | 20.00±0.12 | 13.67±0.07 | 1.45±0.05 | 4.52±0.16 | 14.55±0.13 | 10.04±0.11 | 1.32±0.01 | 1.16 ±0.13 | 2.59±0.05 | 3.06±0.09 | 0.56±0.07 |
| 12 | 1 | 11.53±0.06 | 32.11±0.21 | 27.08±0.40 | 2.73±0.05 | 11.27±0.11 | 30.16±0.19 | 26.31±0.28 | 2.66±0.10 | 10.24±0.16 | 28.97±0.44 | 24.14±0.14 | 2.16±0.11 |
| | 3 | 10.69±0.22 | 31.01±0.41 | 26.01±0.27 | 2.65±0.03 | 9.08±0.12 | 26.22±0.30 | 24.11±0.10 | 2.43±0.08 | 5.62±0.14 | 17.61±0.18 | 14.20±0.33 | 1.65±0.07 |
| | 6 | 9.78±0.16 | 28.67±0.23 | 24.70±0.31 | 2.51±0.03 | 7.96±0.21 | 22.94±0.25 | 21.80±0.07 | 2.25±0.01 | 3.63±0.23 | 9.21±0.25 | 8.01±0.46 | 1.29±0.03 |
| | 12 | 8.81±0.12 | 26.10±0.14 | 22.19±0.25 | 2.10±0.06 | 6.75±0.11 | 19.23±0.17 | 19.24±0.17 | 1.96±0.00 | 1.91±0.13 | 4.64±0.18 | 4.14±0.36 | 0.78±0.08 |

* Mean of 4 replications
** Ground wheat
" Shorts, wheat germ, and coarse particles of flour

tively, immediately after treatment; however, after 12 months, residues were decreased to 28.05, 20.17, and 6.44 ppm in the bran fractions milled from wheat stored at -5, 10, and 20 C, respectively. Corresponding residues were 20.09, 17.07, and 5.55 ppm in the middlings and 2.09, 1.65, and 0.81 ppm in the flour.

#### 4.2.2.3 Comparison of Treatments

The percentage residue degradation of malathion and fenitrothion in whole grain and milled fractions during 12 months of storage of wheat treated at 8 and 12 ppm and stored at three different temperatures are shown in Figures 16 and 17. In whole grain, fenitrothion was lost at a slower rate than malathion at each period of assessment and at each dosage rate and storage temperature tested, except for insecticide applied at 8 ppm to wheat stored at -5 C. After 12 months, residue analysis showed malathion and fenitrothion had degraded 15.2 and 23.1%, respectively. At the end of storage, less malathion than fenitrothion was found in the bran milled from wheat stored at -5, 10, or 20 C. Higher malathion residues than fenitrothion were present in the middlings and flour milled from wheat stored at -5, or 10 C, but at 20 C, more fenitrothion than malathion was recovered in the middlings and flour.

The results of other workers (Schesser et al. 1958; Strong et al. 1961; Eichler and Knoll, 1974; Kadoum and LaHue, 1977; Kadoum et al. 1978; Mensah et al. 1979; Alnaj et al. 1979) were similar to the present study and have shown that the bran and middlings contain the largest insecticide residues; very small amounts of residues were found in the flour at various time intervals after insecticide application to wheat stored at 20 C or higher.

## TABLE 12

TABLE III. Mean* ± SD fenitrothion residues (ppm) found on wheat and milled fractions during 1, 3, 6, and 12 months of storage of wheat treated at 8 or 12 ppm and stored at various temperatures.

| Dosage (ppm) | Storage Period (months) | Storage Temperature (C°) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -5 | | | | 10 | | | | 20 | | | |
| | | Whole** | Bran | Middlings" | Flour | Whole | Bran | Middlings | Flour | Whole | Bran | Middlings | Flour |
| 8 | 1 | 7.91±0.06 | 27.14±0.86 | 15.21±0.11 | 1.39±0.07 | 7.64±0.26 | 26.66±0.35 | 15.01±0.17 | 1.40±0.03 | 6.70±0.14 | 22.10±0.42 | 14.02±0.06 | 1.29±0.07 |
| | 3 | 7.63±0.09 | 25.51±0.22 | 14.66±0.20 | 1.30±0.03 | 6.31±0.04 | 21.90±0.62 | 13.25±0.18 | 1.40±0.01 | 3.81±0.12 | 9.17±0.13 | 10.80±0.39 | 1.04±0.12 |
| | 6 | 6.98±0.17 | 23.57±0.18 | 14.12±0.09 | 1.31±0.03 | 5.63±0.15 | 18.75±0.19 | 11.75±0.23 | 1.32±0.02 | 2.43±0.10 | 6.30±0.08 | 7.00±0.30 | 0.91±0.09 |
| | 12 | 6.11±0.12 | 21.49±0.33 | 13.01±0.03 | 1.24±0.02 | 4.76±0.08 | 14.95±0.08 | 9.75±0.16 | 1.09±0.06 | 1.65±0.07 | 3.61±0.15 | 4.02±0.22 | 0.55±0.04 |
| 12 | 1 | 11.80±0.05 | 35.31±0.27 | 28.05±0.27 | 2.65±0.04 | 11.33±0.08 | 33.40±0.19 | 26.39±0.22 | 2.46±0.07 | 10.55±0.10 | 31.15±0.16 | 24.87±0.08 | 2.51±0.03 |
| | 3 | 10.91±0.16 | 33.92±0.13 | 24.77±0.23 | 2.48±0.04 | 9.26±0.13 | 27.91±0.11 | 22.22±0.16 | 2.17±0.06 | 5.85±0.24 | 14.91±0.41 | 14.61±0.43 | 1.63±0.09 |
| | 6 | 9.89±0.19 | 30.88±0.18 | 21.89±0.24 | 2.29±0.17 | 7.90±0.20 | 22.19±0.76 | 19.28±0.35 | 1.82±0.10 | 3.99±0.14 | 10.01±0.67 | 10.71±0.05 | 1.16±0.15 |
| | 12 | 8.95±0.19 | 28.05±0.55 | 20.09±0.44 | 2.09±0.06 | 6.87±0.08 | 20.07±0.28 | 17.07±0.21 | 1.65±0.12 | 2.35±0.23 | 6.44±0.33 | 5.55±0.05 | 0.81±0.06 |

* Mean of 4 replications
** Ground wheat
" Shorts, wheat germ, and coarse particles of flour

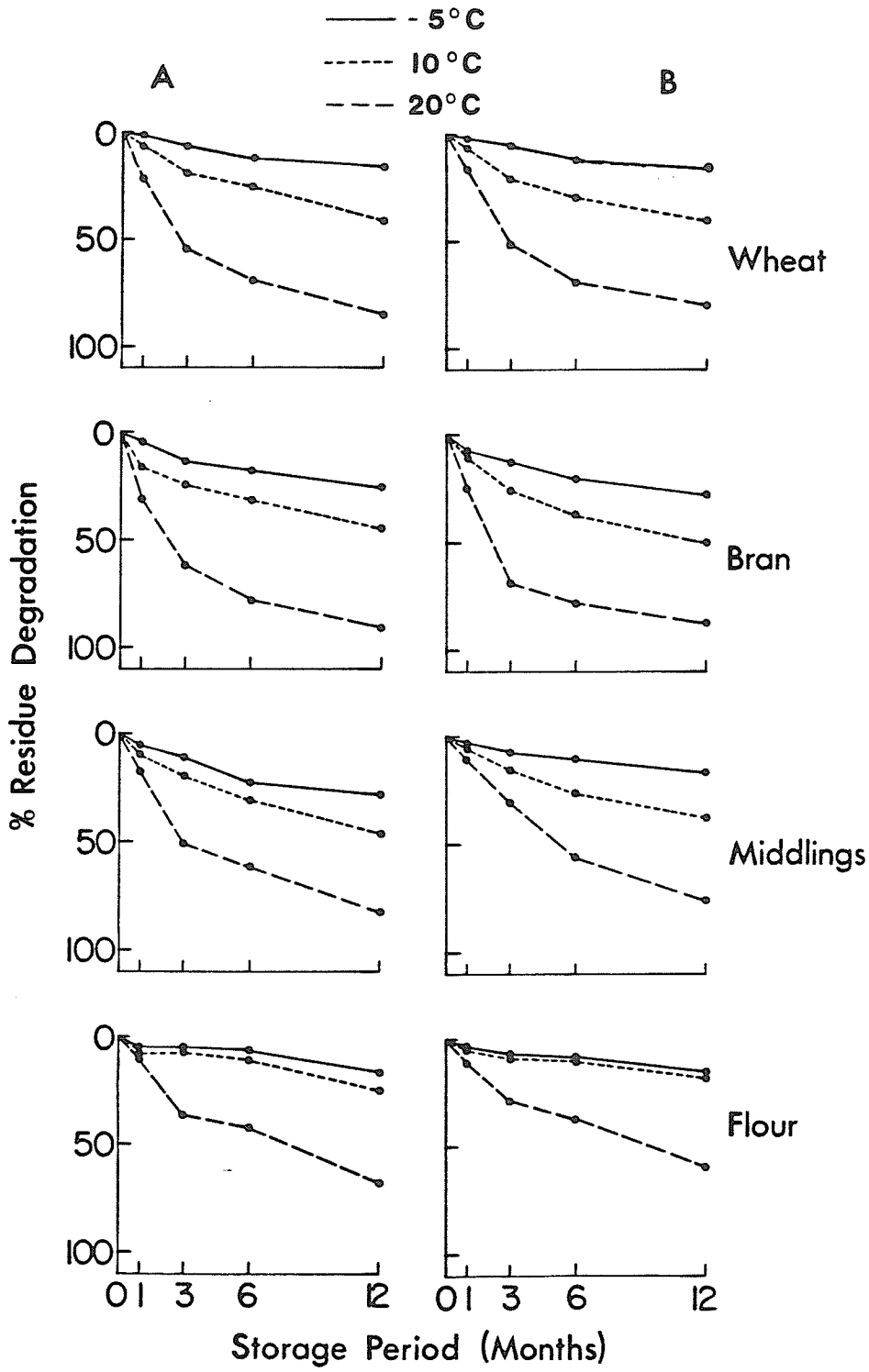



Figure 16: Percentage residue degradation of malathion (A) and fenitrothion (B) in wheat and milling fractions (8 ppm) during 12 months of storage of wheat treated at 8 ppm.

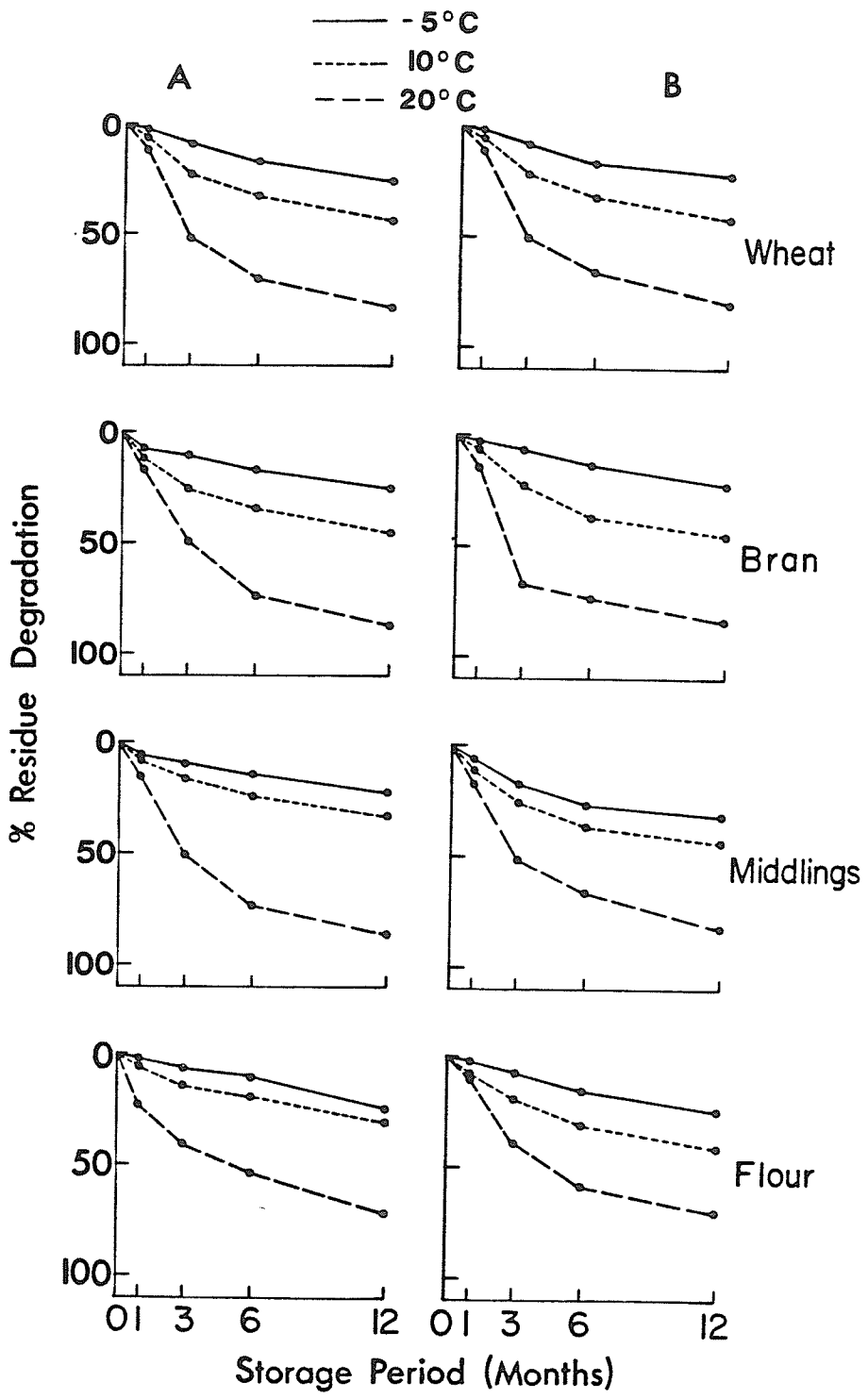



Figure 17: Percentage residue degradation of malathion (A) and fenitrothion (B) in wheat and milling fractions (12 ppm) during 12 months storage of wheat treated at 12 ppm.

In all insecticide treatments, higher residues were recovered from bran and middlings than from flour. Insecticide residues found in milled fractions decreased generally with the increase of storage temperature and duration of storage of treated wheat. The greatest loss on whole wheat occurred, generally, during the first three months after treatment. The largest amounts of insecticide contained in the milled fractions were found immediately and after one month's storage, which indicate a rapid penetration of the insecticides through the pericarp of the grain.

Application of the insecticide at both dosage rates initially resulted in residue accumulation in the bran above the tolerance level (20 ppm of either insecticide) established by the joint FAO/WHO Committee on Pesticide Residues (FAO/WHO 1974, 1976 a,b). Also, malathion applied at 12 ppm resulted in residues higher than the recommended residue limits (2 ppm malathion and 3 ppm fenitrothion) in white flour (FAO/WHO 1974, 1976 a,b, 1980). However, the residues progressively declined to within tolerance limits after 12 months storage except for bran and flour milled from wheat treated with malathion at 12 ppm stored at -5 C. Since bran and middlings contained higher insecticide residues than flour, it is possible that foodstuffs and animal feeds derived from these fractions could pose a significant toxicological hazard if corrective measures are not taken, e.g., cooking. However, bran is often mixed with other ingredients for animal feeds thus reducing the overall concentrations that may be consumed; but unprocessed bran, consumed by humans, could contain excessive levels of residues.

The presented data indicates that fenitrothion residues persisted in and penetrated into wheat similarly to malathion at any of the dosages rates tested and at all storage temperatures used.

#### 4.2.2.4 Insecticide mass penetrated into the endosperm

The above discussion indicates that residue levels of malathion and fenitrothion in the flour fractions milled from wheat stored at -5, -10, and 20 C were generally below the permitted tolerance level except at -5 C. Proportional insecticide distribution in the flour was calculated by multiplying insecticide residue level in the flour fraction at a given time by the percentage yield of flour milled from the wheat kernels (i.e. 71%). The percentage insecticide present in the endosperm was calculated by dividing the value obtained above by the total insecticide residue level detected in whole grain, i.e.;

$$\% \text{ insecticide mass in the flour} = \frac{\text{residue level in the flour X 71\%}}{\text{residue level in whole grain}}$$

The above calculations were made for malathion and fenitrothion in flour fractions milled from wheat treated at 8 and 12 ppm and stored at -5, 10, and 20 C for 12 months (Tables 13 and 14). The data indicate that 15.7 - 16.8% and 12.9 - 14.9% of the initial applied malathion and fenitrothion, respectively, penetrated into the endosperm immediately after treatment. At the end of the storage period, there was a slight increase of the percent insecticide mass in the flour milled from wheat stored at -5 C. As the temperature increases, percent insecticide mass in the flour increased. As high as 34% and 29% malathion and fenitrothion, respectively, were found in the flour after 12 months storage of

wheat. This was probably due to higher degradation rate of the compound in the bran fraction (where enzymes exist) compared to much slower degradation of the insecticide in the flour.

Calculations on percent insecticide residues in the bran fraction milled from wheat stored at 20 C revealed that as high as 56 and 54%, respectively, were found immediately after treatment. This was decreased to 36% for both compounds after 12 months storage.

The data indicates that insecticide mass penetrating into the flour is significant, although the overall residue level in the flour is well below the permitted tolerance level.

TABLE 13

Percent remaining malathion present in the flour milled from wheat stored at various temperatures

| Dose (ppm) | Storage Period (Months) | Storage Temperature (C) | | |
|---|---|---|---|---|
| | | -5 | 10 | 20 |
| 8 | 0 | 15.7 | 15.7 | 15.7 |
| | 1 | 15.1 | 16.1 | 17.9 |
| | 3 | 15.9 | 18.3 | 22.0 |
| | 6 | 16.5 | 19.1 | 29.7 |
| | 12 | 15.4 | 20.7 | 34.3 |
| 12 | 0 | 16.8 | 16.8 | 16.8 |
| | 1 | 16.8 | 16.7 | 17.9 |
| | 3 | 17.6 | 19.0 | 20.8 |
| | 6 | 18.2 | 20.0 | 25.2 |
| | 12 | 16.9 | 20.6 | 28.9 |

TABLE 14

Percent remaining fenitrothion present in the flour milled from wheat stored at various temperatures

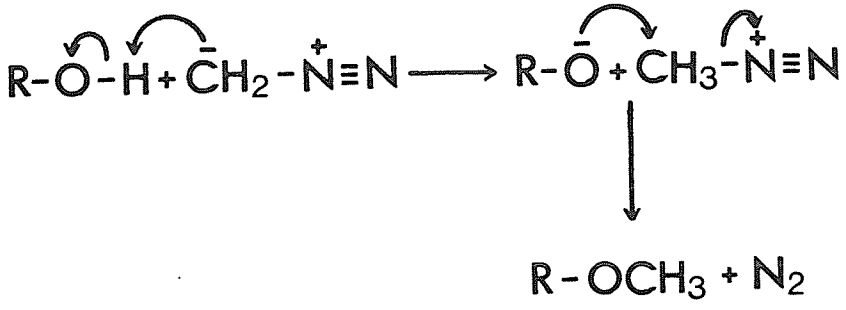
| Dose (ppm) | Storage Period (Months) | Storage Temperature (C) | | |
|---|---|---|---|---|
| | | -5 | 10 | 20 |
| 8 | 0 | 12.9 | 12.9 | 12.9 |
| | 1 | 12.5 | 13.0 | 13.6 |
| | 3 | 12.1 | 15.7 | 19.4 |
| | 6 | 13.3 | 16.6 | 26.6 |
| | 12 | 14.4 | 16.2 | 23.6 |
| 12 | 0 | 15.9 | 15.9 | 15.9 |
| | 1 | 15.9 | 15.4 | 16.8 |
| | 3 | 16.1 | 16.6 | 19.7 |
| | 6 | 16.4 | 16.3 | 20.6 |
| | 12 | 16.5 | 17.0 | 24.5 |

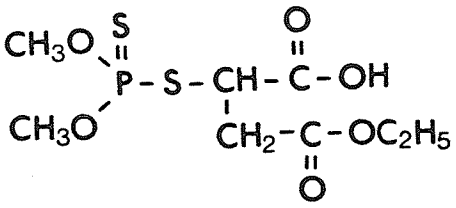
## 4.3 ANALYTICAL METHODS DEVELOPMENT AND QUANTITATIVE DETERMINATION OF MALATHION AND FENITROTHION AND THEIR METABOLITES IN STORED WHEAT

### 4.3.1 Derivatization of malathion and fenitrothion metabolites with diazoalkanes

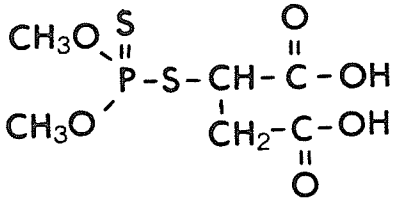
Most metabolites of malathion and fenitrothion (Figure 18) are not chromatographable under GLC conditions used for either malathion or fenitrothion because of their low volatility. This can be increased by converting polar groups such as OH, SH, COOH, to their ethers or esters. Alkylation using diazoalkanes is often employed to esterify carboxylic acids, (Quinon and Hobbs, 1958; Cook and Moore, 1976), dialkyl phosphate derivatives (Stanely, 1966;John and Lisk, 1968, Shafik and Enos, 1969) and phenols (Hilgetag and Martini, 1972; Khan, 1975).

The reaction is quantitative and takes place under mild conditions in an unreactive medium such as ether or alcohol (Hilgetag and Martini, 1972). The reaction mechanism involves protonation of the diazoalkane carbon (Smith, 1937); the alkylation reaction may be written for diazomethane as follows:

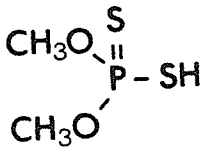




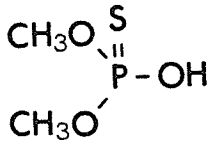
(1) MMA



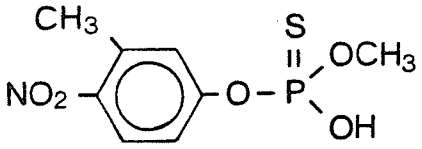
(2) MDA



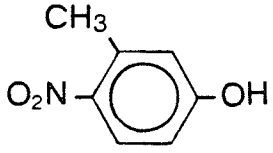
(3) DMPDTA



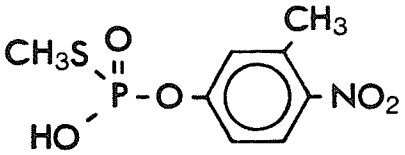
(4) DMPTA



(5) DMF



(6) MNP



(7) DMSMF

Figure 18: Metabolites of malathion (1, 2, 3, and 4) and fenitrothion (4, 5, 6, and 7) that are not directly chromatographable by GLC

Diazomethane and diazoethane were successfully employed in this study for alkylating various metabolites of malathion and fenitrothion.

### 4.3.2 Analytical method development for malathion and its metabolites in wheat

A rapid and simple method has been developed which enables simultaneous GLC analysis of malathion and its four major metabolites in wheat.

#### 4.3.2.1 Standard calibration curves

Figure 19 shows the linearity of the FPD/GLC response to the methyl esters of malathion metabolites; namely, malathion monoacid (MMA), malathion diacid (MDA), dimethyl phosphorothioic acid (DMPTA), and dimethyl phosphorodithioic acid (DMPDTA). These compounds gave a linear response over a range of 0.2 ng to 10 ng and quantification was based on interpolation of sample peak heights and linear regression equation within the linear range of each compound respectively.

#### 4.3.2.2 Extraction efficiency studies

1. One step extraction process:

   Table 15 presents results obtained from acidified acetone extraction of three replicates of 25 gram samples of wheat fortified with a mixture of M, MMA, MDA, DMPDTA, and DMPTA. Recoveries for the five compounds were each greater than 90%.

   The rapid and convenient extraction method, used in this study, gave the highest results when compared to hexane extraction of malathion (62 $\pm$ 3.8%) and Soxhlet extraction which gave a high recovery of malathion but took more time (Norris and Kuchar,

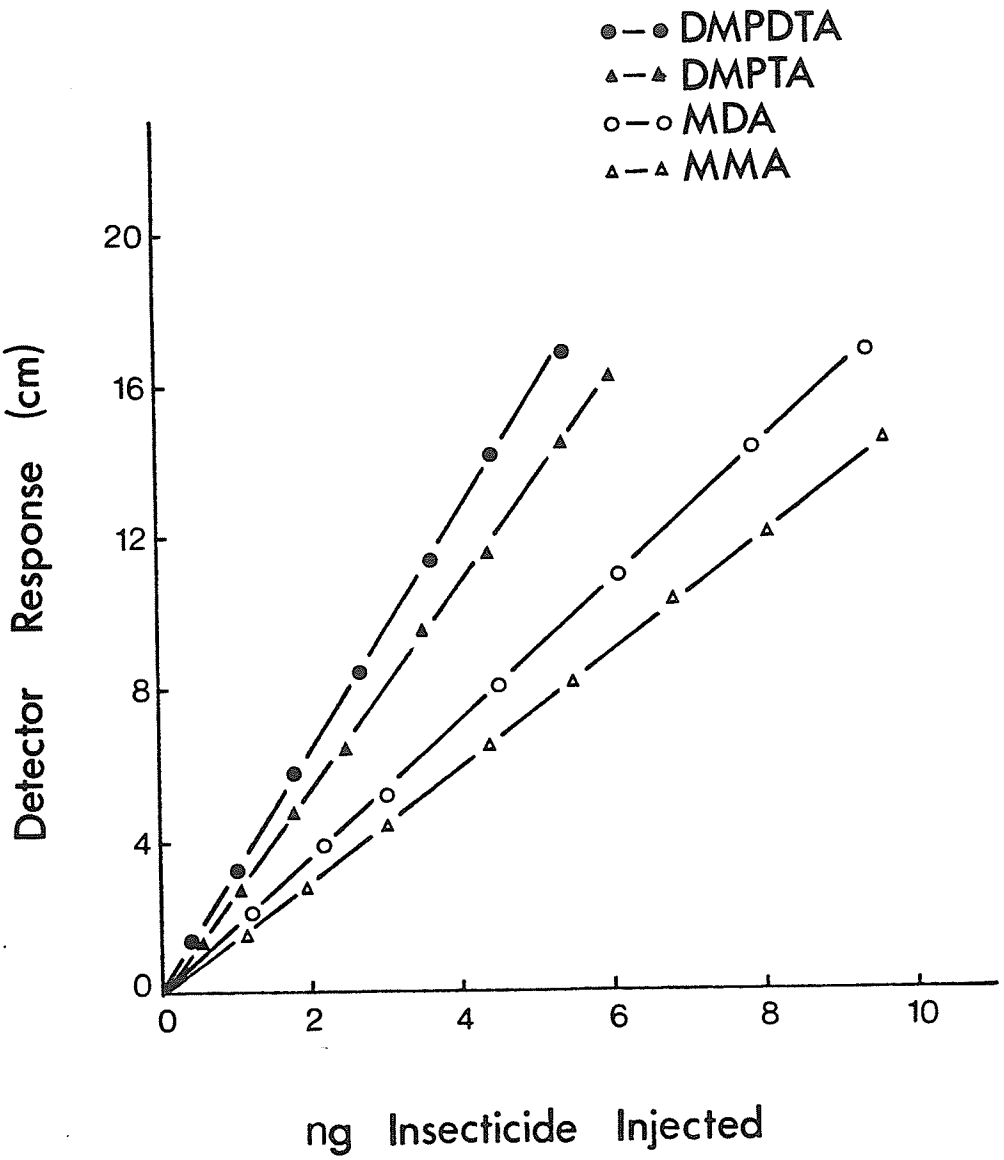



Figure 19: FPD-GLC linearity response to the methyl esters of malathion metabolites

TABLE 15

Recovery data for malathion and metabolites from fortified wheat using the one step extraction process

| Compound | Fortification level (ppm) | % Recovery* |
|---|---|---|
| Malathion | 5.0 | 99.1±0.7 |
| | 1.0 | 98.8±1.0 |
| | 0.5 | 98.5±1.6 |
| | 0.1 | 97.9±1.9 |
| Malathion monoacid | 5.0 | 96.6±2.3 |
| | 1.0 | 96.3±2.8 |
| | 0.5 | 95.8±3.0 |
| | 0.1 | 95.0±3.1 |
| Malathion diacid | 5.0 | 97.2±2.4 |
| | 1.0 | 96.8±2.0 |
| | 0.5 | 95.7±3.2 |
| | 0.1 | 95.2±3.0 |
| Dimethyl phosphorodithioic acid | 5.0 | 94.1±3.1 |
| | 1.0 | 93.9±3.2 |
| | 0.5 | 94.2±2.8 |
| | 0.1 | 93.8±3.1 |
| Dimethyl phosphorothioic acid | 5.0 | 95.6±2.6 |
| | 1.0 | 94.8±2.9 |
| | 0.5 | 94.2±3.0 |
| | 0.1 | 93.9±3.1 |

* Average of three determinations

1959). The addition of 1 mL HCl to acidify the acetone used for extraction increased the percentage recovery for the acidic metabolites only. Therefore, as expected, it is not necessary to acidify the acetone which will be used for extracting malathion only.

The silica gel column cleanup described previously was performed only when a large number of samples were to be analyzed. This was found necessary to avoid saturation of the GLC column with lipids that would alter its separation performance. Figure 20 shows typical chromatograms for malathion and the derivatives of its metabolites.

2. Extraction plus separation of the acidic metabolites (two step procedure):

The above one step extraction procedure may be used for malathion and its metabolites excluding malaoxon which has the same GLC retention time as does (MMA) (Figure 21). However, the second extraction method (two step) while lengthy, involves separation of neutral compounds (e.g., malaoxon) from acidic metabolites (e.g., MMA) (Figure 9), thus enabling the analysis of malathion and its metabolites including malaoxon (Figure 21). The recoveries obtained for these compounds using the two step extraction method (Table 16) compare closely to those obtained using the one step extraction procedure.

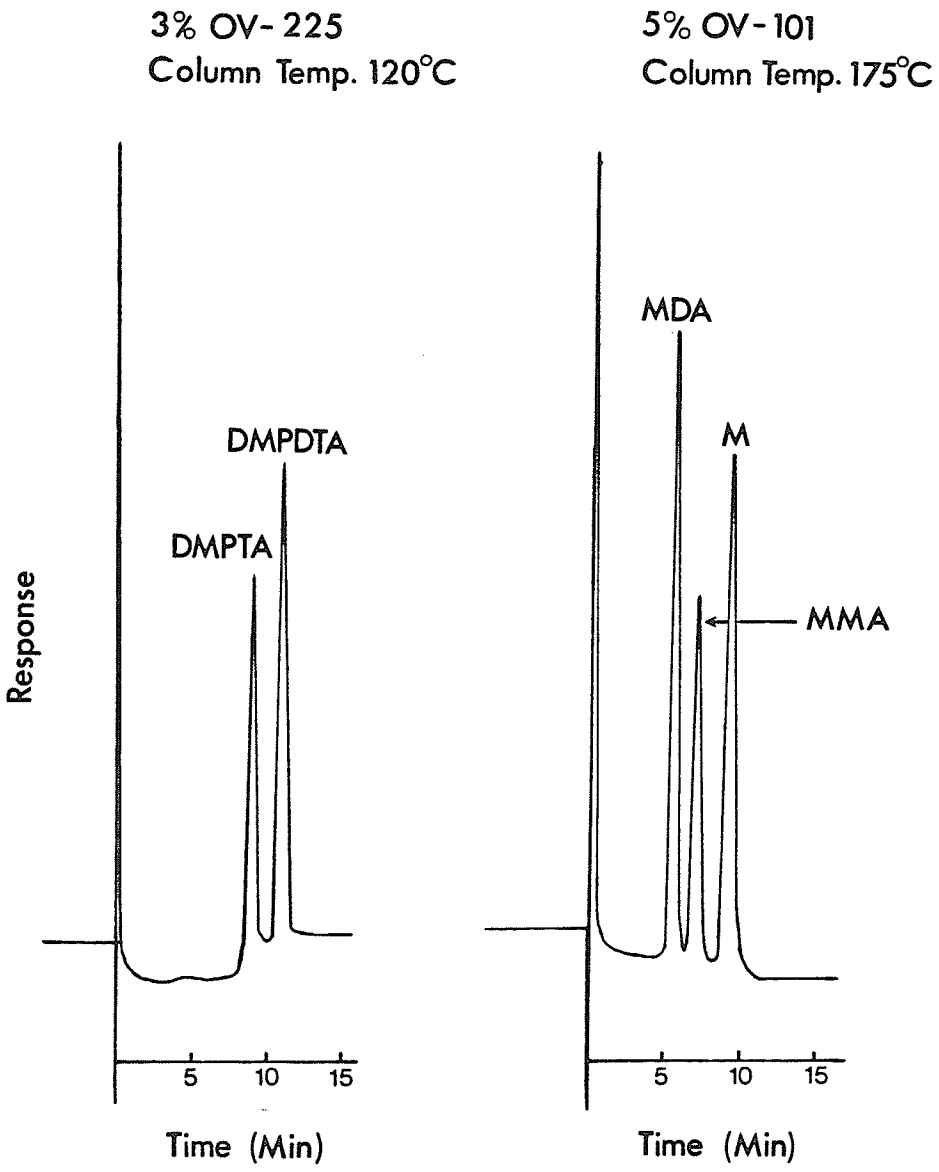



Figure 20: Chromatograms of malathion and the methylated esters of its metabolites from fortified wheat extract

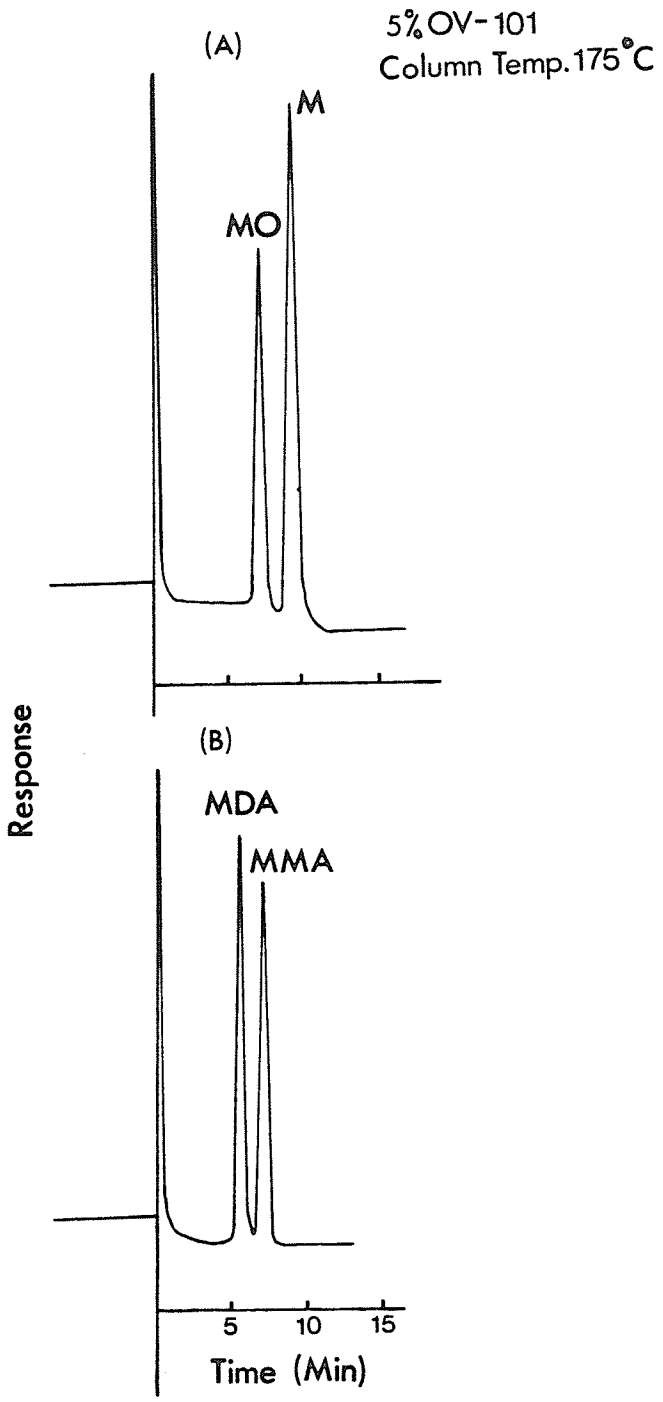



Figure 21: Chromatograms of malathion and metabolites after separation of neutral (A) and acidic (B) compounds

TABLE 16

Recovery data using the second (two step) extraction* method for malathion and its metabolites from wheat fortified at 1.0 ppm

| Compound | % Recovery** |
|---|---|
| Malathion | 98.2±1.3 |
| Malaoxon | 95.7±1.4 |
| Malathion monoacid | 95.3±2.1 |
| Malathion diacid | 95.6±3.1 |
| Dimethyl phosphorodithioic acid | 92.0±2.0 |
| Dimethyl phosphorothioic acid | 91.8±1.9 |

* Extraction incorporating separation of the acidic metabolites

** Average of three determinations

#### 4.3.2.3 Gas-liquid chromatographic analysis

Most organophosphorus pesticides are analyzed at present by GLC using a flame photometric detector (FPD) which is very selective and sensitive to phosphorus containing compounds such as those studied here. In this study, a GLC column containing Chromosorb W coated with either 3% OV-101 or 5% OV-225 was used for the separation of malathion from its derivatized metabolites. Table 17 gives limits of detection and retention times for a mixture of malathion and five of its metabolites.

Under the operating conditions it was possible to separate all five compounds by making two injections into the GLC from the extract obtained from the first extraction method. One injection was made at 80 or 120 C

TABLE 17

Retention times and limits of detection of malathion and its metabolites

| Compound | Retention time (min) | Limits of detection (ng) |
|---|---|---|
| Malathion | 10.0 | 0.05 |
| Malathion monoacid* | 7.2 | 0.20 |
| Malathion diacid* | 5.3 | 0.20 |
| Malaoxon | 7.2 | 0.50 |
| Dimethyl phosphorothioic acid* | 9.4** | 0.20 |
| Dimethyl phosphorodithioic acid* | 11.2** | 0.30 |

* Methylated compound
** 3% OV-225, Column temperature 120 C.

on OV-101 or OV-225, respectively to resolve DMPTA, DMPDTA. The second injection was made at 175 C on OV-101 to separate MMA, MDA, and M (Figure 20). Alternatively, one injection may be made to separate all five compounds on OV-101 by employing temperature programming (75 - 95 C, 2 C/min; 95 - 180 C, 5 C/min).

GLC analysis of extracts (3 fractions) obtained from the second (two step) extraction procedure (i.e., separation of acidic (2 fractions) and neutral compounds) was performed under the some operating conditions. Injection of the ether fraction at 80 C resulted in separation of methylated DMPTA and DMPDTA. Injection of the methylene chloride extract on OV-101 at 175 C gave a good resolution of malathion and malaoxon while injecting the ethyl acetate extract at the same temperature resulted in separation of methylated MMA and MDA (Figure 21 ). As seen malaoxon has an identical retention time to that obtained for the methylated MMA. Therefore, the separation of neutral compounds from the acidic metabolites by partitioning with solvents was, other than enabling determination of all metabolites, investigated to confirm the identity (to be discussed in detail later) of the peak at retention time of 7.2 min as either malaoxon or MMA.

### 4.3.3 Analytical methods development for fenitrothion and its metabolites in wheat

The rapid, simple and reliable method described below has been developed for the extraction and simultaneous analysis of fenitrothion and its major metabolites in stored wheat.

#### 4.3.3.1 Standard calibration curves

Figure 22 shows the linearity of the FPD/GLC response to fenitrothion metabolites; namely, fenitrooxon (FO), S-methyl fenitrothion (SMF), O-demethyl fenitrothion (DMF), O-demethyl S-methyl fenitrothion (DMSMF), and dimethyl phosphorothioic acid (DMPTA). These compounds gave linear response over a range of 0.5 ng to 7 ng. The linear range of the electron capture (ECD/GLC) response to 3-methyl-4-nitrophenol (MNP) (Figure 22) was 0.3 ng to 10 ng.

#### 4.3.3.2 Extraction efficiency studies

Table 18 shows results obtained from acidified acetone extraction of three 25 g replicates samples of wheat fortified with a mixture of F, FO, SMF, DMF, DMSMF, DMPTA, and MNP. Average recovery for all compounds ranged from 90.2 to 97.8% at the four fortification levels tested.

This rapid and convenient extraction method may be employed if the fenitrothion residue level is present in small amounts. If fenitrothion is present in high concentrations, this would alter the separation (by cochromatographing with DMF), and consequently, the quantification of DMF (Figure 23). Dilution of the sample would result in an overall DMF concentration below its detection limit. Such a problem could be overcome by the use of the second (two step) method of extraction which is based on separation of neutral compounds, e.g., fenitrothion from acidic metabolites, e.g., DMF, followed by making two injections into the GLC to resolve each group separately (see below).

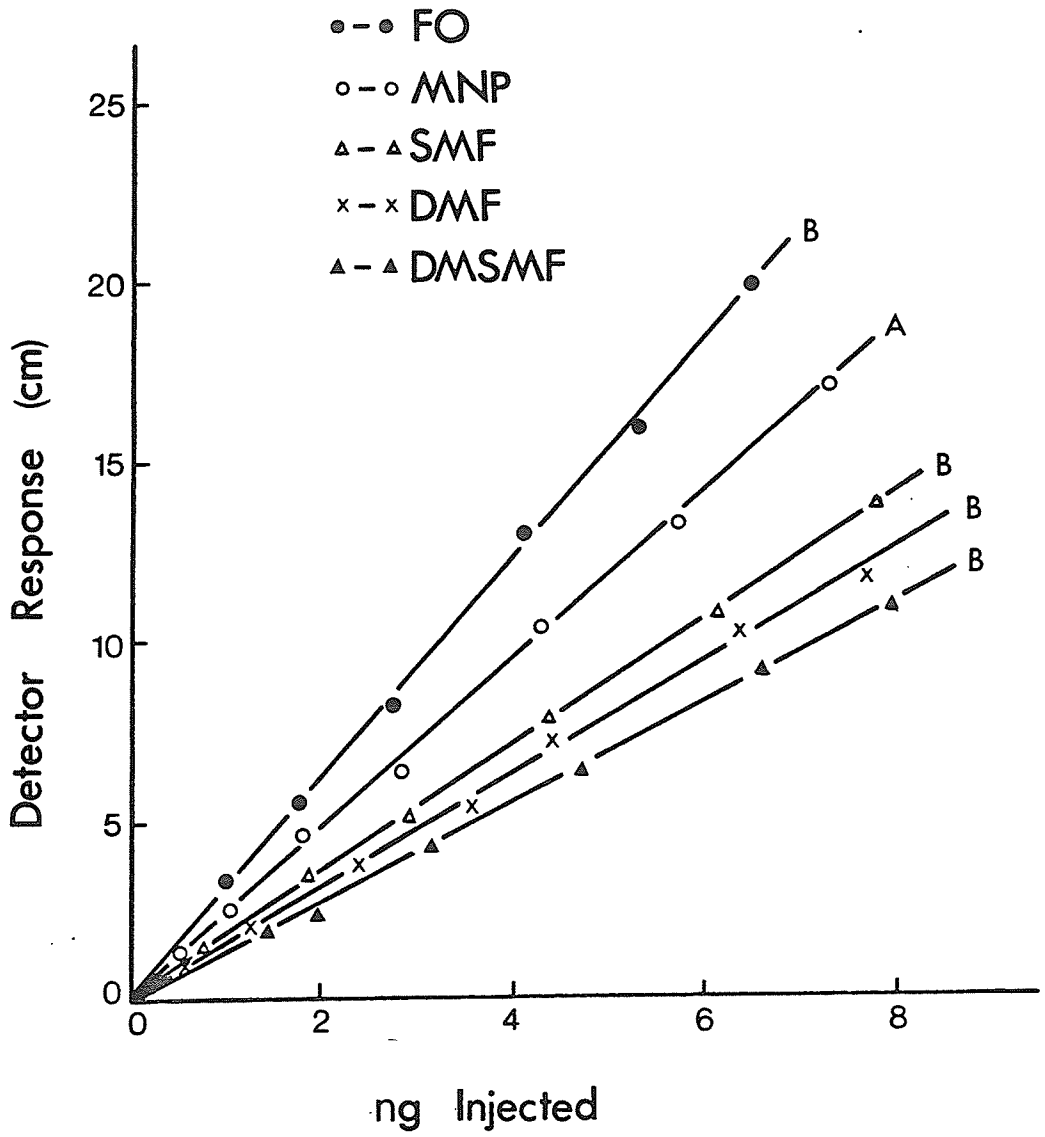



Figure 22: Linearity response of EC (A) or FPD (B) GLC to several metabolites of fenitrothion

TABLE 18

Recovery data for fenitrothion and its metabolites from fortified wheat samples

| Compound | Fortification level (ppm) | % Recovery* |
|---|---|---|
| Fenitrothion | 5.0 | 97.8±0.9 |
| | 1.0 | 97.2±1.1 |
| | 0.5 | 97.0±0.8 |
| | 0.1 | 96.7±2.2 |
| Fenitrooxon | 5.0 | 96.9±2.1 |
| | 1.0 | 96.5±2.1 |
| | 0.5 | 96.1±1.8 |
| | 0.1 | 95.8±2.0 |
| O-demethyl fenitrothion | 5.0 | 92.4±1.8 |
| | 1.0 | 92.1±2.0 |
| | 0.5 | 91.8±1.9 |
| | 0.1 | 91.0±2.0 |
| S-methyl fenitrothion | 5.0 | 93.0±1.1 |
| | 1.0 | 93.1±1.7 |
| | 0.5 | 91.1±1.6 |
| | 0.1 | 90.8±2.8 |
| 3-methyl-4-nitrophenol | 5.0 | 92.6±2.3 |
| | 1.0 | 91.7±1.8 |
| | 0.5 | 90.9±0.9 |
| | 0.1 | 90.2±1.3 |
| O-demethyl S-methyl fenitrothion | 5.0 | 93.3±1.3 |
| | 1.0 | 93.1±2.1 |

* Average of three determinations

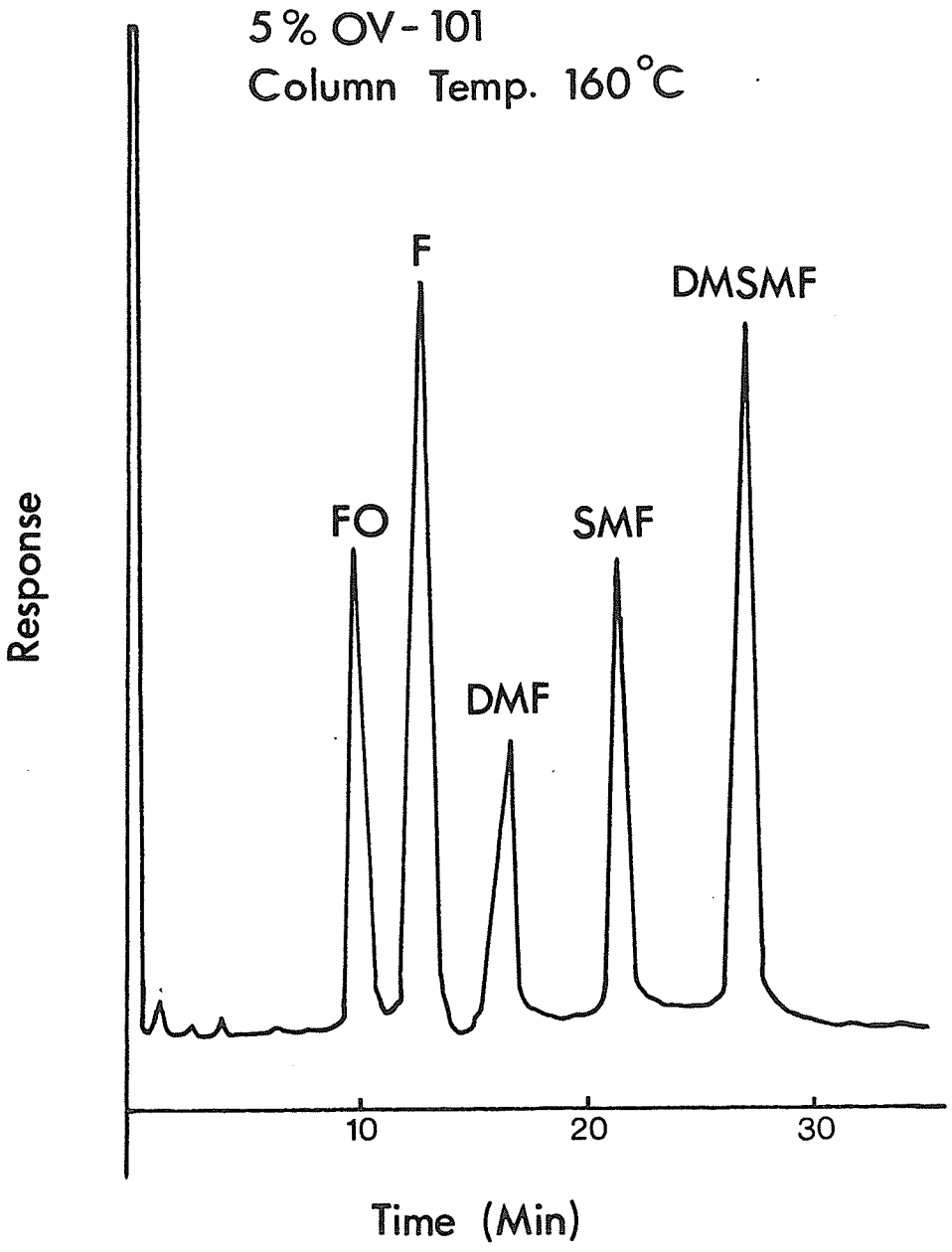



Figure 23: Chromatogram of fenitrothion and metabolites from fortified wheat extract

#### 4.3.3.3 Gas-liquid chromatographic analysis

Figure 23 shows typical FPD/GLC response to fenitrothion and four of its metabolites using the column at 160 C packed with Chromosorb W coated with 5% OV-101. EC/GLC response to MNP is also shown in Figure 24 using the column at 120 C packed with Chromosorb W coated with 3% OV-101. Table 19 presents detection limits and retention times for fenitrothion and its metabolites.

GLC analysis of extracts obtained from the second extraction procedure was performed under the same operating conditions. Three peaks resulted when the methylene chloride extract was injected. These peaks were FO, F, and SMF. Injection of the methylated ethyl acetate extract resulted in two peaks: DMF and DMSMF (Figure 25).

Such a separation of the two groups of compounds (i.e., neutral and acidic) enables the quantitation of DMF if fenitrothion residue level is present in high concentration.

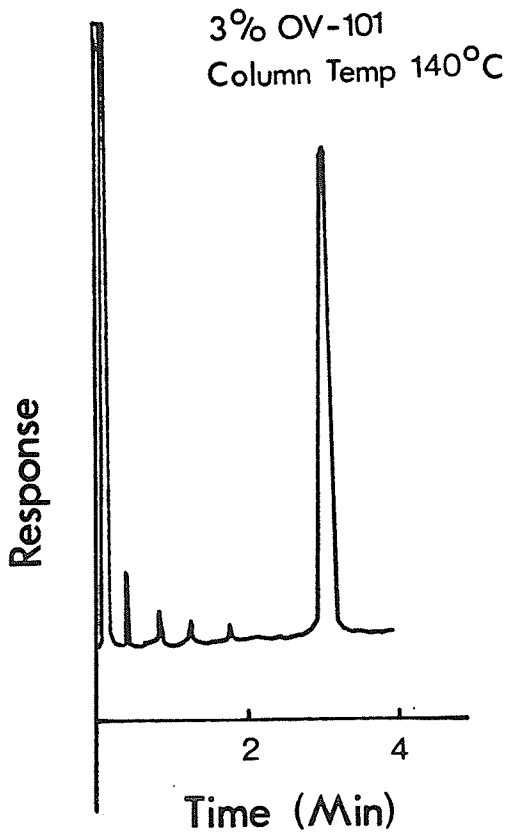



Figure 24: Chromatogram of MNP from fortified wheat extract

TABLE 19

Retention times and limits of detection of fenitrothion and its metabolites

| Compound | Retention time (min) | Limits of detection (ng) |
|---|---|---|
| Fenitrothion | 15.5 | 0.05 |
| Fenitrooxon | 11.5 | 1.0 |
| O-demethyl fenitrothion* | 20.0 | 1.0 |
| S-methyl fenitrothion | 25.6 | 0.5 |
| O-demethyl S-methyl fenitrothion* | 32.2 | 1.0 |
| 3-methyl-4-nitrophenol | 2.6** | 0.3 |

* Ethylated compound
** 3% OV-101, Column temperature 140 C.

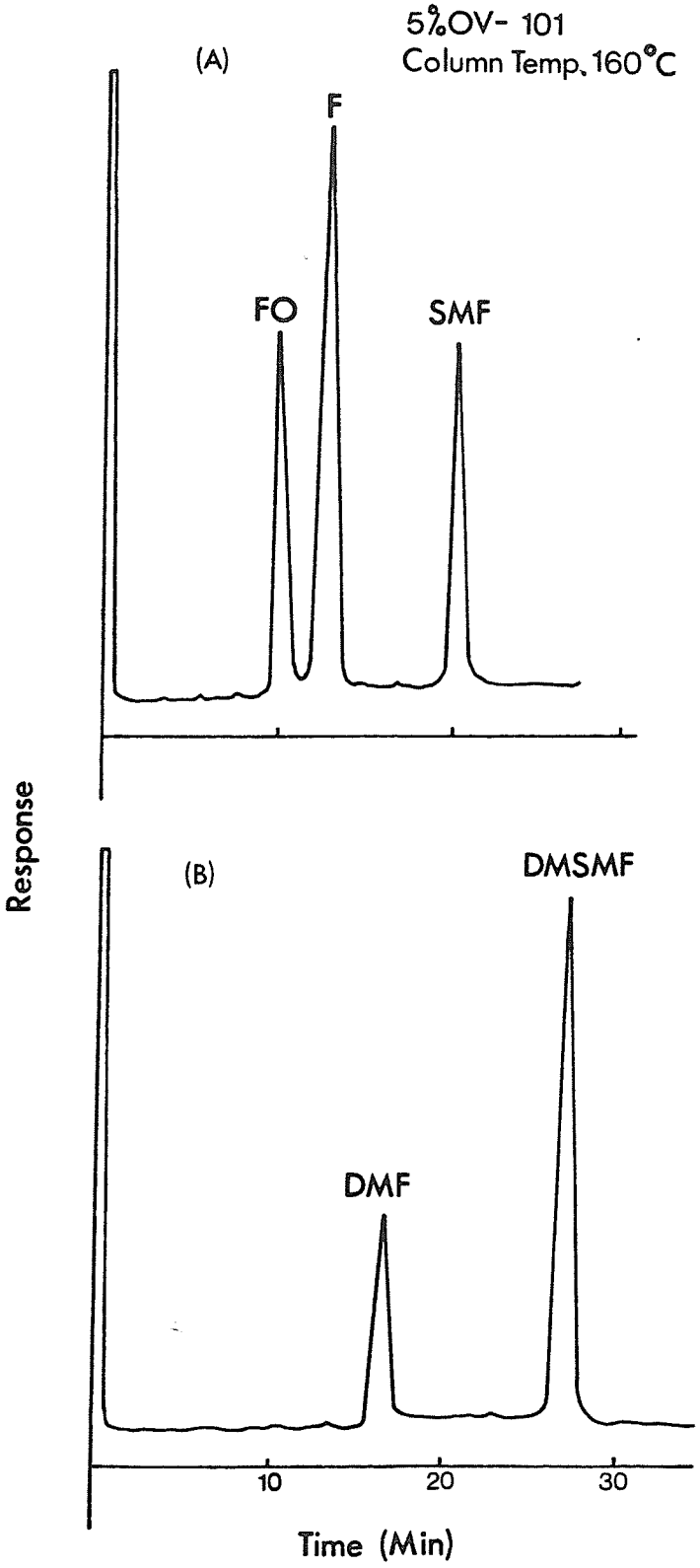



Figure 25: Chromatograms of fenitrothion and metabolites after separation of neutral (A) and acidic (B) compounds

### 4.3.4 Quantitative determination of malation and fenitrothion and metabolites in stored wheat

#### 4.3.4.1 Quantification of malathion metabolites

Table 20 and Figure 26 show residues of malathion metabolites found on wheat stored at 20 C during the 12-month study. Malathion residue levels shown in Table 11 for wheat treated at 12 ppm and stored at 20 C are plotted in Figure 26. Malathion monoacid (MMA) and dimethyl phosphorodithioic acid (DMPTA) were the major metabolites found during the study. The highest amount of DMPDTA (1.11 ppm) was detected after three months storage; it decreased to 0.26 ppm by the end of the study. As high as 1.85 ppm MMA was found in treated wheat stored for 6 months. This level started to decrease with the appearance of MDA consistent with conversion of MMA to MDA. At the end of storage, the MMA level had decreased to 0.48 ppm while the MDA level was 0.65 ppm. Rowlands (1964) identified malathion metabolites by TLC as DMPDTA and MDA but could not identify MMA. However, he used wheat of 18% moisture content which may be the factor for the fast conversion of MMA to MDA (water is a prerequisite for enzymic activity) during the first few months.

No malaoxon above the detection limit of 0.1 ppm was detected in wheat (eight months old) throughout the experiment. Rowlands (1966 b) showed that, when freshly harvested wheat was treated with malathion, production of malaoxon was initially rapid and continued for three to four weeks. A sample of the same bulk treated at one week after harvest produced malaoxon over one week and treatment of similar samples at two to eight weeks after harvest showed almost no oxidative activity.

Figure 27 suggests degradation pathways of malathion in stored wheat. It appears that DMPDTA may be formed from both malathion and malathion monoacid.

TABLE 20

Mean* ± SD residues of malathion metabolites (ppm) found on wheat after 1, 3, 6 and 12 months storage at 20 C

| Sampling Period (mo) | MO | MMA | MDA | DMPDTA |
|---|---|---|---|---|
| 1 | ND** | 0.66±0.09 | ND" | 0.55±0.15 |
| 3 | ND | 1.22±0.07 | ND | 1.11±0.21 |
| 6 | ND | 1.85±0.16 | 0.11±0.06 | 0.86±0.24 |
| 12 | ND | 0.48±0.17 | 0.65±0.14 | 0.26±0.10 |

* Mean of four determinations

** Not detected < 0.01 ppm

" Not detected < 0.05 ppm

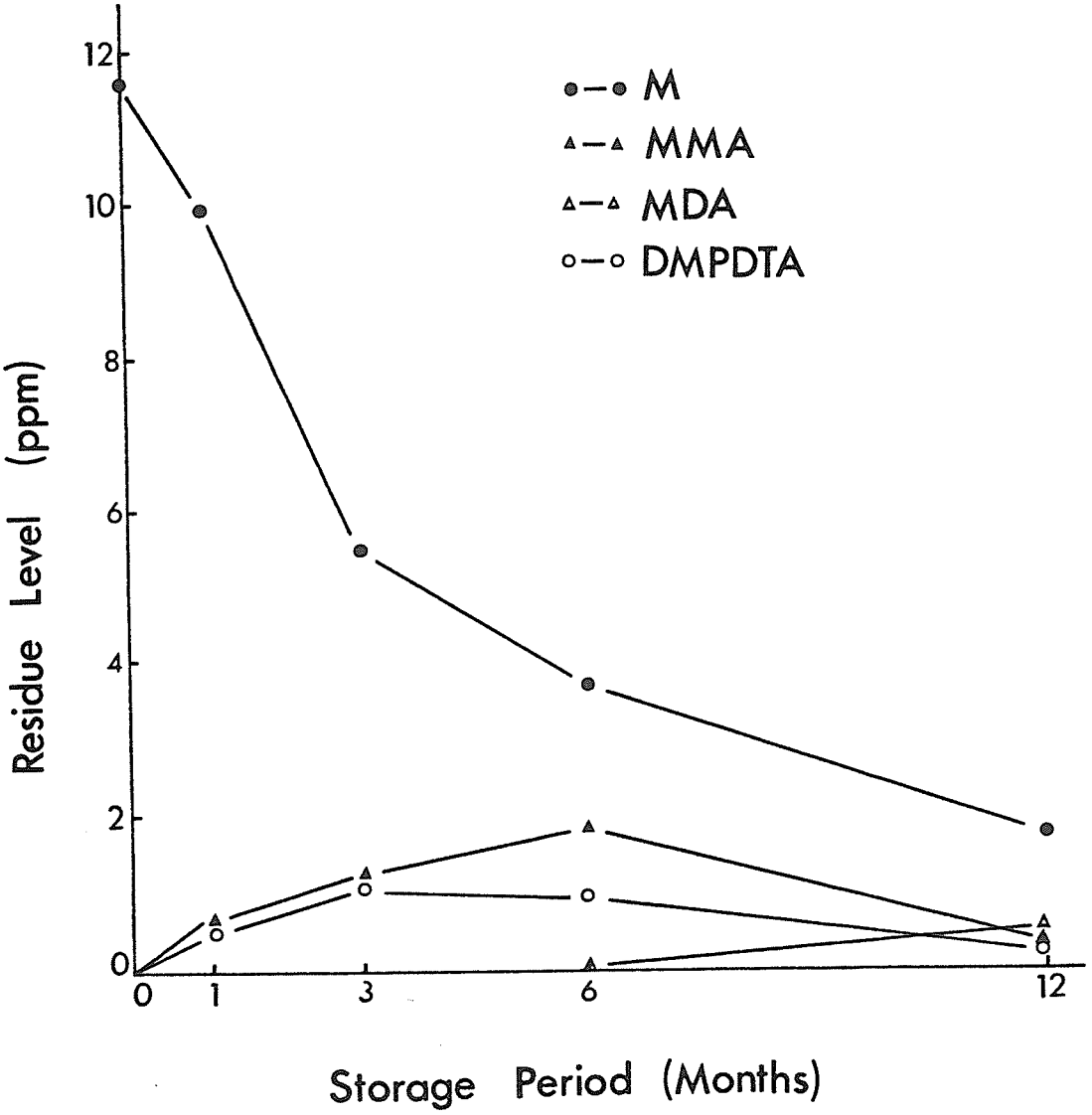



Figure 26: The breakdown and metabolic products of malathion on wheat stored at 20 C

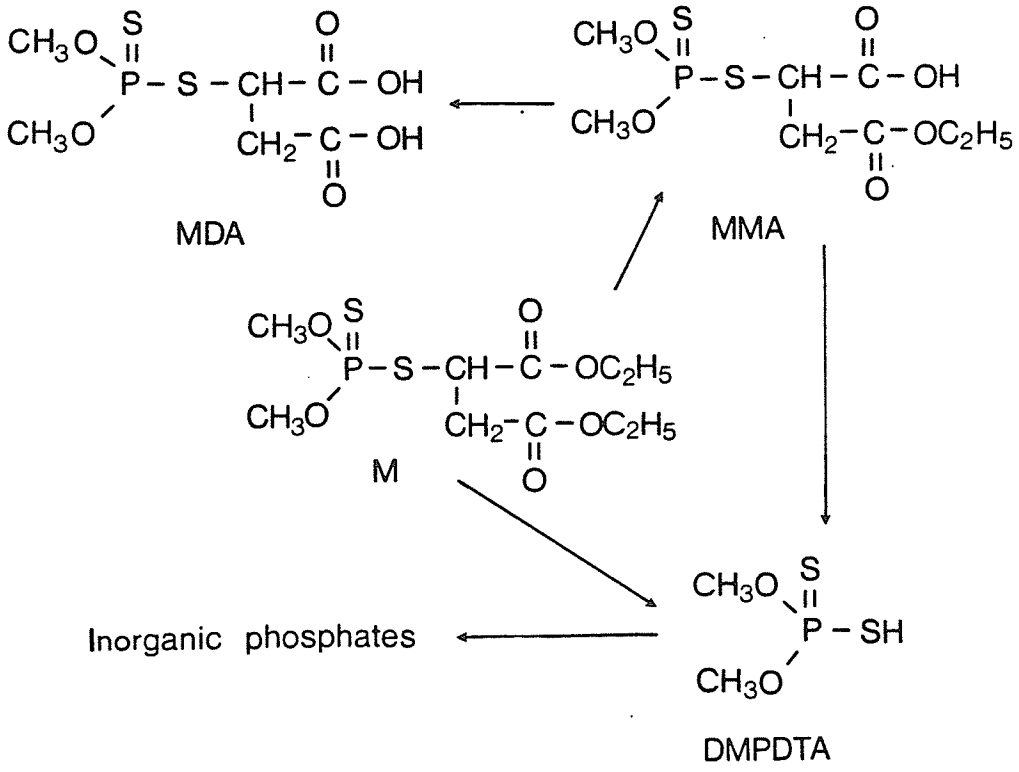



Figure 27: Suggested degradation pathways of malathion in stored wheat

#### 4.3.4.2 Quantification of fenitrothion metabolites

Table 21 and Figure 28 present residue levels of fenitrothion metabolites found in wheat during 12 months storage. Fenitrothion residue levels obtained from Table 12 for wheat treated at 12 ppm and stored at 20 C are also shown in Figure 28. O-demethyl fenitrothion (DMF), 3-methyl-4-nitrophenol (MNP) and dimethyl phosphorothioic acid (DMPTA) were the main metabolites. The highest levels of DMF and DMPTA (2.01 and 0.55 ppm, respectively) were found after 6 months storage; they decreased to 0.98 ppm DMF and 0.21 ppm DMPTA at the end of storage. MNP showed an initial value of 0.38 ppm after one month storage but continued to increase with the duration of storage. After 12 months, MNP level reached 0.96 ppm.

No fenitrooxon or S-methyl fenitrothion, above the detection limits of 0.1 and 0.15 ppm, respectively, was detected throughout the study period.

Figure 29 suggests degradation pathways of fenitrothion in stored treated grain. It appears that production of MNP is probably from fenitrothion and O-demethyl fenitrothion. The maximum DMF residue level was found after 6 months storage. This level started to decrease with gradual increase of MNP residue level, presumably from the degradation of DMF as well as fenitrothion.

TABLE 21

Mean* ± SD residues of fenitrothion metabolites (ppm) found on wheat after 1, 3, 6 and 12 months storage at 20 C

| Sampling period (months) | FO | DMF | SMF | DMPTA | MNP |
|---|---|---|---|---|---|
| 1 | ND** | 0.56±0.22 | ND" | 0.25±0.08 | 0.38±0.11 |
| 3 | ND | 1.46±0.11 | ND | 0.45±0.13 | 0.55±0.13 |
| 6 | ND | 2.01±0.28 | ND | 0.55±0.09 | 0.76±0.14 |
| 12 | ND | 0.98±0.14 | ND | 0.21±0.11 | 0.96±0.21 |

* Mean of four determinations

** Not detected < 0.01 ppm

" Not detected < 0.05 ppm

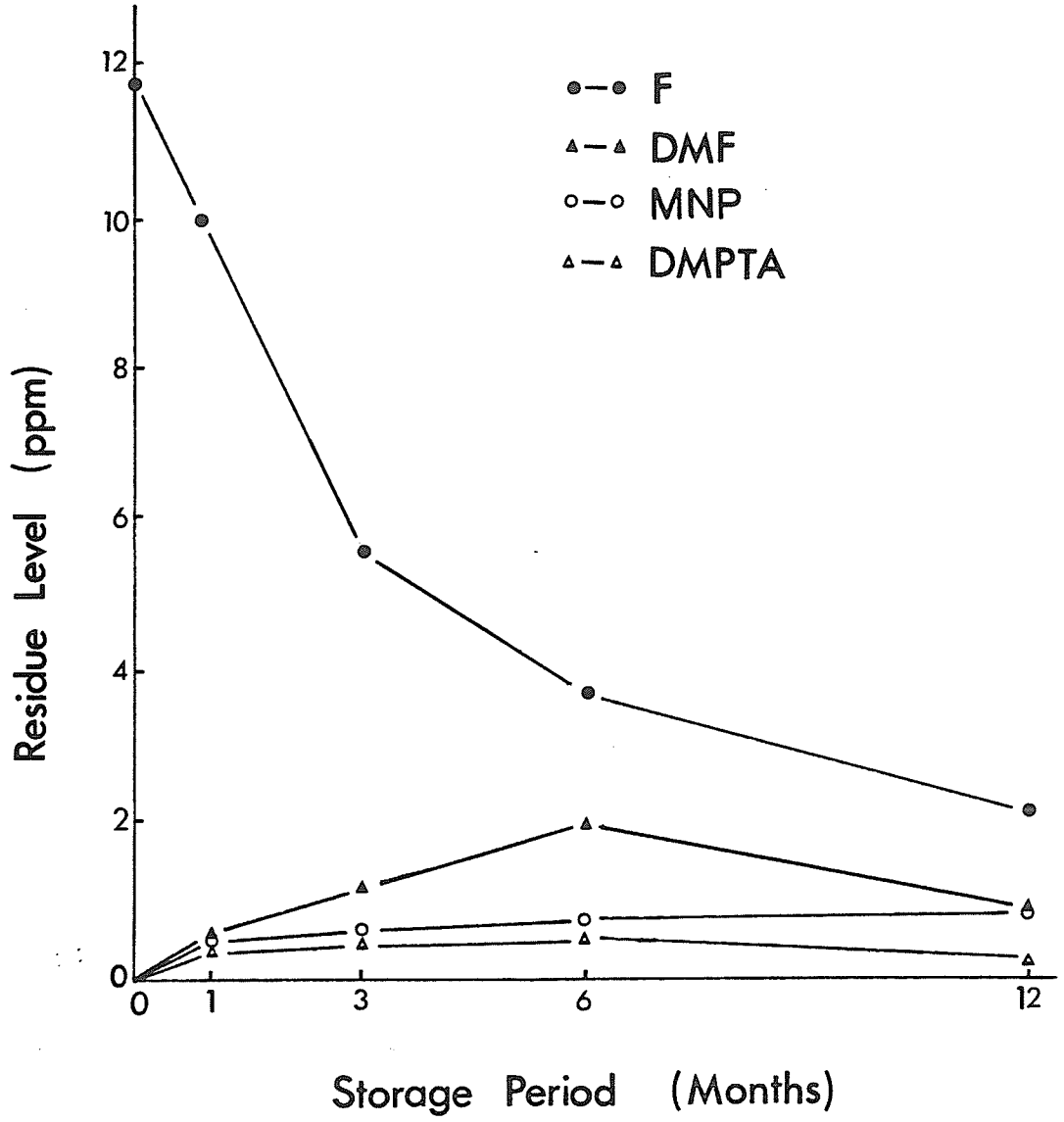



Figure 28: The breakdown and metabolic products of fenitrothion on wheat stored at 20 C

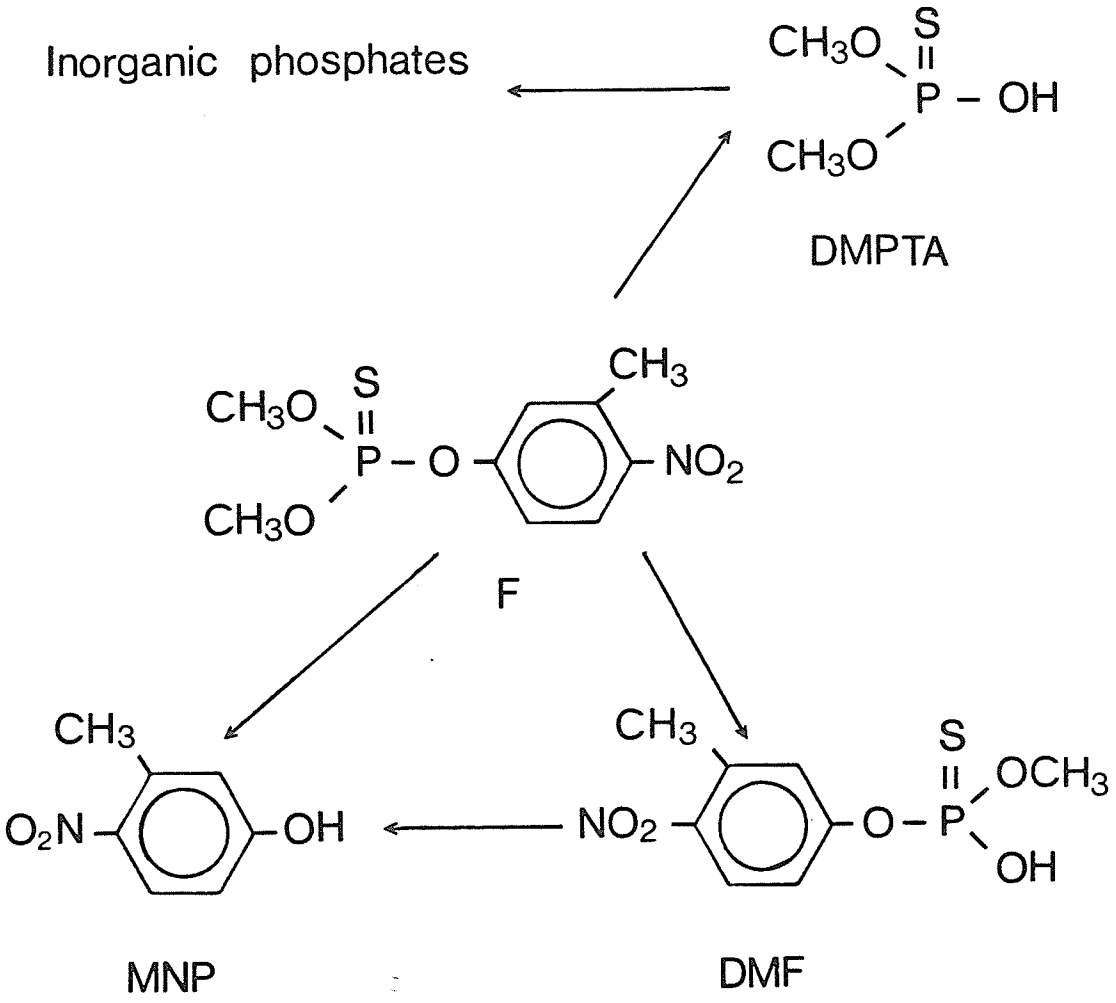



Figure 29: Suggested degradation pathways of fenitrothion in stored wheat

### 4.3.5 Confirmation tests

Two different techniques were employed to identify and confirm malathion and fenitrothion metabolites found in stored treated wheat.

#### 4.3.5.1 Chemical derivatization

Stored wheat samples treated with malathion or fenitrothion were extracted by the two step extraction method. Ethyl acetate fractions, containing malathion monoacid and malathion diacid or O-demethyl fenitrothion, were concentrated and derivatized with either diazoethane or diazomethane, respectively. Aliquots of the alkylated extracts were injected along with injections of parent compounds standards into the FPD/GLC.

Retention times obtained for the ethylated MMA and MDA coincided with that for malathion, and for methylated DMF coincided with that for fenitrothion. The findings indicated the conversion of the dealkylated metabolites found in stored grain to their parent compounds.

This test confirms the results discussed earlier on the production of MMA, MDA, and DMF during storage of treated wheat.

#### 4.3.5.2 Thin-layer chromatography

1. Malathion and Metabolites:

   Solutions of malathion and metabolites mixture, treated wheat extract, and extract from untreated control were chromatographed on silica gel. When the chromatoplate was sprayed with the ethanolic solution of 2,6-dibromo-N-chloro-p-benzoquinoneimine reagent and placed in the oven (110 C) for five minutes, the sepa-

rated components became visible as orange spots against a light background. Table 22 lists the $R_f$ values measured for each compound using two solvent systems. Satisfactory separation (Figure 30) of these components was obtained.

Comparison of $R_f$ values measured for the standard mixture with those obtained for the treated wheat extract indicated that MMA and DMPDTA were present in the sample. MDA could not be identified by the system used; this could be attributed to the existence of the compound at a level below the TLC detection limit. On a second TLC plate, half of the separated components of the treated wheat extract was covered with a glass plate before spraying. The spots were located by matching the $R_f$ values of the standards with those of MMA and MDA from the wheat extract, scraped off, and the compounds were extracted in ethanol, and then derivatized with diazoethane. GLC results indicated that similar retention times were obtained for derivatized MMA, derivatized MDA and the parent compound, malathion, confirming the presence of both metabolites in the treated wheat.

2. Fenitrothion and Metabolites:

Solutions of fenitrothion and metabolites, treated wheat extract, and extract from an untreated control were chromatographed on a silica gel TLC plate. When the chromatoplate was sprayed with an aqueous solution of $TlCl_3$ followed by the solution of p-N,N-dimethylaminocinnamaldehyde, the separated components appeared as reddish spots against a light background. Table 23 lists the $R_f$ values measured for the compounds which had good separations (Figure 31) in the solvent system employed.

TABLE 22

$R_f$ values for malathion and its metabolites in two solvent systems

| Compound | $R_f$ | |
|---|---|---|
| | Solvent system 1 | Solvent system 2 |
| Malathion | 0.86 | 0.54 |
| Malaoxon | 0.72 | 0.22 |
| Malathion monoacid | 0.69 | 0.41 |
| Malathion diacid | 0.26 | 0.27 |
| Dimethyl phosphorothioic acid | 0.05 | 0.06 |
| Dimethyl phosphorodithioic acid | 0.02 | 0.07 |

Solvent system 1- benzene: acetic acid (4:1, v/v)
Solvent system 2- benzene: ether: acetic acid (8:2:1, v/v)

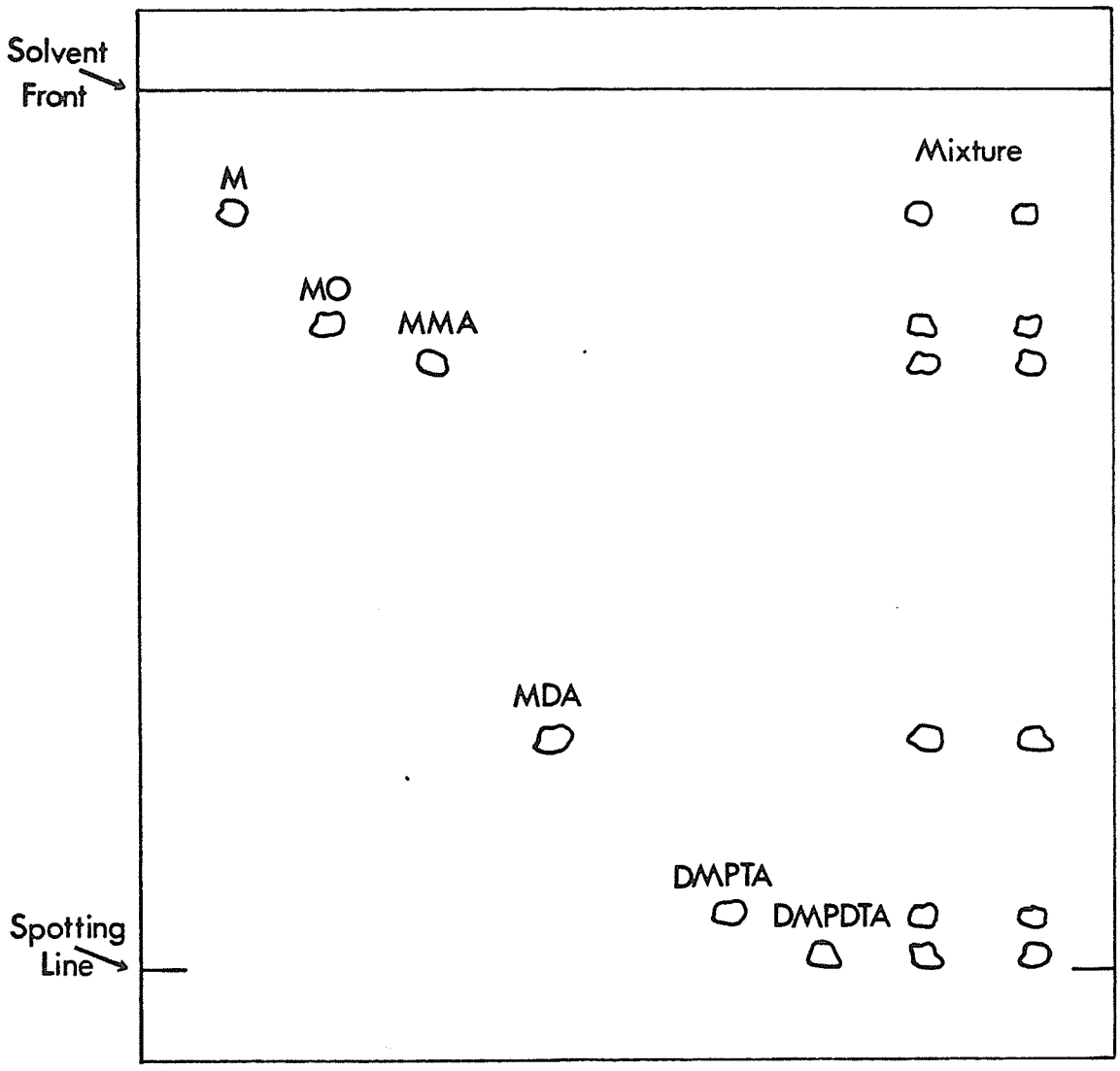



Figure 30: Typical chromatogram for the separation of malathion and its metabolites

TABLE 23

$R_f$ values for fenitrothion and its metabolites

| Compound | $R_f$ |
|---|---|
| Fenitrothion | 0.51 |
| 3-methyl-4-nitrophenol | 0.41 |
| S-methyl fenitrothion | 0.32 |
| Fenitrooxon | 0.15 |
| O-demethyl fenitrothion | 0.00 |

Solvent system- ether: isooctane (7:3, v/v)

Comparison of $R_f$ values calculated for the standards with those obtained for the treated wheat extract confirmed that DMF, MNP and DMPTA were found in the grain sample. The spots for DMF were located as before and scraped off, extracted in methanol, and derivatized with diazomethane. Results by GLC revealed that the methylated spot had a similar retention time to fenitrothion, confirming the presence of DMF in the grain sample.

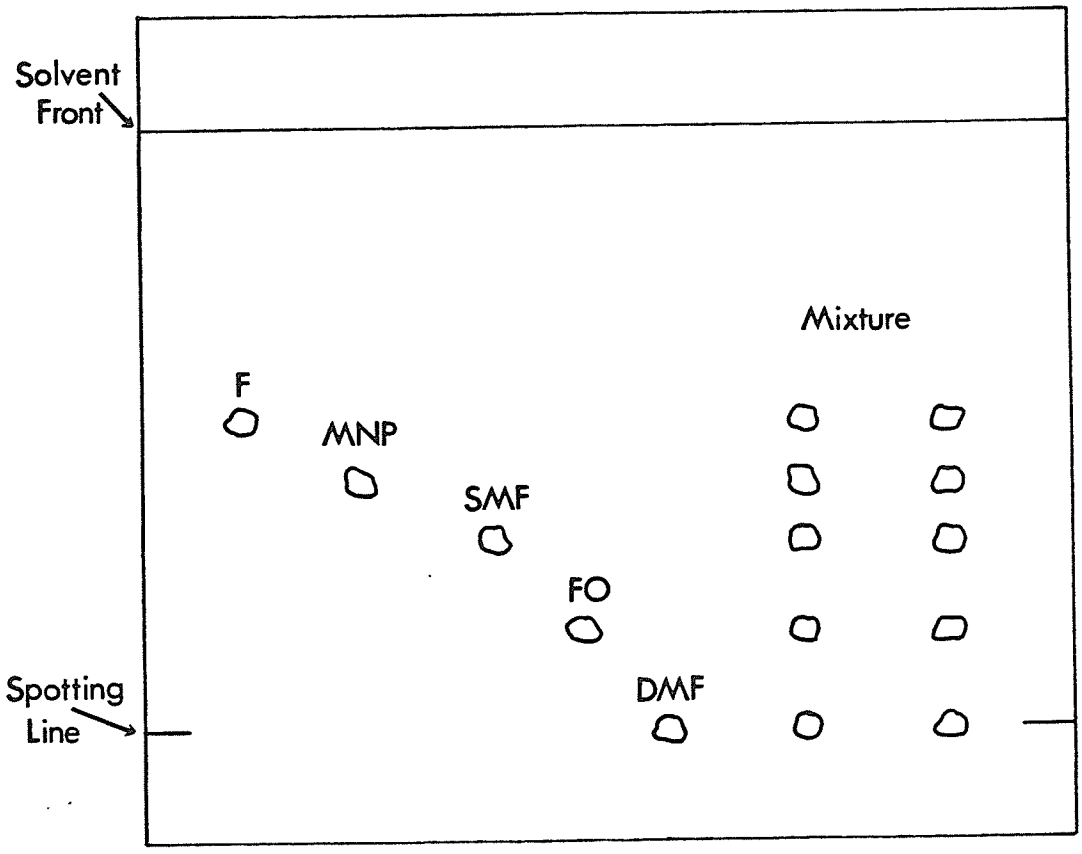



Figure 31: Typical chromatogram for the separation of fenitrothion and its metabolites

## Chapter V

## SUMMARY AND CONCLUSION

Treatments of stored grain with residual contact insecticides is a recommended farm practice to confer long-term protection against insect infestation. Although persistent residues of insecticides on grain may be useful from the standpoint of insect pest control, the problem of residues, both of the original insecticides and their break-down products, poses possible toxic hazard to the consumer. Laboratory studies were therefore conducted to determine residue levels and residue distribution of malathion and fenitrothion in treated wheat stored at various temperature similar to those experienced in Western Canada.

Wheat (12.5% moisture content) was treated with water based emulsions of malathion and fenitrothion and stored at -35, -20, -5, 5, 10, 20, and 27 C for 72 weeks. Residue analysis using FPD/GLC showed that very little loss of both insecticides occurred on wheat that had been stored at -35 and -20 C for 72 weeks. Fenitrothion appeared to be more persistent than malathion particularly at low storage temperatures. At the end of storage period, 74, 59, 26, 5, and 4% of the initial deposit of malathion remained on wheat stored at -5, 5, 10, 20, and 27 C, respectively. Corresponding values for fenitrothion were: 82, 65, 44, 10, and 4%.

The chemical data coupled with the efficacy data reported by Weaving (1975) and Hyari et al. (1977) indicates that fenitrothion would be a useful alternative for malathion-resistant strains of stored-grain pests under simulated Canadian prairie conditions.

In a second study, malathion and fenitrothion were applied at 8 and 12 ppm to wheat (12.5% mc) to determine and compare residue distribution in fractions milled from wheat stored at -5, 10, and 20 C over a period of 12 months. High levels of insecticide residues were contained in the bran and middlings. Less malathion than fenitrothion was found in the bran after 12 months of wheat at the three temperatures tested. Comparatively smaller amounts of insecticide residues were found in the flour than in the bran or middlings. Less fenitrothion than malathion was present in flour.

Application of both insecticides at both dosage rates initially resulted in residue accumulation in the bran above the maximum tolerance level (20 ppm of either insecticide). Also, malathion applied at 12 ppm and fenitrothion applied at 8 and 12 ppm resulted in residues higher than the recommended residue limits (2 ppm malathion and 1 ppm fenitrothion) in the flour fraction. However, the residues progressively declined to within tolerance limits except for bran and flour milled from wheat treated with malathion or fenitrothion and stored at -5 C.

Finally, research was conducted to develop rapid, reliable and sensitive methods for the simultaneous quantitative analysis of malathion or fenitrothion and their metabolites in stored wheat. Acidified acetone was used to extract malathion and four of its metabolites. Analysis of extracts was conducted using FPD/GLC after derivatization with diazomethane. The use of a column packed with 5% OV-101 on Chromosorb W allowed the separation of dimethyl phosphorothioic acid and dimethyl phosphorodithioic acid (at 80 C) and malathion diacid, malathion monoacid and malathion (at 175 C). However one injection of the extract may be

made to simultaneously separate all compounds on OV-101 by employing temperature programming (75 - 95 C, 2 C/min; 95 - 180 C, 5 C/min). Recoveries of malathion and its metabolites from fortified wheat were greater than 90%.

A second method was developed for the analysis of fenitrothion and five of its metabolites. Aliquots of the extract was analyzed for the 3-methyl-4-nitrophenol using ECD/GLC. The use of column packed with 5% OV-101 on Chromosorb W allowed the simultaneous separation of fenitrothion and four of its metabolites (demethyl fenitrothion, fenitrooxon, S-methyl fenitrothion, and O-demethyl S-methyl fenitrothion). These compounds were analyzed by FPD/GLC. Recoveries of all compounds were greater than 90%.

Reproducibility of both methods was good with recoveries decreasing at lower levels, these lower recoveries being attributed to the adsorption and not poor efficiency of the methods.

These developed methods were employed to quantitatively determine major metabolites of malathion and fenitrothion found in grain stored at 20 C for 12 months. Dimethyl phosphorodithioic acid, malathion monoacid, and malathion diacid were the metabolites of malathion found. Major metabolites of fenitrothion present were demethyl fenitrothion, 3-methyl-4-nitrophenol, and dimethyl phosphorothioic acid. Confirmation of these metabolites were carried out by chemical derivatization plus FPD/GLC and by thin-layer chromatography.

BIBLIOGRAPHY

Abe, T., Y. Fujimoto, T. Tatsuno, and J. Fukami. 1979. Separation of methyl parathion and fenitrothion metabolites by liquid chromatography. Bull. Environ. Contamin. Toxicol. 22: 791-795.

Acton, F.E. and W. Hertel. 1966. Malathion levels in wheat and wheat products. Fd. Technol. Aust. 18: 77-79.

Aharoni, A.H. and R.D. O'Brien. 1968. The inhibition of acetylcholinesterase by anionic organophosphorus compounds. Biochem. 7: 1538-1543.

Allessandrini, M.E. 1965. Determination of the persistence and fate of various insecticides in or on wheat during storage, milling and during baking or cooking of the products made from the treated wheat. Final Technical Report, USDA Project NO. E15-AMS-8.

Alnaji, L.K. and A.M. Kadoum. 1979. Residues of methyl phoxim in wheat and milling fractions. J. Agric. Food Chem. 27: 583-584.

Anonymous. 1977. Canada Dept. of National Health and Welfare. Office of Consolidation of the Food and Drugs Act and of the Food and Drug Regulations with Amendments to Oct. 7, 1977. Ottawa.

Approved Methods of the American Assoc. Cereal Chem. 1976. 53: 413-421.

Ardley, J.H. and R. Sticka. 1977. The effectiveness of fenitrothion and malathion as grain protectants under bulk storage conditions in New South Wales, Australia. J. Stored Prod. Res. 13: 159-168.

Bender, M.E. and J.R. Westman. 1976. The toxicity of malathion and its hydrolysis products to the eastern mud minnow, Umbra pygmaea (DeKay). Chesapeake Sci. 17: 125-128.

Bengston, M., M. Connell, J. Desmarchelier, M. Phillips, J. Snelson, and R. Sticka. 1974. Field trials with four new grain protectants. Technical Report No. 6 to Australian Wheat Board 12 pp.

Bhatia, S.K., T.D. Yadav, and P.B. Mookherjee. 1971. Malathion resistance in Tribolium castaneum (Herbst) in India. J. Stored Prod. Res. 7: 227-232.

Champ, B.R. and M.J. Campbell. 1970. Insecticide resistance in Australian Tribolium castaneum (Herbst)- I. A test method for detecting insecticide resistance. J. Stored Prod. Res. 6: 53-70.

Champ, B.R. and M.J. Campbell-Brown. 1970. Insecticide resistance in Australian Tribolium castaneum (Herbst) (Coleoptera, Tenebrionidae)-II. Malathion resistance in Eastern Australia. J. Stored Prod. Res. 6: 111-131.

Champ, B.R. and D.E. Dyte. 1976. Report of the FAO Global survey of pesticide susceptibility of stored grain pests. FAO Plant Prodn. Protect. Ser. No. 5: 297 pp.

Champ, B.R. and G.K. Girish. 1974. Pesticidal resistance detected in India. Bull. Grain technol. 12: 82-84.

Champ, B.R., R.W. Steele, B.G. Glenn, and K.D. Elmis. 1969. A comparison of malathion, diazinon, fenitrothion and dichlorvos for control of Sitophilus oryzae (L.) and Rhyzopertha dominica (F.) in wheat. J. Stored Prod. Res. 5: 21-48.

Cheng, Y.Y., P. Linko, and M. Milner. 1958. Glutamic acid decarboxylase in wheat grains. J. Sci. Food Agric. 9: 333-336.

Cochrane, W.P., M. Lanouette, and R. Greenhalgh. 1979. Quantitation of the contaminants in technical grade fenitrothion by reverse phase high pressure liquid chromatography. J. Assoc. Off. Anal. Chem. 62: 1222-1230.

Cook, G.H. and J.C. Moore. 1976. Determination of malathion, maloxon, and mono- and dicarboxylic acids of malathion in fish, oyster, and shrimp tissue. J. Agric. Food Chem. 24: 631-634.

Coulon, J. 1966. Influence of grain humidity on the effectiveness of some insecticides used for the treatment of stored cereals. Rev. Appl. Entomol. 57: 349- 355.

Crisp, S. and K.R. Tarrant. 1971. Determination of residues of dichlorvos and malathion in wheat grain by gas-liquid chromatography. Analyst 96: 310-313.

Deong, E.R. 1948. Chemistry and uses of insecticides. Reinhold Publishing Corporation, New York pp. 1-266.

Desmarchelier, J. 1978. Loss of fenitrothion on grains in storage. Pest. Sci. 9: 33-38.

Desmarchelier, J., M. Bengston, M. Connell, W. Minett, B. Moore, M. Phillips, J. Snelson, R. Sticka, and K. Tucker. 1977. A collaborative study of residues on wheat of chlorpyrifosmethyl, fenitrothion, malathion, methacrifos and pirimiphosmethyl. I. Method development. Pest. Sci. 8: 473-483.

Devlin, R.M. 1969. Plant Physiology. Reinhold Publishing Corporation, New York, Ch 6: 81-93.

Donald, A.W. 1958. Protectants for farm-stored grains. Proceedings-Tenth International Congress of Entomology 4: 73-76.

Drozd, J. 1975. Chemical derivatization in gas chromatography. J. Chromatogr. 113: 303-356.

Dyte, C.E. 1970. Insecticide resistance in stored product insects with special reference to Tribolium castaneum Trop. Stored Prod. Res. 20: 13-18.

Dyte, C.E. and D.G. Blackman. 1965. The red flour beetle and malathion . Pest. Infest. Res. 1964: 40-41.

Dyte, C.E. and D.G. Blackman. 1970. The spread of insecticide resistance in Tribolium castaneum (Herbst). J. Stored Prod. Res. 6: 255-261.

Dyte, C.E. and D.G. Blackman. 1972. Laboratory evaluation of organophosphorus insecticides against susceptible and malathion -resistant strains of Tribolium castaneum (Herbst). J. Stored Prod. Res. 8: 103-109.

Eichler, D. and H.A. Knoll. 1974. Degradation of bromophos in stored wheat. Proc. 1st International Working Conf. Stored Prod. Entomol. Oct. 7-11, Savannah,Ga., U.S.A., p. 582-590.

Engel, C. 1947. The distribution of enzymes in resting cereals. I. Distribution of the saccharogenic amylase in wheat, rye and barely. Biochem. Biophys. Acta 1: 42-45.

Engel, C. 1948. Distribution of amylase, proteinase and esterase in resting cereals. Rec. Trav. Chim. 64: 318-322.

FAO/WHO. 1967. Evaluation of some pesticide residues in food. FAO/PL:CP/15; WHO/Food Add./67. 32: 237.

FAO/WHO. 1968a. Pesticide residues. Report of the 1967 Joint Meeting of the FAO Working Party and the WHO Expert Committee. FAO/PL:1967/M/11; Who Tech. Rep. Ser. 391: 43 p.

FAO/WHO. 1968b. 1967 evaluation of some pesticide residues in food. FAO/PL: 1967/M/11/ 1; WHO/Food Add./68. 30: 242 p.

FAO/WHO. 1969a. Pesticide residues in food. Report of the 1968 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and WHO Expert Committee on Pesticide Residues. FAO Agr. Studies No. 78; WHO Techn. Rep. Ser. 417: 40 p.

FAO/WHO. 1969b. 1968 evaluations of some pesticide residues in food. FAO/PL: 1968/M/9/ 1; WHO/Food Add./69.35: 293 p.

FAO/WHO. 1973a. Pesticide residues in food. Report on the 1972 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on the Pesticide Residues. FAO Agr. Studies No. 90; WHO Techn. Rep. Ser. No. 525: 47 p.

FAO/WHO. 1973b. 1972 evaluations of some of pesticide residues in food. FAO/AGP/ 1972/ M/9/1; WHO Pesticide Residues Ser. No. 2: 587 p.

FAO/WHO. 1974. 1973 evaluations of some pesticide residues in food. AGP, 1974/M/11, WHO Techn. Rep. Ser. No. 545: 484 p.

FAO/WHO. 1976a. Pesticide residues in food. Report of the 1975 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Plant Prodn. Protect. Ser. No. 1; WHO Techn. Rep. Ser. No. 592: 45 p.

FAO/WHO. 1976b. 1975 evaluations of some pesticide residues in food. FAO/AGP:1975/M/ 13; WHO Pesticide Residues Ser. No. 5: 409 p.

FAO/WHO. 1980. 1979 evaluation of some pesticide residues in food. FAO Plant Prodn. Protect. Ser. No. 12; 289p.

Godavari Bai, S. 1964. Persistance of malathion residues on treated food grains under different conditions of storage and processing. Pesticides (Symp. on Pesticides, Dec. 21-22, 1964, Mysore). Academy of Pest Control Sciences, Mysore, India. 1968. 310-315.

Green, A.A. and P.S. Tyler. 1966. A field comparison of malathion, dichlorvos and fenitrothion for the control of Oryzaephillus surirramensis infesting stored barley. J. Stored Prod. Res. 1: 223-228.

Green, A.A., P.S. Tyler, J. Kane and D.G. Rowlands. 1970. An assessment of bromophos for the protection of wheat and barley. J. Stored Prod. Res. 6: 217-228.

Greenhalgh, R. 1975. Fenitrothion: The effects of its use on environmental quality and its chemistry. N.R.C. Publication No. NRCC 14104: 103-108.

Greenhalgh, R., K.L. Dhawan, and P. Weinberger. 1980. Hydrolysis of fenitrothion in model and natural aquatic systems. J. Agric. Food Chem. 28: 102-105.

Gunther, F.A. and L.R. Jeppson. 1960. Modern insecticides and world food production. London, Chapman and Hall, Ch. 6: 76-104.

Gunther, F.A., D.L. Lindgren and R.C. Blinn. 1958. Biological effectiveness and persistence of malathion and lindane used for protection of stored wheat. J. Econ. Entomol. 51: 843-844.

Harary, I., S.R. Korey and S. Ochoa. 1953. Biosynthesis of dicarboxylic acids by $CO_2$ fixation. VII. Equilibrium of malic enzyme reaction. J. Biol. Chem. 203: 595-600.

Hayward, L.A.W. 1962. Ground nuts. West African Stored Res. Report. 1961: 12-15.

Hilgetag, G. and Martini, A. 1972. Preparative organic chemistry. Wiley and Sons, New York, pp. 1-368.

Hyari, S., A.M. Kadoum and D.W. LaHue. 1977. Laboratory evaluation of emulsifiable and encapsulated formulations of malathion and fenitrothion on soft red winter wheat against attack by adults of four species of stored-product insects. J. Econ. Entomol. 70: 480-482.

John, L.E. and D.J. Lisk. 1968. Determination of hydrolytic metabolites of organophosphorus insecticides in cow urine using an improved thermionic detector. J. Agric. Food Chem. 16: 48-49.

Kadoum, A.M. 1969. Extraction and cleanup methods to determine malathion and its hydrolytic products in stored grains by gas-liquid chromatography. J. Agric. Food Chem. 17: 1178-1180.

Kadoum, A.M. and D.W. LaHue. 1969. Effect of hybrid, moisture content, foreign material, and storage temperature on degradation of malathion residues in grain sorghum. J. Econ. Entomol. 62: 1161-1164.

Kadoum, A.M. and D.W. LaHue. 1972. Degradation of malathion on viable and sterilized sotghum grain. J. Econ. Entomol. 65: 479-500.

Kadoum, A.M. and D.W. LaHue. 1974. Penetration of malathion in stored corn, wheat, and sorghum grain. J. Econ. Entomol. 67: 477-478.

Kadoum, A.M. and D.W. LaHue. 1975. Degradation of malathion in corn, wheat, and sorghum grain of high moisture content. J. Econ. Entomol. 69: 205-206.

Kadoum, A.M. and D.W. LaHue. 1977. Degradation of malathion in wheat and milling fractions. J. Econ. Entomol. 70: 109-110.

Kadoum, A.M. and D.W. LaHue. 1979. Degradation of malathion on wheat and corn of various moisture contents. J. Econ. Entomol. 72: 228-229.

Kashi, K.P. 1972. An appraisal of fenitrothion as a promising grain-protectant. Intern. Pest Control 14: 20-22.

Khan, S.U. 1975. Chemical derivatization of herbicide residues for GLC. Res. Rev. 21-43.

Koivistionen, P. 1961. Studies on the disappearance of malathion from plant material. Ann. Acad. Sci. 51: 1-3.

Kovacicova, J., V. Batora and S. Truchlik. 1973. Hydrolysis rate and *in vitro* anticholinesterase activity of fenitrothion and S-methyl fenitrothion. Pest. Sci. 4: 759-763.

Kretovitch, V.L. 1945. Physiologico-biochemical bases for the storage of wheat. Akad. Nauk. SSSR. Inst. Biokhim. 1: 78-84.

LaHue, D.W. 1976. Grain protectants for seed corn. J. Econ. Entomol. 69: 652-654.

LaHue, D.W. and E.B. Dicke. 1976. Evaluating selected protectants for shelled corn against stored-grain insects. U.S. Department of Agriculture Marketing Research Report No. 1058: 9 pp.

LaHue, D.W. and E.B. Dicke. 1977a. Evaluation of selected insecticides applied to high moisture sorghum grain to prevent stored insect attack. U.S. Department of Agriculture Marketing Research Report No. 1063: 10 pp.

LaHue, D.W. and E.B. Dicke. 1977b. Candidate protectants for wheat against stored-grain insects. U.S. Department of Agriculture Marketing Research Report No. 1080: 11 pp.

LaHue, D.W. and A.M. Kadoum. 1979. Residual effectiveness of emulsion and encapsulated formulations of malathion and fenitrothion against four stored grain beetles. J. Econ. Entomol. 72: 234-237.

Lemon, R.W. 1967. Laboratory evaluation of malathion, bromophos and fenitrothion for use against beetles infesting stored products. J. Stored Prod. Res. 2: 197-210.

Levi, I. and T.W. Nowicki. 1974. Rapid screening method for simultaneous determination of organochlorine and organophosphate pesticide residues in wheat by gas-liquid chromatography. J. Assoc. Off. Anal. Chem. 57: 924-929.

Lockwood, L.M., S.K. Majumder and D.R. Lineback. 1974. Degradation of organophosphate pesticides in cereal grains during milling and cooking in India. Cereal Sci. Today 19: 330-334.

Lores, E.M. and D.E. Bradway. 1977. Extraction and recovery of organophosphorus metabolites from urine using an anion exchange resin. J. Agric. Food Chem. 25: 75-79.

Malathion Panel. 1973. The determination of malathion and dichlorvos residues in grain. Analyst 98: 19-24.

Martin, H. and C.R. Worthing. 1977. Pesticide manual. 5th Edn. British Crop Protection Council 593 p.

Martin, J.H. and W.H. Leonard. 1967. Priciples of field crop production. Collier-MacMillan, Canada, ch 15: 391-448.

McGaughey, W.H. 1971. Malathion on milling fractions of three varieties of rough rice: duration of protection and residue degradation. J. Econ. Entomol. 64: 1201-1204.

McGaughey, W.H. 1972. Malathion treatment of rough rice re-evaluated. Rice J. 75: 16-20.

Mensah, G.W.K. and F.L. Watters. 1979. Comparison of four organophosphorus insecticides on stored wheat for control of susceptible and malathion-resistant strains of the red flour beetle. J. Econ. Entomol. 72: 456-461.

Mensah, G.W.K., F.L. Watters and G.R.B. Webster. 1979. Insecticide residues in milled fractions of dry and tough wheat treated with malathion, bromophos, iodofenphos, and primiphos-methyl. J. Econ. Entomol. 72: 728-731.

Minett, W., R.S. Belcher and E.J. O'Brien. 1968. A critical moisture level for malathion breakdown in stored wheat. J. Stored Prod. Res. 4: 179-181.

Nishizawa, Y. 1976. Chemistry of sumithion. Res. Rev. 60: 1-10.

O'Brien, R.D. 1960. Toxic phosphorus esters. London, Academic Press pp. 1-325.

O'Brien, R.D. 1976. Acetylcholinesterase and its inhibition. in Insecticide Biochemistry and Physiology. C.F. Wilkinson, ed., Plenum Press, New York, pp. 271-326.

Parkin, E.A. and R. Forster. 1962. The rust-red flour beetle and malathion. Pest. Infest. Res. Rep. 1961: 38-39.

Parkin, E.A., E.I.C. Scott and R. Forster. 1962. The resistance of field strains of beetles. Pest Infest. Res. 1961: 34-35.

Pasalu, I.C., G.K. Girish and K. Krishnamurthy. 1974. Status of insecticide resistance in insect pests control of stored products. Bull. Grain Technol. 12: 50-59.

Pietere, A.H. and G.G.M. Schuten. 1974. Investigation on insecticide resistance in Tribolium castaneum (Herbst), T. confusm Duv. and Sitophilus zeamais Motsch. in small maize cribs in Malawi. Trop. Agric. 51: 63-67.

Quinon, L.D. and M.E. Hobbs. 1958. Analysis of the nonvolatile acids in cigarette smokeby gas chromatography of their methyl esters. Anal Chem. 30: 1400-1405.

Rainsford, K.D. 1978. Toxicity in the brain of organophosphate insecticide: Comparison of the toxicities of metabolites with parent compounds using an intracerebral injection method. Pest. Biochem. Physiol. 8: 302-316.

Roan, C.L. and B.P. Srivastava. 1965. Dissipation of diazinon residues in wheat. J. Econ. Entomol. 58: 996-997.

Rowlands, D.G. 1964. The degradation of malathion on stored maize and wheat grains. J. Sci. Food Agr. 15: 824-827.

Rowlands, D.G. 1965a. The in vitro and in vivo oxidation and hydrolysis of malathion by wheat grain enzymes. J. Sci. Food Agr. 16: 325-328.

Rowlands, D.G. 1965b. Effect of triphenyl phosphate on the phosphatase degradation of malathion in vitro and the metabolism of triphenyl phosphate. Pest. Infest. Res. 1964: 29-30.

Rowlands, D.G. 1966a. The metabolism of bromophos in stored wheat grains. J. Stored Prod. Res. 2: 1-5.

Rowlands, D.G. 1966b. The activation and detoxification of three organic phosphorothionate insecticides applied to stored wheat grains. J. Stored Prod. Res. 2: 105-110.

Rowlands, D.G. 1966c. The in vitro and in vivo metabolism of dimethoate in stored wheat and sorghum grains. J. Sci. Food Agr. 17: 90-98.

Rowlands, D.G. 1967. The metabolism of contact insecticides in stored grains. Res. Rev. 17: 105-177.

Rowlands, D.G. 1968. The metabolism of DDT in stored wheat grains J. Stored Prod. Res. 4: 183-190.

Rowlands, D.G. 1970a. Metabolic activity towards insecticide substrates in the germ of wheat grains. Pest. Infest. Res. 1969: 34-36.

Rowlands, D.G. 1970b. The metabolic fate of dichlorvos on stored wheat grains. J. Stored Prod. Res. 6: 19-24.

Rowlands, D.G. 1971. The metabolism of contact insecticides in stored grains. Res. Rev. 34: 91-161.

Rowlands, D.G. and J.S. Bramhall. 1977. The uptake and translocation of malathion by the stored wheat grain. J. Stored Prod. Res. 13: 13-22.

Rowlands, D.G. and D.F. Horler. 1967. Penetration of malathion into wheat grains. Insecticide and Fungicide Conf. 1: 331-335.

Rowlands, D.G., R.K. Sharma and P.R. Dean. 1968. Nature of the oxidative activity in the seed coat. Pest Infest. Res. 1967: 29-34.

Schesser, J.H., W.E. Priddle and E.P. Farrell. 1958. Insecticidal residue in milling fractions from wheat treated with methoxychlor, malathion and lindane. J. Econ. Entomol. 51: 516-518.

Seitz, L.M., D.B. Sauer, H.E. Mohr and R. Burroughs. 1975. Weathered grain sorghum: Natural occurrence of alternariols and storability of the grain. Phytopathol. 65: 1261-1263.

Shafik, M.T. and H.F. Enos. 1969. Determination of metabolic and hydrolytic products of organophosphorus pesticide chemicals in human blood and urine. J. Agric. Food Chem. 17: 1186-1189.

Shafik, M.T., D.E. Bradway, and H.F. Enos. 1971. A cleanup procedure for the determination of low levels of alkyl phosphates, thiophosphates, and dithiophosphates in rat and human urine. J. Agric. Food Chem. 19: 885-889.

Shafik, M.T., D.E. Bradway, H.F. Enos and A.P. Yobs. 1973. Human exposures to organophosphorus pesticides. A modified procedure for the gas-liquid chromatographic analysis of alkyl phosphate metabolites in urine. J. Agric. Food Chem. 21: 625-629.

Singer, T.P. 1948. Mechanisms of enzyme inhibitions by sulphahydryl reagents. J. Biol. Chem. 174: 11-18.

Sinha, R.N. 1971. Spoilage of farm-stored grain by molds, insects and mites in Western Canada. Canada Dept. Agr. Publ. No. 1437: 8 p.

Smith, L.I. 1937. Aliphatic diazo compounds, nitrones, and structurally analogous compounds. Chem. Rev. 23: 193-285.

Spiers, R.D. and J.L. Zettler. 1969. Toxicity of three organophosphorous compounds and pyrethrins to malathion-resistant Tribolium castaneum (Herbst). J. Stored Prod. Res. 4: 279-283.

Spiers, R.D., L.M. Redlinger and H.P. Boles. 1967. Malathion resistance in the red flour beetle. J. Econ. Entomol. 60: 1373-1374.

Stanley, C.W. 1966. Derivatization of pesticide-related acids and phenols for gas chromatographic determination. J. Agric. Food Chem. 14: 321-323.

Strong, R.G. and D.E. Sbur. 1960. Influence of grain moisture content and storage temperature on the effectiveness of malathion as agrain protectant. J. Econ. Entomol. 53: 341-346.

Strong, R.G. and D.E. Sbur. 1964. Influence of grain moisture content and storage temperature on the effectiveness of five insecticides as grain protectants. J. Econ. Entomol. 57: 44-50.

Strong, R.G. and D.E. Sbur. 1965. Evaluation of insecticides as protectants against pests of stored grain and seeds. J. Econ. Entomol. 57: 544-548.

Strong, R.G. and D.E. Sbur. 1968. Evaluations of insecticides for control of stored-product insects. J. Econ. Entomol. 61: 1034-1041.

Strong, R.G., D.E. Sbur and R.G. Arndt. 1961. Influence of formulation on the effectiveness of malathion, methoxychlor, and synergized pyrethrum protective sprays for stored wheat. J. Econ. Entomol. 54: 489-501.

Talcott, R.E., N.M. Mallipud, N. Umetsu and T.R. Fukuto. 1979. Inactivation of esterases by impurities isolated from technical malathion. Toxicol. Appl. Pharmacol. 49: 107-112.

Tyler, P.S. and A.A. Green. 1968. The effectiveness of fenitrothion and malathion as grain protectants uder severe practical conditions. J. Stored Prod. Res. 4: 119-126.

Tyler, P.S., S.G. Bristew, D.F. Horler and J.E. Clements. 1966. Protective treatment of grain, laboratory experiments. Pest. Infest. Res. 1966: 26-27.

Varca, L.M., E.D. Magallona, and J.A. Pasco. 1975. Gas chromatography of organophosphate insecticide residues in stored grain. I. Comparative performance of two detection systems illustrated with malathion and primiphos-methyl. Phillip. Entomol. 3: 55-60.

Volpe, G.G. and V.N. Mallet. 1980. Development of an analytical method for fenitrothion and five derivatives in water using XAD resins and gas-liquid chromatography (GLC). Intern. J. Environ. Anal. Chem. 8: 291-301.

Watters, F.L. 1959. Effects of grain moisture content on residual toxicity and repellency of malathion. J. Econ. Entomol. 52: 131-134.

Watters, F.L. and G.W.K. Mensah. 1979. Stability of malathion applied on stored wheat for control of rusty grain beetles. J. Econ. Entomol. 72: 794-797.

Weaving, A.J.S. 1975. Grain protectants for use under tribal storage conditions in Rhodesia. Comparative toxicities of some insecticides on maize and sorghum. J. Stored Prod. Res. 11: 65-70.

Wilkin, D.R., S.L. Aggarwal and K.P. Thomas. 1973. Treatment of grain: admixture of iodofenphos. Pest. Infest. Res. Rep. 1970: 110-111.

Zettler, J.L. 1974. Malathion resistance in Tribolium castaneum collected from stored peanuts. J. Econ. Entomol. 67: 339-340.

Zettler, J.L. 1975. Malathion resistance in strains of Tribolium castaneum collected from rice in the U.S.A. J. Stored Prod. Res. 11: 115-117.