PHOTODEGRADATION OF MALATHION ON STORED GRAIN

by

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INTRODUCTION

Malathion, O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate, is an organophosphorous insecticide introduced by the American Cyanamid Company in 1950. Unlike other organophosphorous insecticides available at that time, malathion exhibited relatively low mammalian toxicity coupled with high insecticidal activity. Desirable qualities such as low toxicity to man and animals, short residual life as compared to many of the chlorinated hydrocarbon insecticides, and effectiveness in controlling a wide range of insect pests have made malathion one of the most widely used insecticides. Malathion is applied for the control of insect pests of field crops, fruits and vegetables, domestic animals and poultry, and common household pests. In addition, one of its primary uses is as a stored grain protectant. Because malathion is one of the few contact insecticides recommended for the control of stored grain pests of edible grain and grain products, considerable research has been carried out to determine its effectiveness, persistence, and metabolic fate. Although the effects of varying storage conditions such as temperature, relative humidity, and grain moisture content have been thoroughly investigated, very little is known of the effects of light on malathion residues in stored grain.

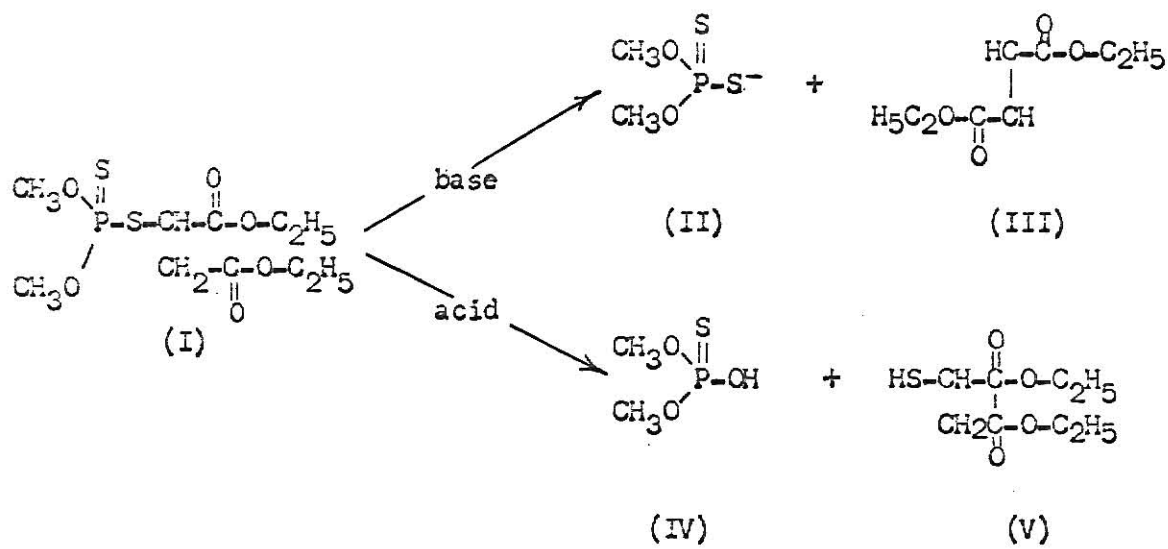
The present research was carried out to study malathion photodegradation on stored wheat and sorghum grains. Specifically, this investigation included effects of different regions of the electromagnetic spectrum (infrared, solar visible region, and near and far ultraviolet light) upon deposits of malathion applied to wheat grain, sorghum grain, and glass beads. Irradiated malathion residues on surfaces or substrates were analyzed at various time intervals of light exposure to determine the malathion degradation products and the relative

photodegradative activity of the different lights.

REVIEW OF LITERATURE

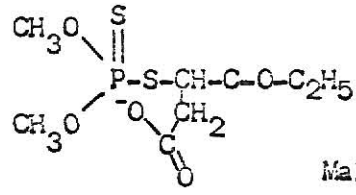
Some General Aspects of Malathion Degradation

The degradation of malathion (and its degradation products) is determined to a great extent by its susceptability toward non-enzymic or chemical hydrolysis. Spencer (1968) states that malathion is rapidly hydrolyzed at pH values greater than 7.0 and below 5.0, but is stable in aqueous solutions buffered at pH 5.35. Thus malathion is most stable under slightly acidic conditions and any pH change toward the basic side results in alkaline hydrolysis. Alkaline hydrolysis of malathion (I) yields O,O-dimethyl phosphorodithioate (II) and diethyl fumarate (III), and further hydrolysis may produce such products as O,O-dimethyl phosphorothioate and various products of the side chain moiety. Acid hydrolysis cleaves malathion between the phosphorous and the thiolo sulfur yielding O,O-dimethyl phosphorothionic acid (IV) and diethyl mercaptosuccinate (V) (Koivistoinen, 1951).



Any change in the structure of the malathion molecule may increase or

decrease the relative electronic charge on the phosphorous atom. For this reason, the accumulation of certain malathion degradation products may be a function of their susceptability toward non-enzymic hydrolysis. For example, two of the most commonly detected malathion degradation products are malaoxon (O,O-dimethyl S-(1,2-biscarbo-ethoxy)ethyl phosphorothiolate) and malathion monocarboxylic acid (O,O-dimethyl S-(1-carboethoxy 2-carboxy)ethyl phosphorodithioate). Because the =O of malaoxon is more electrophilic than the =S of malathion, malaoxon is many times more susceptible to nucleophilic attack (by OH^-) and hydrolysis than malathion. O'Brien (1957) reported that solutions of malaoxon in ethanol were rapidly hydrolyzed at pH 10, whereas, malathion was only slowly hydrolyzed in the same medium. On the other hand, the conversion of malathion to its monoacid reduces the electrophilic nature of the phosphorous atom resulting in a more stable molecule. This phenomenon is described as a field effect by O'Brien (1967). In essence, a negative site (carboxyl ion) of the side chain is brought in close proximity to the phosphorous atom, thus reducing its positive charge and susceptability toward nucleophilic attack. In general, accumulation of some degradation products and the rapid disappearance of other malathion degradation products can at least partially be explained by inductive and field effects of groups attached to the phosphorous atom.

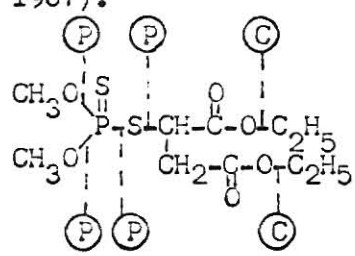


Malathion β-Monoacid

Enzyme catalyzed reactions of malathion can generally be divided into two main categories: oxidation and hydrolysis. The in vivo activation of

malathion to its oxygen analog, malaoxon, by biological systems is of twofold importance. Toxicologically, this conversion in vertebrates and insects results in production of a powerful cholinesterase inhibitor which is about 10,000 times more potent than the parent compound (O'Brien, 1967). Thus, malathion, like several other thionophosphate insecticides, is a latent anti-cholinesterase agent and only exhibits toxic properties after its metabolic conversion to malaoxon. In contrast to the activation of malathion in vertebrates and insects, production of malaoxon in plants may be thought to be a degradative mechanism. Rowlands (1964) indicated that oxidation of malathion resulting from plant enzymic action is unlikely to increase toxicity and may even accelerate hydrolytic degradation.

Unlike the activation reaction just discussed, hydrolytic reactions of malathion are degradative. Hydrolysis of malathion at any of the sites indicated in the figure below results in compounds considered to be relatively non-toxic (O'Brien, 1967).



Points of degradation of malathion: circled P, phosphatases; circled C, carboxyesterases (O'Brien, 1967).

Degradation of malathion in vertebrates and insects generally follows attack by either phosphatases, carboxyesterases, or both. Phosphatases as defined by O'Brien (1967) are any enzymes capable of hydrolyzing any phosphorous ester or anhydride bond, including P-O-C, P-F, P-S and others. In the case of malathion, phosphatases may either demethylate to produce O-demethyl malathion or attack the phosphorothiolo linkage yielding either O,O-dimethyl

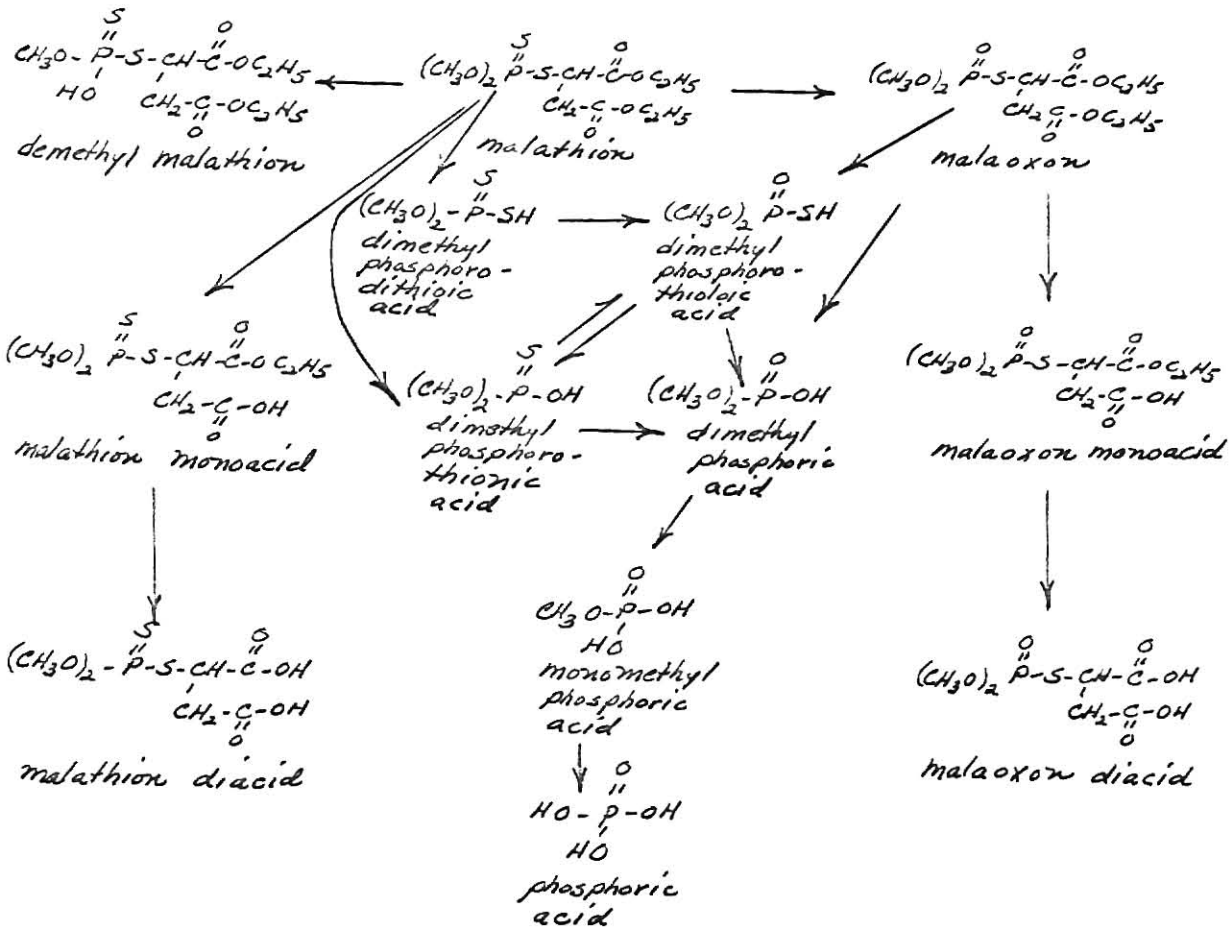
phosphorodithioate or O,O-dimethyl phosphorothioate. Apparently phosphatases capable of demethylation are much less common and degradation by this pathway generally constitutes a minor route of metabolism, especially in plants. In general, combined effects of phosphatases and non-enzymic action result in such end products as dimethyl phosphate, monomethyl phosphate, and inorganic orthophosphate.

Much of malathion's success as an insecticide can be attributed to its susceptability to detoxification by carboxyesterases. Carboxyesterase attack produces the relatively non-toxic monoacid and diacid derivatives of malathion. Because mammals and various other organisms can rapidly degrade malathion by this pathway, they generally are not poisoned. In contrast to this phenomenon, many insects lack sufficient carboxyesterase activity and are victims of the buildup of toxic quantities of malaoxon. Malaoxon can be degraded by similar mechanisms, however, the carboxyesterases involved are most likely different.

Plants are capable of detoxifying malathion by carboxyesterases also. In fact, metabolism of malathion by this route may be the prime reason for disappearance of malathion residues in some plants (Koivistoinen, 1961).

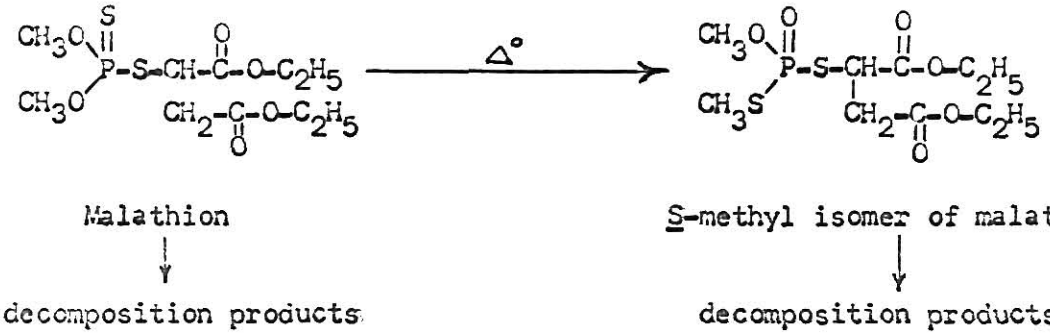
Other aspects of malathion metabolism, such as its synergism with other organophosphorous compounds, insect resistance mechanisms, and degradative pathways in specific organisms have been discussed by Spiller (1961), O'Brien, (1960, 1967), and Durham (1967). The following diagram is the general metabolic pathways of malathion.

METABOLIC PATHWAYS OF MALATHION



Thermal Isomerization and Decomposition of Malathion

Malathion undergoes isomerization and decomposition when subjected to highly elevated temperatures.



Metcalf and March (1953) investigated isomerization of parathion, methyl parathion, isopropyl parathion, and malathon (malathion). They reported about 90% isomerization after heating malathion for 24 hours at 150°C. The resulting products consisted of an orange-brown liquid soluble in chloroform and a colorless, cloudy material immiscible in chloroform. The main chloroform soluble product was tentatively identified as the S-methyl isomer of malathion (O,S-dimethyl phosphorodithioate of diethyl mercaptosuccinate) on the basis of total sulfur and thiono sulfur content and paper chromatographic analysis.

O'Brien (1956) studied the isomerization of thiophosphates and concluded that the nature of thermal degradation products formed depended on the severity of conditions. Products varying from light brown to charred black resulted when malathion was heated from temperatures ranging from 115°C (18 hours) to 180°C (4 hours). A liquid phase soluble in organic solvents and a solid phase soluble in water were observed. As the temperature was raised the proportion of the liquid phase decreased with a corresponding increase of the solid phase. Identification of the isomerization product as the S-methyl thiolo phosphate of malathion was accomplished by means of infrared spectral analysis.

McPherson and Johnson (1956) studied the thermal decomposition products of five phosphorothioate insecticides, parathion, methyl parathion, chlorothion, malathion, and insecticide 4124 (dicapthon). They subjected small samples of these insecticides to maximum temperatures of 270°C and observed a two step degradation process. Methyl parathion allowed to proceed through the first step resulted in the production of dimethyl sulfide, sulfur dioxide, and a mixture of aryl polymetaphosphates. A second, and at times explosive, decomposition step produced a black carbonaceous residue. Malathion, which does not contain a nitrophenyl group, apparently does not undergo a second decomposition step. The estimated decomposition times of the four insecticides at varying temperatures was presented. Values for malathion are as follows:

Temperature, °C	Decomposition time, days
65	925
80	163
100	20
115	5

Although elevated temperatures such as those used above are not likely to be attained under field conditions, consideration of this phenomenon is of great importance when synthesizing malathion and/or its degradation products.

In addition to the thermal decomposition products reported by McPherson and Johnson, several other oxidation and degradation products formed by heating thiophosphates have been reported. Metcalf and March (1956) detected 8 compounds after heating parathion for 24 hours at 150°C. Compounds tentatively identified were O-ethyl O,O-bis(p-nitrophenyl)-phosphorothioate, the S-ethyl isomer, O,O-diethyl p-nitrophenyl phosphate (paraoxon), and p-nitrophenol. Methyl parathion subjected to the same conditions was degraded to 4 compounds including the S-methyl isomer and the oxygen analog, methyl paraoxon.

Effects of Light on Organophosphorous Insecticides

Early in the development of organophosphorous insecticides it was noted that radiant energy had a definite effect on their persistence and degradation. Subsequent studies have shown that oxidation to more potent *in vitro* cholinesterase inhibitors occurs. However, the toxicity of these light products to insects and mammals is much reduced due to their increased susceptability to detoxification within the organism. Hence, the importance of photochemical changes to organophosphorous insecticides lies primarily in the stability and persistence of parent compounds.

Payton (1953) was one of the early investigators of organophosphate photodegradation. He exposed aqueous salt emulsions of parathion to ultraviolet light and noted an increase in anti-cholinesterase activity. Control samples kept in darkness or exposed to electric incandescent light were unchanged.

J.W. Cook and co-workers have contributed several publications concerning the photodegradation of organophosphorous insecticides. Cook (1954) noted that exposure of systox and isosystox to light forms products more hydrophilic than the parent compounds. The photoconversion product of systox was not an anti-cholinesterase agent, however, the light product of isosystox was a strong cholinesterase inhibitor. On the basis of infrared absorption spectra, Cook concluded that light conversion products of systox and isosystox retained the P=S and P=O linkages, and that some other linkage was involved.

Cook (1955) treated twelve organophosphorous insecticides with *N*-bromosuccinimide or exposed them to ultraviolet light and noted that all of the thiono and dithiophosphates were converted to more potent *in vitro* cholinesterase inhibitors. The compounds formed were generally more soluble in water than oil. In addition, it was noted that the ethoxy compounds tested

were apparently more susceptible to the change than were the methoxy compounds.

Cook and Pugh (1959) determined that the residues of trithion, ethion, thimet, and to a lesser degree malathion after exposure to far ultraviolet light may not consist of the parent compounds or their simple oxidation products. Exposure of the insecticides to a germicidal lamp for 20-30 minutes produced less polar compounds. However, prolonged exposure resulted in the formation of more highly polar light products.

Frawley (1958) exposed parathion applied on glass plates to ultraviolet light for various intervals of time up to 16 hours and analyzed the degradation products for anti-cholinesterase activity and toxicity to houseflies and rats. Exposure to ultraviolet light produced paraoxon, and other degradation products which as a mixture were greater in vitro cholinesterase inhibitors, but were less toxic to the organisms tested. The light products were more hydrophilic than the parent compound as was indicated by paper chromatographic analysis.

Mitchell (1960, 1961) determined the relative stability of 141 pesticides to far ultraviolet light. By utilization of paper chromatographic analysis, he classified the compounds into three general groups: those undergoing little change, those completely or nearly completely degraded, and those showing intermediate change between the two extremes. It was noted that many of the organophosphorous insecticides are changed to a number of degradation products, but none of the parent compounds was completely degraded. The dithio and thiophosphates, malathion, chlorothion, delnav, diazinon, guthion, methyl parathion, parathion, systox, trithion, and dimethoate were all classified in the intermediate group.

El-Refai (1960) investigated the far ultraviolet light photodegradation of malathion and identified several malathion degradation products. Compounds identified were: O,O-dimethyl hydrogen phosphorodithioic acid, O,O-dimethyl

hydrogen phosphorothioic acid, phosphoric acid, O,O-dimethyl-S-(1,2-bis-carboxy)-ethyl phosphorothioate or malathion diacid, and the oxygen analog malaoxon.

In a more intensive study of the photodegradation phenomenon, Walters (1968) determined effects of specific regions of the electromagnetic spectrum upon deposits of malathion and four other organophosphorous insecticides applied to glass surfaces. The different spectral regions utilized were infrared (7000-9000Å), solar visible region (4300-6400Å), near ultraviolet (3900-4300Å), and far ultraviolet (2200-2600Å). In addition, deposits of malathion on glass plates and bean leaf surfaces were exposed to direct sunlight and greenhouse light.

The relative activity or energy of the various light sources was reflected by the time required to produce malaoxon and other degradation products. The relative sequence determined was as follows: direct sunlight = green house light > infrared > far ultraviolet > near ultraviolet > solar visible region. Apparently the infrared and the far ultraviolet regions were most effective inducing oxidation of malathion to malaoxon and subsequent degradation to inorganic phosphoric acid. Identification of malathion, malaoxon and detection of two unknown spots on cellulose thin-layer chromatographic plates was reported. One of the unknowns was thought to be the S-methyl isomer of malathion. This compound was characterized as a sulfur containing cholinesterase inhibitor with polarity intermediate to malathion and malaoxon. The author indicated that the other unknown was possibly the monoacid or diacid derivative of malathion, however, little supporting evidence was given. Paper chromatographic analysis was used to identify the following hydrolytic products of malathion: O,O-dimethyl phosphorodithioate, O,O-dimethyl phosphorothioate, dimethyl phosphate, monomethyl phosphate, and phosphoric acid. An unknown

intermediate in polarity between dimethyl and monomethyl phosphate was not identified. Deposits of malathion on glass plates kept in darkness showed only malathion and malaoxon after 14 days.

Malathion Metabolism in Stored Grains

Numerous studies have determined the biological effectiveness (Lindgren, 1954; Strong and Spur, 1960; King et al., 1962; Lemond, 1966) and the residual life of malathion in stored grains (Rowlands, 1964; Rowlands and Clements, 1965; LaHue, 1965, 1966, 1967, 1968; and Kadoum and LaHue, 1969). These studies have determined the rate of malathion degradation in stored grains under a wide variety of storage conditions. However, very few investigations of metabolic pathways of malathion breakdown in stored grains have been reported. D.G. Rowlands (1967) reviewed the metabolism of contact insecticides in stored grain, however, outside of this publication, little information on malathion metabolism in stored grains is available.

Rowlands (1964) studied the degradation of malathion in stored maize and wheat grains and concluded that malathion metabolism followed a hydrolytic rather than an oxidative route. Both enzymic and non-enzymic degradation was noted. O,O-dimethyl phosphorodithioic acid was detected in malathion treated dead (autoclaved) and living wheat and in living maize. In addition, malathion monoacid and diacid were detected in maize, whereas, only the diacid was found in wheat. No oxidation products, malaoxon or its metabolites, were detected. Rowlands concluded that when malathion is applied to stored wheat or maize it slowly breaks down by chemical and enzymic hydrolysis. Hydrolytic attack was between the thiolo sulfur and the diethyl succinate moiety or at one or both of the ethyl esters of the side chain. In addition to grain phosphatase activity producing O,O-dimethyl phosphorodithioate, carboxyesterase

activity leading to the production of malathion monoacid and malathion diacid was noted. The rate of malathion breakdown on living wheat was greater than on dead wheat or living maize.

Rowlands and Clements (1965) investigated malathion degradation in two varieties of rice brans containing significantly different (10% and 0.8%) levels of fatty acids. Analysis of malathion and its degradation products in living and autoclaved brans after 3, 6, and 12 months of storage revealed that breakdown occurred more rapidly in bran having a high fatty acid content. Metabolites detected were O,O-dimethyl phosphorodithioate and greater amounts of O,O-dimethyl phosphorothioate. Since only slightly smaller quantities of these degradation products were detected in autoclaved samples, the authors suggested that hydrolysis depended on the acid content, although both enzymic and non-enzymic degradation was noted. No evidence indicated carboxyesterase activity producing malathion monoacid or malathion diacid.

Rowlands (1965) determined by *in vitro* and *in vivo* methods that wheat grain oxidase systems were capable of activating malathion to malaoxon. Furthermore, the possible inhibition of hydrolytic enzymes (capable of degrading malathion) by malaoxon was studied. Malaoxon was shown to have no inhibitory effect on the carboxyesterase and acid phosphatase enzyme systems of wheat grain. Anti-cholinesterase metabolites other than malaoxon were identified as malaoxon monoacid and malaoxon diacid. Although these compounds are less potent inhibitors than malaoxon, it was noted that these compounds were probably present in greater quantities and resulted from the hydrolysis of malaoxon.

Rowlands (1966) studied enzyme systems of wheat concerned with the metabolism of the organophosphorous insecticides malathion, bromophos, and dimethoate. Dissection of individual grains into pericarp, seedcoat, starchy

endosperm, and germ and subsequent *in vitro* study enabled the author to locate the activity of different enzyme systems. Oxidative activity was most pronounced in the seedcoat fraction, whereas, slight activity was noted in the outer pericarp and germ and none was found in the starchy endosperm. He also discovered that the thiol-oxidase activity in wheat capable of oxidizing malathion and bromophos is greatest immediately after harvest, but rapidly declines within a few weeks. Since much of the oxidative activity of wheat is associated with the phenol-oxidase systems responsible for the ripening, tinting, and hardening of the seed coats, the author indicated either a direct or indirect link of these systems with the thiol-oxidation of phosphorothionate insecticides. Thus the age of grains may greatly affect the persistence of malathion and other phosphorothionate insecticides. Changes in grain moisture content did not seem to affect oxidation of malathion or bromophos to their corresponding oxons.

Hydrolase activity capable of hydrolyzing malathion and bromophos was found to be greatest in the seed coat and germ, however, traces of slight activity were located in the outer pericarp and the starchy endosperm. Moisture content of wheat grains affected the hydrolysis of diethyl succinate and bromophos. Wheat with moisture content of 18% showed the highest esterase and acid phosphatase activity, whereas, grain at moisture levels of 11, 12 and 14% were roughly equal in activity, but significantly less than grain at 18%. No relationship between grain age and hydrolase activity was observed.

Decarboxylase activity capable of degrading malathion monoacid and diacid and dimethoate acid (*O,O*-dimethyl *S*-carboxymethyl phosphorodithioate) was determined by *in vitro* studies with dimethoate acid. Decarboxylation activity was predominant in the germ, although traces of activity were located in the

seed coat. A relationship between moisture content and carboxylase activity was noted. The decarboxylation of dimethoate acid was greatest at a grain moisture content of 18%, less at 14% and only traces of activity were observed at levels of 11 and 12%.

The author pointed out that moisture content of grain is not the only factor affecting the metabolism of organophosphorous insecticides in stored grain. The penetration rate of the insecticide into the grain to specific areas of enzyme activity may effect the rate of degradation. Penetration of the insecticide is a function of moisture content, the polarity and solubility of the insecticide, the type of formulation and the method of application. Other factors such as temperature and relative humidity are also important. The rapid penetration of bromophos as compared to the slower penetration of malathion was given as a possible explanation for the more rapid breakdown of bromophos, which reaches the inner areas of hydrolase activity faster.

MATERIALS AND METHODS

Light chambers and sources of light. Four light chambers lined with aluminum foil, and equipped with appropriate lamp fixtures were constructed from cardboard boxes according to Walters (1968). Each of three chambers was equipped with two fluorescent lamp fixtures (Midwest Chandelier Company, Kansas City, Mo.), and a fourth with a standard porcelain light socket for insertion of a large infrared light bulb. The four sources of light irradiation used were identical to those used by Walters: plant GRO or natural sunlight simulation lamp, F15T8/GRO (Westinghouse Electric Corp., Bloomfield, N.J.); near ultraviolet light lamp or blacklight, F15T8/BL; far ultraviolet light lamp or germicidal lamp, G15T8; and a reflector infrared red bowl lamp, R-40 (all three manufactured by General Electric Corp., Cleveland, Ohio). The

Table 1. Light intensity and temperatures within light chambers.

Type of light	Peak Spectrum (Å)	Intensity of light (footcandles)	Temperature (°C)
far ultraviolet	2537 1/	71±3	36±1
near ultraviolet	3200-4000 1/	74±3.5	35±1
plant GRO	6550 2/	105±4	35±1
infrared	11000 2/	560±26 3/	119±2 3/

1/ Information obtained from General Electric lamp supply catalog.

2/ Determined with model SR spectroradiometer.

3/ Measured within a 6 inch radius from the center of the light chamber. Measurements for the other light chambers represent the entire chamber.

relative intensity of light in each chamber was measured with a model 756 Weston Illumination Meter (Weston Instruments, Newark, N.J.). The photo-electric probe was positioned at several locations at the bottom of each chamber. The relative intensity of light at different locations within the chambers equipped with fluorescent lamps did not vary more than 10%, however, the intensity within the infrared light chamber decreased considerably 6 inches beyond the center of the chamber. Table 1. lists the light intensity and temperatures of the four light chambers. The peak spectrum for each lamp was obtained from the manufacturers or determined with a model SR spectro-radiometer (Instrumentation Specialties Company, Lincoln, Neb.).

Treatment of stored grain with malathion. A standard solution of 10.0 mg/ml of malathion was prepared by dissolving analytical grade malathion (99.3%) in redistilled acetone. Twenty grams of wheat or sorghum grain, 12.5±.05% moisture content, was treated in a 50 ml beaker by slowly pipetting 0.2 ml of 10.0 mg/ml malathion solution over the surface. The grain was then thoroughly mixed with a glass stirring rod to obtain homogeneous distribution and evaporate the solvent. An initial deposit of 100 parts per million was thus attained. Glass beads (3 mm diameter) were treated in the same manner as the grain. After treatment, each sample was transferred to a petri dish (9 cm diameter) and immediately placed in the appropriate light chamber. Samples of wheat and sorghum grains and glass beads were exposed to the four lights and analyzed by thin-layer chromatography (TLC) for malathion degradation products at time intervals of 0, 1, 5, 10, 15, 20, 25, and 30 days. Control samples of wheat, sorghum and glass beads kept in darkness at 35°C and 119°C were analyzed at the same time intervals.

Removal of malathion residues from glass beads. Light exposed and control

glass bead samples were transferred to 125 ml erlenmeyer flasks and shaken with three successive 50 ml aliquots of redistilled acetone. The extract and combined rinses were transferred to a 250 ml round-bottomed flask for evaporation to near dryness under vacuum. The malathion and degradation products were then quantitatively transferred to a 15 ml graduated centrifuge tubewith three successive aliquots of acetone and concentrated with the aid of a stream of nitrogen to an appropriate volume for TLC spotting.

Extraction and cleanup of malathion residues in stored grains. The method of Kadoum (1969) was used for extraction and cleanup of malathion and its degradation products in 20 gram samples of wheat and sorghum grains. Preliminary experiments with sorghum grain samples fortified with 200 and 2000 micrograms of various malathion metabolites indicated that the cleanup procedure does not permit quantitative recovery of dimethyl phosphate and orthophosphate. Therefore, a fraction of the crude extract was analyzed for these two compounds without any cleanup. The isopropanol:ammonium hydroxide solvent system and silica gel TLC plates (see Table 2) were used to separate interfering grain extractives from the aforementioned phosphates. The TLC analysis for the other malathion degradation products required that the grain extracts be cleaned-up. After cleanup, the recovery of the potassium salts of O,O-dimethyl phosphorodithioate and O,O-dimethyl phosphorothioate were estimated to be 75-85% at both concentrations tested. Estimates ranging from 85-100% were obtained for malathion, malaoxon, malathion monoacid, malathion diacid, and K^+ salt of O-demethyl malathion at both concentrations. Estimation of quantities of the various malathion degradation products was accomplished by spotting varying amounts of the cleaned-up samples on silica gel TLC plates, developing with solvent systems 1-4 (see Table 2), spraying with chromogenic

reagent, and comparing the detected spots with known quantities of analytical standards.

Extraction and cleanup procedure. A 20 gram sample of grain was blended with 100 ml of acidified acetone (1 ml 2N HCl/100 ml) using a Sorvall omni-mixer driven at top speed for five minutes. After filtering and rinsing with an additional 100 ml of acetone, the crude extract was transferred to a 500 ml round-bottomed flask and evaporated to near dryness under vacuum. The residue was then transferred to a separatory funnel with the aid of 10 ml of hexane, and vigorously shaken with 100 ml of water. The layers were allowed to separate and the lower aqueous layer was drawn off into a second separatory funnel. The hexane layer was partitioned with 4 successive 20 ml aliquots of 80% acetonitrile saturated with hexane, and each aliquot was collected in a 250 ml round-bottomed flask. The acetonitrile was removed by vacuum distillation and the residual water was transferred with the aid of 10 ml of hexane to a separatory funnel, and shaken with an additional 100 ml of water. After the layers had separated, the lower layer was drawn off and discarded. The hexane layer, which contains malathion and malaoxon, was collected in a 15 ml graduated centrifuge tube, evaporated to dryness with a stream of nitrogen, and the residue taken up with an appropriate volume of redistilled acetone for TLC spotting.

The water soluble malathion metabolites were extracted from the water in a second separatory funnel with ethyl acetate. After addition of 1 ml of 2N HCl and 30 ml of redistilled ethyl acetate, the separatory funnel was shaken vigorously. After the layers had separated, the water layer was drawn off and discarded. The ethyl acetate fraction was dried by passing through a column of anhydrous sodium sulfate (5 grams), collected in a 100 ml round-bottomed flask and reduced to dryness under vacuum. The residue obtained

consists of the hydrolytic products of malathion, and was dissolved in acetone, transferred to a 15 ml centrifuge tube and concentrated to an appropriate volume for thin-layer chromatographic analysis.

Analytical standards. Analytical grade standards of malathion and seven possible malathion degradation products obtained from the American Cyanamid Company, Princeton, N.J., were used for TLC identification of malathion degradation products. Standard solutions of malathion, malathion half-ester (monoacid), malathion diacid, K^+ salt of O-demethyl malathion, Na^+ dimethyl phosphate, and orthophosphate were prepared in redistilled acetone at concentrations of 10.0 and 1.0 mg/ml. K^+ salt of O,O-dimethyl phosphorodithioate and K^+ salt of O,O-dimethyl phosphorothioate were prepared in 80% ethanol at concentrations of 10.0 mg/ml because of their limited solubility in acetone. Solutions of 1.0 mg/ml of these two compounds were prepared for TLC spotting by diluting 0.5 ml of the ethanol solutions to 5 ml with redistilled acetone. In addition, to the standard solutions of the individual malathion degradation products, various mixtures of the standards at concentrations of 1.0 mg/ml in acetone were utilized. These standards were prepared daily. The use of mixed standards saved considerable time.

Thin-layer chromatography methods. Silica gel (MN silica gel N-HR) and cellulose (cellulose powder MN 300) layers on plastic plates available from Brinkman Instruments Inc., Westburry, N.Y., and Eastman silica gel plates (Type K301R2 without fluorescent indicator) manufactured by Distillation Products Industries, Rochester, N.Y., were used for TLC analysis. Best results were obtained with Brinkman plates, however, only Eastman silica gel plates were suitable for solvent systems containing chloroform. Silica gel plates were generally used without reactivation, although plates exposed to

atmospheric moisture were reactivated at 100°C for 15 minutes. All plates were stored in a dessicator cabinet prior to use. No washing of plates was necessary for solvent systems #1, #2, and #3 (see Table 2.), although washing of both cellulose and silica gel plates with acetone and water (1:1) according to El-Refai and Hopkins (1965) followed by drying at 100°C for 30 minutes gave best results for plates developed with solvent systems #4 and #5.

The separation of malathion and malathion degradation products on silica gel plates with the hexane and ether solvent systems (see Table 2.) was often poor if large (12 x 11 x 4 inches) chromatographic chambers were used. Therefore, half-gallon mason jars, which resulted in good resolution, were used. The semi-quantitative estimation of malathion and some of its

Table 2. Thin-layer chromatographic solvent systems used for the separation of malathion and malathion degradation products.

No.	Solvent system	Layer	Detection reagents
1	Benzene:hexane: acetic acid (40:40:20)	Brinkman silica gel	DCQ or Bromophenol blue
2	Petroleum ether: ether:acetic acid (60:40:1)	Brinkman silica gel	DCQ or Bromophenol blue
3	Chloroform:methanol: 30% ammonium hydroxide (75:25:4)	Eastman silica gel	DCQ or Hanes-Isherwood
4	Isopropanol: 30% ammonium hydroxide (85:15)	Brinkman silica gel	DCQ or Bromophenol blue or Hanes-Isherwood
5	Immobile-N,N dimethyl formamide:acetone (15:85) Mobile-Benzene: isooctane (15:85)	Brinkman cellulose	Bromophenol blue

degradation products was accomplished by spotting varying amounts of the cleaned-up samples on TLC plates, developing in an appropriate solvent system, spraying with chromogenic reagent, and comparing the spots produced in Rf value, color, color intensity, and size with spots produced by known amounts of analytical standards.

Chromogenic sprays. DCQ (2,6-dibromo-N-p-quinoneimine),(Menn et al., 1957; Kadoum, 1970): Dried chromatograms were sprayed with a 0.5% w/v solution of DCQ in redistilled acetone. Instead of heating for 20 minutes at 100°C (Kadoum, 1970), the heating step was reduced to 5 minutes, after which, the chromatogram was exposed to bromine vapor until formation of red or yellow spots on a white background. Different distinct colors were observed for the various malathion degradation products and was of considerable aid in spot identification. Colors ranged from bright red (malathion, malathion monoacid, malathion diacid), to brownish red (K^+ O-demethyl malathion) to pinkish red (K^+ O,O-dimethyl phosphorodithioate and K^+ O,O-dimethyl phosphorothioate),whereas, malaoxon produced a bright yellow color. K^+ O,O-dimethyl phosphorothioate appeared as a brownish red spot after the heating step and changed to a pinkish red spot after treatment with bromine vapor.

DCQ was used for detection of malathion and its sulfur containing degradation products on silica gel TLC plates. The limits of detection were 0.1-1.0 micrograms of the degradation products used. DCQ in acetone rapidly breaks down at room temperature, however, storage of the solution in a freezer maintains its effectiveness for up to one week. Chromatogram spots detected by this method rapidly fade at room temperature in the presence of light, but storage of plates in a freezer slows fading and spots can be

readily seen several months after treatment.

Silver nitrate-bromophenol blue, (Getz, 1962): Chromatograms were dried at 50°C for 15 minutes, sprayed with the reagent (9 parts silver nitrate, 1.0% w/v in 75 ml acetone and 25 ml water; 1 part bromophenol blue, 0.4% w/v in acetone), and reheated for 10 minutes at 50°C. Cooled plates were then immersed in 0.01% w/v aqueous citric acid solution until formation of blue spots. The immersing technique was not suitable for Eastman silica gel plates, but they could be sprayed with 0.1% w/v aqueous citric acid. The silver nitrate-bromophenol blue reagent must be prepared daily, although the components may be stored in a refrigerator one to two weeks without loss of effectiveness.

Cholinesterase spray, (El-Refai and Hopkins, 1965): This method was used for characterization of unknown malathion degradation products. Samples dissolved in acetone were spotted on cellulose TLC plates or Whatman #1 filter paper. These plates were then sprayed with enzyme indicator solution (10 ml human serum, 30 ml water, 1 ml 0.1N NaOH, and 4 ml 1.2% bromothymol blue in 0.1N NaOH) until the surface was moist, incubated at 80°F for 30 minutes, then sprayed with substrate solution (2% w/v aqueous acetylcholine iodide). Within a few minutes areas of inhibition appeared as blue spots on a yellow background. Serum from centrifuged whole human blood was used as a source of acetylcholinesterase.

Hanes-Isherwood reagent, (1949): This reagent was prepared by adding 2 grams of ammonium molybdate to 10 ml perchloric acid (60%) and 16.6 ml of diluted concentrated HCl (1 part conc. HCl diluted to 10 parts with water) and dilution with water to a final volume of 200 ml. Chromatograms were sprayed with the reagent until the surface was moist, heated for 15 minutes

at 85°C, and then exposed to hydrogen sulfide fumes (produced by reacting solid sodium sulfide with concentrated HCl) until formation of blue spots on a light brownish gray background. This reagent was used for detection of dimethyl phosphate and phosphoric acid on silica gel chromatograms.

Characterization of unknown malathion degradation products. In order to characterize some of the unknown photodegradation products of malathion, especially those produced by infrared light, 200 mg of analytical grade malathion was place in a 5 ml volumetric flask, stoppered, and placed in an oven at 150°C for 24 hours. After the heating period, the resulting residue was dissolved in distilled chloroform, and stored in a freezer until TLC analysis. Metcalf and March (1953) indicated that this method produces about 90% isomerization of malathion, so that, detection of the S-methyl isomer of malathion and other thermal degradation products was expected.

Characterization of some of the unknown malathion photodegradation products as acetylcholinesterase inhibitors was accomplished by using the method of El-Refai and Hopkins (1965). After separation of malathion degradation products on silica gel TLC plates, various areas of the plates were scraped off and thoroughly shaken with 10 ml of redistilled acetone. After centrifuging, the acetone extracts were concentrated and spotted on cellulose TLC plates and Whatman #1 filter paper and the cholinesterase spray detection method was carried out as previously described.

Tentative identification of some unknown malathion degradation products was accomplished by dissolving the residue from light exposed malathion in 8% w/v aqueous sodium bicarbonate. An aliquot of the sample dissolved in acetone was evaporated to dryness and then taken up with sodium bicarbonate solution. This method converted acids of some malathion photodegradation

products to their respective salts (Rowlands, 1965). The sodium bicarbonate solution was then directly spotted on silica gel TLC plates and developed with appropriate solvent systems. DCQ was used as the detection reagent.

RESULTS AND DISCUSSION

Thin-layer chromatographic separation and detection of malathion and its degradation products. Figures 1 through 5 show the separation of malathion and its degradation products detected throughout this study. Malathion and eight degradation products were identified with analytical standards, while thirteen unknown compounds were also detected. By using several solvent systems of increasing polarity complete separation of malathion degradation products was obtained.

The DCQ detection reagent was found to be the most effective means of compound detection. Not only was it sensitive to submicrogram quantities of malathion and its degradation products, but the differential color previously mentioned was a valuable aid in compound identification. DCQ detected only sulfur containing compounds. Therefore, dimethyl phosphate and orthophosphate were detected with the Hanes-Isherwood reagent. The Hanes-Isherwood reagent was insensitive (5 microgram limits) to dimethyl phosphate, however, the method was quite sensitive to orthophosphate as less than 0.5 micrograms was readily detected. Although solvent systems used with silica gel TLC plates were used for separation of all malathion degradation products, the presence of high concentrations of malathion sometimes masked detection of malaoxon and unknown #5 (see Figs. 1 and 2). This problem was partially solved by using cellulose TLC plates and the N,N-dimethyl formamide solvent system. Complete separation of

Fig. 1. Thin-layer chromatographic separation of malathion and its degradation products. Brinkman silica gel TLC plates developed with solvent system #1, hexane:benzene:acetic acid (40:40:20). Detection with DCQ and bromine vapor. Spots represent about 5 micrograms.

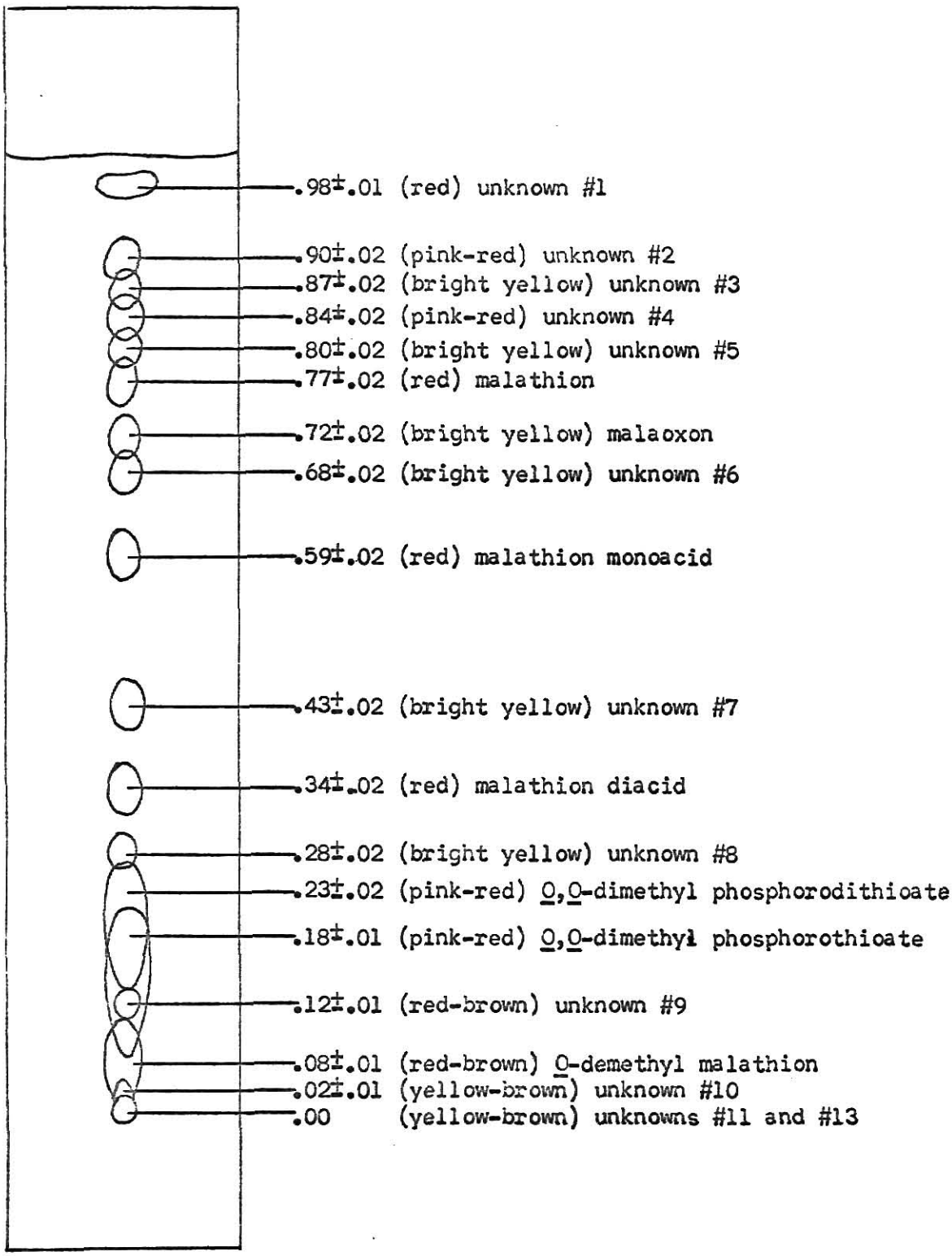


Fig. 2. Thin-layer chromatographic separation of malathion and its degradation products. Brinkman silica gel TLC plates developed with solvent system #2, petroleum ether:ether:acetic acid (60:40:1). Detection with DCQ and bromine vapor. Spots represent about 5 micrograms.

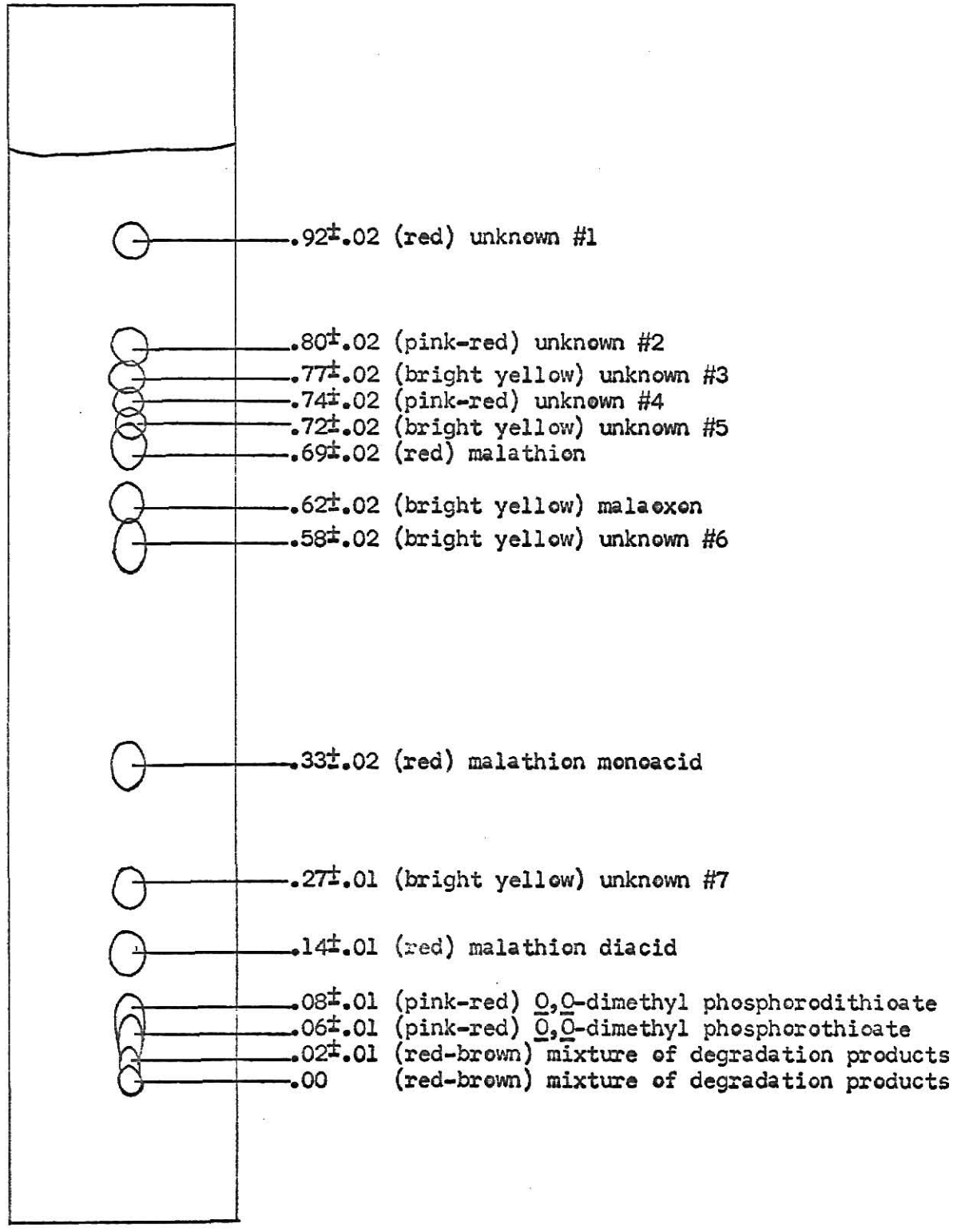


Fig. 3. Thin-layer chromatographic separation of malathion and its degradation products. Eastman silica gel TLC plates developed with solvent system #3, chloroform:methanol:30% ammonium hydroxide (75:25:4). Detection with DCQ and bromine vapor and Hanes-Isherwood reagent. Compounds detected only with Hanes-Isherwood reagent indicated by (*). Spots represent about 5 micrograms.

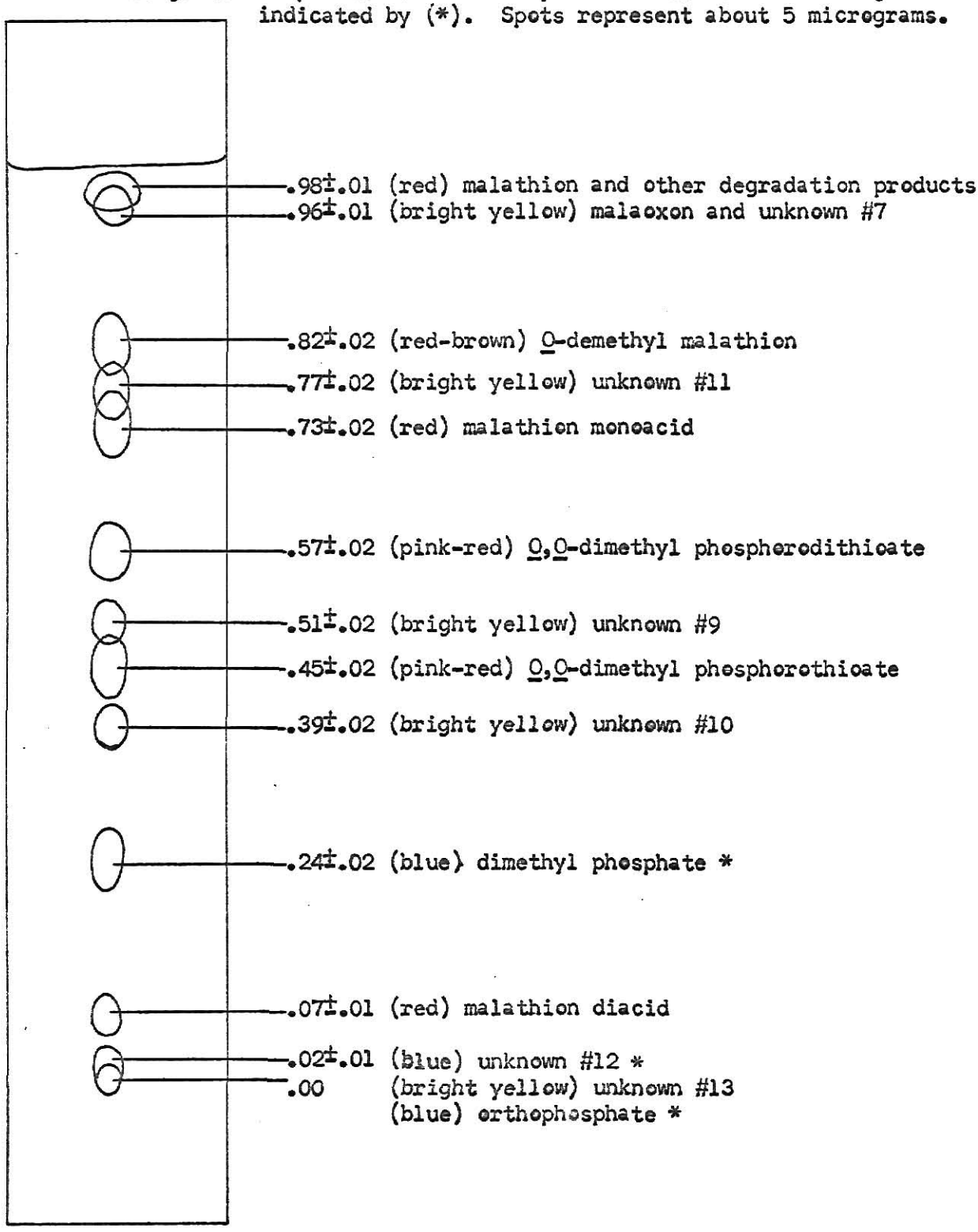


Fig. 4. Thin-layer chromatographic separation of malathion and its degradation products. Brinkman silica gel TLC plates developed with solvent system #4, isopropanol:30% ammonium hydroxide (85:15). Detection with DCQ and bromine vapor, and with Hanes-Isherwood reagent. Compounds detected only with Hanes-Isherwood reagent indicated by (*). Spots represent about 5 micrograms.

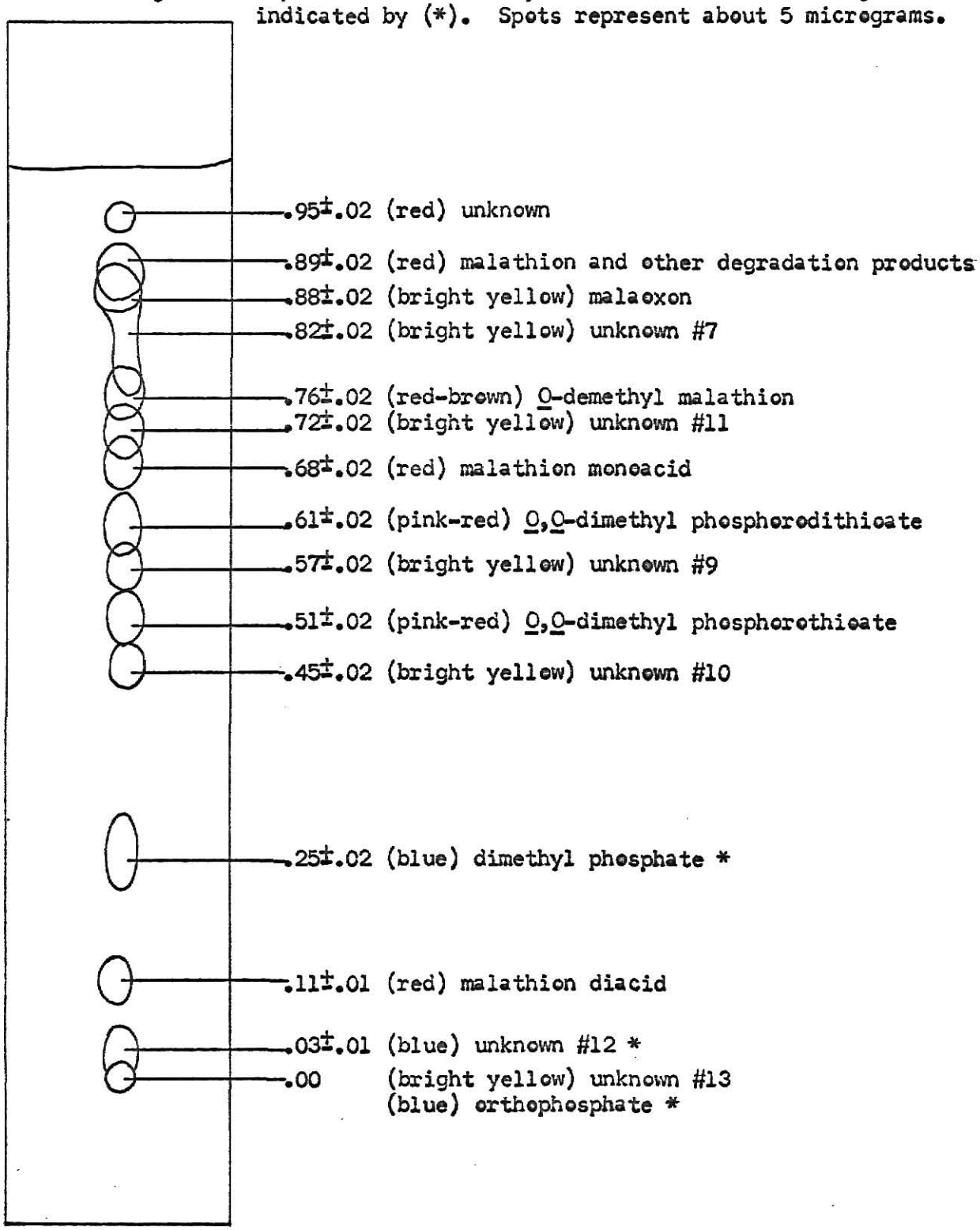
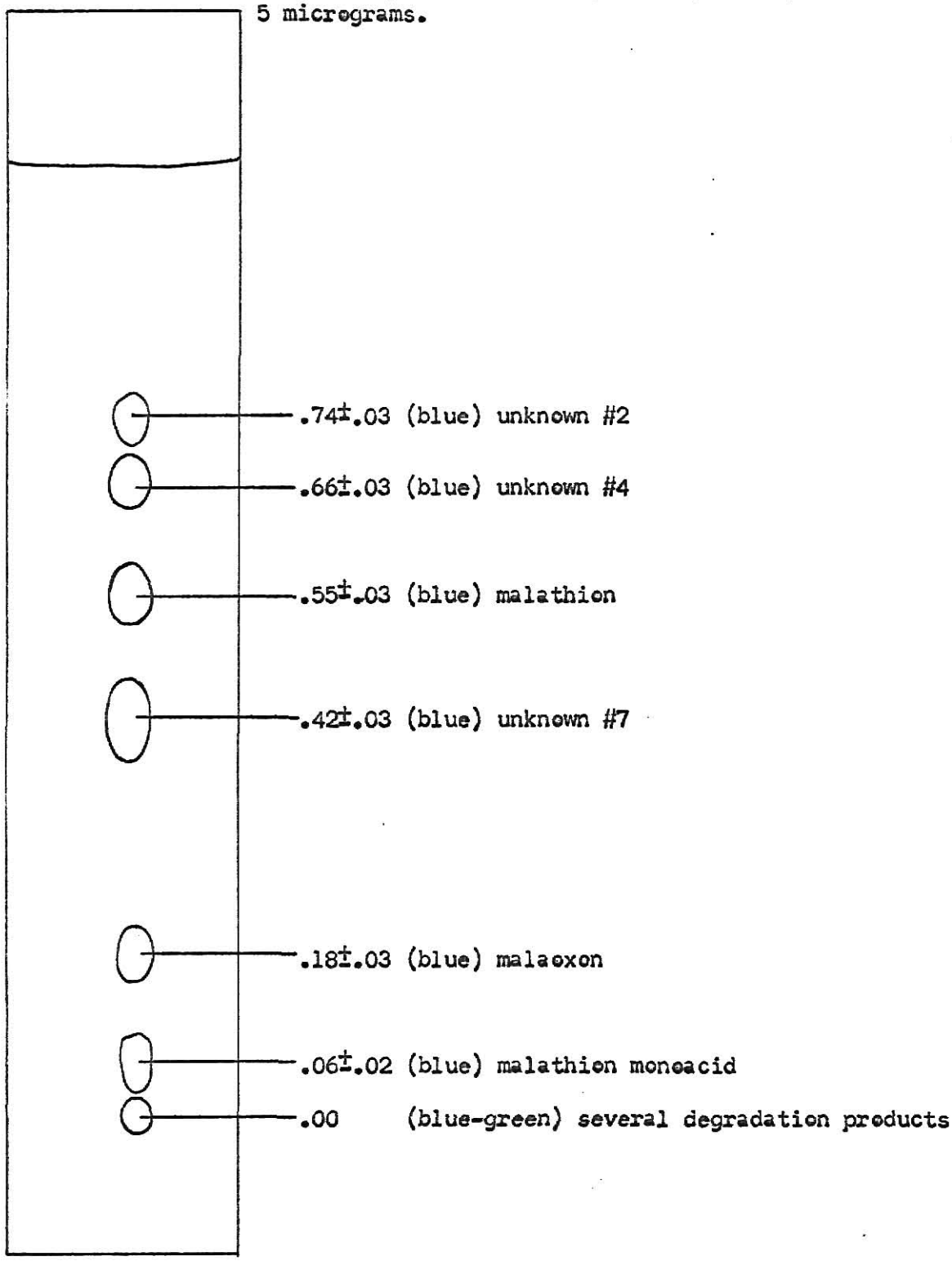


Fig. 5. Thin-layer chromatographic separation of malathion and its degradation products. Brinkman cellulose TLC plates developed with solvent system #5, immobile phase-15% N,N-dimethyl formamide in acetone, mobile phase-15% benzene in isooctane. Detection with silver nitrate-bromophenol blue reagent. Spots represent about 5 micrograms.



malathion and malaoxon was obtained with the aforementioned system. The silver nitrate-bromophenol blue reagent was used with this solvent system at 1-2 microgram detection levels.

Interfering sorghum and wheat grain extractives. Thin-layer chromatographic methods used in this study were quite effective in separating and detecting malathion and its degradation products. Generally, compounds detected in glass bead samples were also detected in corresponding grain samples. However, the presence of grain extractives, which were not removed by the cleanup method was a limiting factor in detection of some malathion degradation products. The highest concentration of grain extractives migrated closely with the solvent front in all solvent systems. These grain extractives interfered with detection of malathion degradation products with higher Rf values than malathion. Unknown #1 was completely masked, whereas, sometimes the other spots indicated were distorted, thus making identification difficult. Another extractive, which remained at the origin with all solvent systems, prevented detection of unknown #13 with DCQ. Unknown #13 and this grain extractive did not react with the Hanes-Isherwood reagent. Hence, orthophosphate, which coincided in Rf with these two compounds, was detected in grain samples without interference from either material. In addition to the aforementioned grain extractives, which were present in both types of grain samples, wheat samples also contained an extractive that nearly coincided in Rf with malathion monoacid. This extractive produced a reddish brown color with DCQ, while all other extractives produced yellowish brown colors. Malathion monoacid produces a red color with DCQ, and was detected as a red spot partially masked by the top of the spot produced by the wheat grain extractive.

Characterization of unknown malathion degradation products. Unknown compounds detected have been numbered from #1 through #13 for discussion purposes. Rf values for unknowns and analytical standards are shown in Figs. 1 through 5. With exception of unknown #7, which was partially extracted into the hexane fraction, all of these compounds were detected in the aqueous fraction of the cleanup method. Some properties of unknowns follow.

Unknown #1: Small amounts of this compound, which produced a red color with DCQ and bromine vapor, were detected in glass bead samples exposed to all four lights. Although this compound was not detected in any grain samples, its presence may not have been detectable due to interfering grain extractives. Unknown #1 was soluble in acetone and exhibited greater mobility on silica gel chromatograms than malathion. Since unknown #1 was detected with DCQ and the bromophenol blue reagent, which are specific for sulfur, it is probably a sulfur containing compound.

Unknown #3: This unknown produced a bright yellow color with DCQ and was detected in samples exposed to high temperatures. Both infrared light exposed and control samples kept in darkness at 119°C contained this malathion degradation product. The absence of this compound in other light exposed and control samples indicated that this compound might possibly be an isomer, oxidation, or rearrangement product of malathion produced by high temperature. Unknown #3 was soluble in organic solvents such as chloroform, acetone, and ethyl acetate. Since malaoxon was the only analytical standard that produced a yellow color with DCQ and also was the only phosphorothiolate, unknown #3 could possibly be a phosphorothiolate. However, a negative reaction to the cholinesterase inhibitor spot test was obtained. Like unknown #1, this compound exhibited higher Rf values on silica gel chromatograms than malathion.

Unknown #5: This compound was mainly detected in samples exposed to far ultraviolet light, although it was detected in three glass bead samples exposed to infrared light. Detection of this compound was possibly limited by high concentrations of malathion which had close Rf values in solvent systems #1 and #2 (see Figs. 1 and 2). Unknown #5 was soluble in acetone and ethyl acetate and was also extracted from hexane into water. Like unknown #3 and malaoxon, it produced a bright yellow color with DCQ. Therefore this compound is possibly a phosphorothiolate. A negative reaction to the cholinesterase spot test was obtained.

Unknown #6: Unknown #6 was detected only in the malathion heated for 24 hours at 150 C and was not detected during light studies. This chloroform soluble compound produced a bright yellow color with DCQ, and exhibited Rf values nearly coinciding with malaoxon. Results of the cholinesterase inhibitor spot test were inconclusive because of difficulty encountered separating unknown #6 from malaoxon. Apparently this unknown is a thiophosphate and a rearrangement or thermal degradation product of malathion.

Unknown #7: Like unknown #3, this unknown was detected in samples exposed to high temperatures. Unknown #7 produced a yellow color with DCQ and also was detected with the bromophenol blue reagent. It gave a rapid positive reaction to the cholinesterase inhibitor spot test and was the main compound detected in heat treated malathion. Figure 5 shows that it exhibits intermediate polarity between malathion and malaoxon. Since the S-methyl isomer of malathion is a strong cholinesterase inhibitor and would be expected to be formed under the experimental conditions (Metcalf and March, 1953), unknown #7 is possibly this compound. If the yellow color produced with DCQ is specific for phosphorothiolates, then the bright yellow color noted for this compound would further support the conclusion that unknown #7 is the S-methyl isomer.

Unknown #8: Unknown #8 produced a bright yellow color with DCQ and also was detected as an impurity in malaoxon analytical standards. The concentration of this compound in the standard slowly increased with time and a 100 mg sample of malaoxon analytical standard over one year old, which had been stored in a freezer, contained an estimated 50% of this compound. This compound was soluble in organic solvents such as acetone, chloroform, and ethyl acetate and was partitioned into the water fraction of the cleanup procedure. The results of the cholinesterase spot test were inconclusive since a faint but not distinct blue spot was observed on both cellulose TLC plates and Whatman #1 filter paper. Apparently, unknown #8 is a degradation product of malaoxon. The yellow color produced with DCQ indicates that this unknown is possibly a phosphosphorothiolate. Unknown #8 was more polar than malaoxon and is possibly malaoxon monoacid.

Unknown #9: This compound was detected in many of the samples exposed to the lights and those kept in darkness. Unknown #9 produced a bright yellow color with DCQ. In addition, this compound was detected in a 10.0 mg/ml K^+ O,O-dimethyl phosphorodithioate standard solution several months after the solution was prepared in 80% ethanol. Unknown #9 was detected in the aqueous fraction of the cleanup procedure and was soluble in acetone and ethyl acetate. A negative reaction to the cholinesterase spot test was obtained. This compound is possibly a phosphorothiolate and a degradation or rearrangement product of dimethyl phosphorodithioate.

Unknown #10: Unknown #10 was detected as an impurity of the K^+ O,O-dimethyl phosphorothioate analytical standard and is possibly a degradation product of this compound. Detection of unknown #10 as a bright yellow color with DCQ indicates that this unknown is a sulfur containing compound more polar than

dimethyl phosphorothioate. This unknown was detected in the aqueous fraction of the cleanup procedure and was soluble in acetone and ethyl acetate. A negative reaction to the cholinesterase spot test was obtained.

Unknown #11: Unknown #11 can be characterized as a sulfur containing cholinesterase inhibitor which produces a yellow color with DCQ. It was detected in the K^+ O-demethyl malathion analytical standard and exhibits similar mobility on silica gel chromatograms. Possibly this compound is O-demethyl malaoxon. Unknown #11 was one of the main compounds detected in samples exposed to high temperatures and persisted after the complete disappearance of malathion and malaoxon.

Unknown #12: This compound was detected only with the Hanes-Isherwood reagent, which indicated that it is probably a phosphate. Walters (1968) detected monomethyl phosphate on filter paper chromatograms using the same detection reagent and a solvent system of isopropanol and 25% ammonium hydroxide (75:25). In this study, silica gel TLC plates and a solvent system of isopropanol and 30% ammonium hydroxide (85:15) were used. Although Walters reported an Rf of .08 for monomethyl phosphate and an Rf of .03 was noted for unknown #12, the lower Rf values for other malathion degradation products on silica gel chromatograms indicates that unknown #12 is probably monomethyl phosphate. Unknown #12 was not detected in any cleaned-up grain samples, but was detected in crude extracts of some grain samples. Poor experimental recovery of orthophosphate and dimethyl phosphate in cleaned-up grain samples further supports the conclusion that unknown #12 is monomethyl phosphate.

Unknown #13: This unknown appeared as a yellowish brown residue when spotted on silica gel plates even before detection with DCQ reagent. Further detection with DCQ produced a bright yellow color. An impurity in the malaoxon analytical standard chromatographed identically with this compound. It is not known

whether the impurity is a degradation product of malaoxon. If unknown #13 is a degradation product of malaoxon, then it is possibly malaoxon diacid. Both unknown #13 and malathion diacid are polar compounds and exhibited little mobility on silica gel chromatograms. Detection of unknown #13 in grain samples was impossible due to interfering grain estractives.

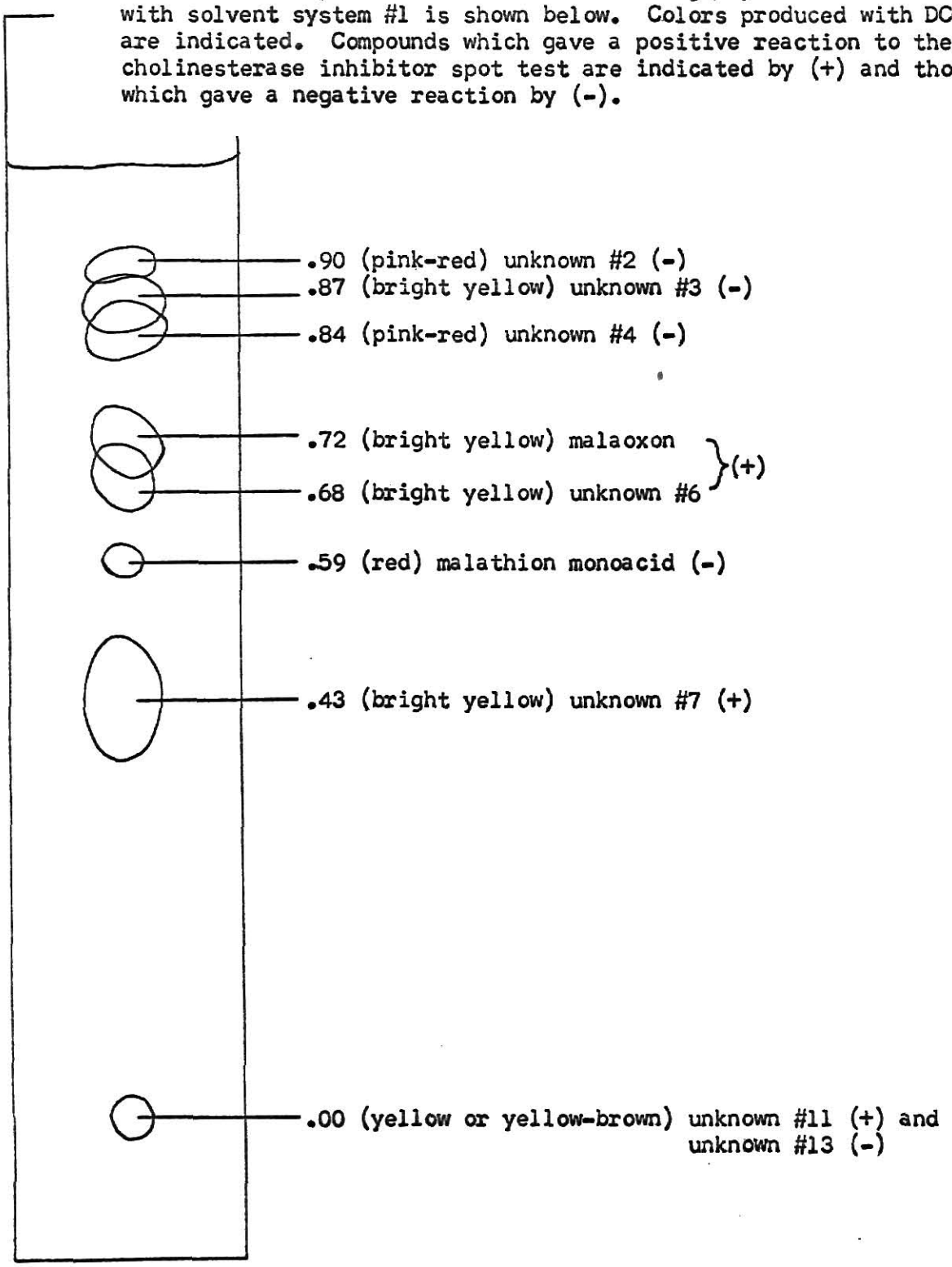
Tentative identification of some unknown malathion degradation products. Unknowns #2 and #4 have been tentatively identified as O,O-dimethyl phosphorothioic acid and O,O-dimethyl phosphorodithioic acid respectively. Early in the study it was noted that unknowns #2 and #4 and the potassium salts of O,O-dimethyl phosphorothioate and O,O-dimethyl phosphorodithioate produced pinkish red colors when detected with DCQ and bromine vapor. In addition, when spotted on silica gel TLC plates and developed with the hexane solvent system, the dithioate standard had a tendency to produce two spots, a spot at Rf .84 and a larger spot at Rf .23. A similar situation was noted with the chloroform solvent system as spots were observed at Rf .98 and Rf .57. The phosphorothioate standard produced only one spot with both solvent systems; a spot at Rf .18 in the hexane system and Rf .45 in the chloroform solvent system. Attempts to convert the potassium salts of the thioate and dithioate to their corresponding acids by dissolving the standards in aqueous HCl, pH 1.0 were unsuccessful. When the acid solutions were spotted on silica gel plates and developed with the two solvent systems, both spots for the dithioate and one spot for the thioate were again detected. Attempts to hydrolyze malathion in acid (ethanolic HCl, pH 1.0, 24 hours at room temperature) and base (ethanolic NaOH, pH 12.0, 24 hours at room temperature) produced only malathion monoacid and and malathion diacid. However, when a sample containing the two unknowns was dissolved in 8% w/v aqueous sodium bicarbonate, spotted

on silica gel TLC plates and developed with the chloroform solvent system only one spot (Rf .57) corresponding to dimethyl phosphorodithioate and one spot (Rf .45) corresponding to dimethyl phosphorothioate were detected. Hence, the spots produced by unknowns #2 and #4 are possibly the corresponding acids of O,O-dimethyl phosphorothioate and O,O-dimethyl phosphorodithioate respectively.

Thermal degradation products of malathion. Thin-layer chromatographic separation of chloroform soluble malathion degradation products produced by heating 200 mg of malathion for 24 hours at 150°C are shown in Fig. 6. The malathion was completely degraded and nine malathion degradation products including malaoxon and malathion monoacid were detected. Unknowns #2 and #4 and malathion monoacid were the only compounds that produced reddish colors with DCQ, whereas the other six compounds all produced bright yellow colors. With exception of unknown #6, all of the compounds were also detected in samples exposed to infrared light or kept in darkness at 119°C. Unknown #7 was the primary heat product and was estimated to be present at twice the concentration as any other degradation product. As previously mentioned unknown #7 is possibly the S-methyl isomer of malathion.

Results of light studies. The results of TLC analysis of irradiated malathion residues on glass beads, wheat and sorghum grains are given in Tables 3 through 6. Tables 7 and 8 list malathion degradation products detected in glass bead, wheat and sorghum grain samples kept in darkness as controls. Estimated quantities of malathion and its degradation products per 100 micrograms of original malathion deposit are indicated. Although many of the compounds detected are unknowns, it was assumed that detection limits of these compounds with DCQ reagent were similar or in a similar range of detection as the analytical standards used. Estimation of quantities of these

Fig. 6. Thin-layer chromatographic separation of malathion degradation products resulting from heating 200 mg of malathion for 24 hours at 150°C and dissolving the remaining residue in 5 ml of chloroform. The tracing of a chromatogram where an equivalent of 400 ug of malathion was spotted on a Brinkman silica gel plate and developed with solvent system #1 is shown below. Colors produced with DCQ are indicated. Compounds which gave a positive reaction to the cholinesterase inhibitor spot test are indicated by (+) and those which gave a negative reaction by (-).



unknowns was based on TLC characteristics of analytical standards. Useful characteristics of standard spots were spot size, coloration, shape and Rf values. Because it was assumed that detection limits of unknowns was in a range of 0.1-1.0 micrograms, the quantities of unknown compounds present in specific samples can be considered a useful indicator, but hardly a quantitative measurement.

TLC analysis revealed that heat producing infrared light (119±2°C) caused the most rapid disappearance of malathion. About 75-80% of malathion applied to grain samples had disappeared after only one day of exposure. In contrast, over 90% of the malathion deposit on glass beads had disappeared in one day. No trace of malathion was detected on glass beads and sorghum grain after 15 days exposure, whereas traces of malathion were last detected on wheat grain after the same exposure period. Oxidation of malathion to malaoxon was the predominant degradative reaction induced by infrared light and was noted on all three types of samples. Subsequent breakdown of malaoxon was indicated by the appearance of an unidentified compound, which possibly was malaoxon monoacid. This unknown (unknown #8) was not detected after the disappearance of malaoxon (15 days exposure) and apparently was further degraded. Unknown #7, which is possibly the S-methyl isomer of malathion was detected in all samples exposed up to 15 days. Unknown #7 was not detected in samples exposed to infrared light more than 15 days indicating that further degradation had taken place. Unknowns #11 and #13 were detected after the complete disappearance of malathion, malaoxon, and unknown #7. As previously mentioned, unknown #11 is possibly O-demethyl malaoxon. If this is the case, then O-demethyl malaoxon could have been formed by the S-demethylation of the S-methyl isomer of malathion, O-demethylation of malaoxon, or O-demethyl-

ation of malathion to O-demethyl malathion followed by oxidation to O-demethyl malaoxon. Since unknown #11 was detected at approximately equal levels after all exposure periods, no indication of its origin was given. Another reaction responsible for the degradation of malathion after exposure to infrared light was hydrolytic degradation of malathion at the phosphorothiolo linkage. Relatively smaller quantities of such hydrolytic products as O,O-dimethyl phosphorothioate and O,O-dimethyl phosphorodithioate were detected. O,O-dimethyl phosphorodithioate and O,O-dimethyl phosphorothioate were detected at 1-2 microgram levels in all three types of samples after 1 and 5 days of exposure to infrared light. Only traces of these two compounds were detected in 10 and 15 day exposed samples. Although neither one of these malathion degradation products was detected after 15 days, the presence of unknowns #9 and #10, which are possibly degradation products of the aforementioned malathion degradation products, were detected after 15 days exposure. Unknown #9 was detected in glass bead, wheat and sorghum grain samples after almost all exposure periods. Its highest concentration was noted at 10 and 15 days. Unknown #10 reached its highest concentration at 5 days and remained relatively constant until its disappearance after 25 days exposure. Hydrolysis of the carboethoxy linkages of the side chain to form malathion monoacid and malathion diacid was another degradation reaction after exposure to infrared light. Malathion monoacid was detected at 1 and 5 day exposure periods at 1-2 microgram levels and dropped off to only trace levels at 10 and 15 days exposure. Malathion diacid was detected at only trace levels in grain samples and slightly higher levels in glass bead samples. Its appearance generally followed a decrease in the monoacid level. Therefore, production of malathion diacid probably resulted from further hydrolysis of the monoacid. Low levels

of O-demethyl malathion were also detected after 1, 5, and 10 days exposure to infrared light. It is not known whether degradation by this route is of major or minor importance. Unknown #13 was detected in all glass bead samples exposed to infrared light, however, interference from grain extractives prevented its detection in grain samples. Its concentration appeared to increase with time and was the main degradation product detected after the complete disappearance of malathion and its primary degradation products. Since unknown #13 did not migrate in any of the solvent systems or even spread out on the chromatograms, it is doubtful whether any degree of accuracy was attained by semi-quantitative estimation. Trace amounts of orthophosphate were detected after 25 days of exposure to infrared light. This indicated that infrared light and/or high temperature had the capability of completely degrading malathion to inorganic orthophosphate. Detection of unknown #11 and #13 with DCQ after 30 days of exposure to infrared light indicated that they were relatively stable and were sulfur containing compounds.

Control samples kept in darkness at 119°C, the same temperature as the infrared light chamber, indicated that although infrared light accelerated degradation of malathion, a similar degradation scheme was produced in the absence of light. Essentially the same degradation products were detected at roughly the same concentrations. This further emphasizes the importance of heat produced by infrared light. Hence, the infrared light induced malathion degradation products as determined in this study can probably best be described as thermal degradation products rather than photodegradation products.

Far ultraviolet light exposed samples contained intact malathion residues after all exposure periods. Malathion applied to glass beads was more than 90% degraded after 30 days, whereas malathion applied to sorghum and wheat

grains was about 80% degraded after the same period of exposure. Control samples kept in darkness at 35°C for 30 days contained an estimated 40-50% of the original malathion deposit. Unlike infrared light, which primarily induced oxidation and isomerization of malathion, degradation of malathion by far ultraviolet light was both hydrolytic and oxidative in nature. Hydrolysis of malathion at the phosphorothiolo linkage was indicated by the presence of two compounds tentatively identified as O,O-dimethyl phosphorothioic acid and O,O-dimethyl phosphorodithioic acid. These compounds and their corresponding salts were detected in nearly all samples throughout the 30 day exposure period. O,O-dimethyl phosphorothioic acid could have also been formed by hydrolysis of malaoxon, however, no indication as to its origin was noted. Unknown #5, which exhibits similar mobility on silica gel chromatograms as malathion was also detected in glass bead samples at slightly lower levels. Only trace amounts of this unknown compound were detected in grain samples, however, the presence of grain extractives sometimes interfered with its detection. Unknowns #9 and #10, which are possibly degradation products of dimethyl phosphorodithioate and dimethyl phosphorothicate respectively, were also detected on glass bead and grain samples. The greatest concentration of unknown #9 was noted after 10 days exposure, whereas lower levels of unknown #10 were detected after all periods of exposure. Hydrolysis of the ethyl esters of the malathion side chain was also noted, but to a lesser degree. Quantities of malathion monoacid and malathion diacid detected after far ultraviolet exposure were greater than after exposure to infrared light. Greater amounts of these two malathion degradation products were also detected in grain samples than were detected in glass bead samples. The difference can probably be attributed to chemical hydrolysis within the grains. Detection

of inorganic orthophosphate and an unknown compound, which could possibly be monomethyl phosphate, with the Hanes-Isherwood reagent indicated that far ultraviolet light exhibits greater hydrolytic degradative activity toward producing secondary malathion degradation products such as dimethyl phosphate, monomethyl phosphate, and orthophosphate than the other three lights. Detection of inorganic orthophosphate and unknown #12, which is possibly monomethyl phosphate, was observed after only 5 days of exposure to far ultraviolet light. Unknown #12 was primarily detected in far ultraviolet light exposed samples, although a few infrared light exposed samples contained this compound.

Far ultraviolet light induced photo-oxidation of malathion was also noted. Malaoxon was detected in all samples exposed to this light, however, lower levels of this malathion oxidation product were detected in grain samples than in glass bead samples. Unknown #8, which is possibly malaoxon monoacid, was detected in nearly all samples. Greater concentrations of this malaoxon degradation product were detected in grain samples indicating that malaoxon was possibly less stable in grain than on glass beads.

O-demethylation of malathion to form O-demethyl malathion was noted in far ultraviolet light exposed samples, however, levels of this compound were considerably lower than previously mentioned degradation products. Unknown #11 was not detected in any of the far ultraviolet light exposed samples.

Plant GRO or artificial sunlight was less active degrading malathion than far ultraviolet light. In addition plant GRO light was found to be only slightly more active degrading malathion than the near ultraviolet light. The earlier appearance of malaoxon and malathion monoacid in plant GRO exposed as opposed to near ultraviolet light exposed samples indicated that plant GRO

light was slightly more active. About 50-60% of the original malathion deposit had disappeared after 30 days exposure to both lights. Malathion was noted to disappear faster on glass bead surfaces than on wheat and sorghum grains. Apparently, malathion remained intact longer on wheat grain than on sorghum grain, however, only slight differences were noted.

Both plant GRO and near ultraviolet light induced degradation of malathion by oxidative and hydrolytic routes. Malaoxon was detected in nearly all samples exposed to plant GRO light, whereas it was present in near ultraviolet light exposed samples only after 15 days exposure. Levels of malaoxon detected in samples exposed to both lights were roughly one-half those noted for samples exposed to far ultraviolet light. Unknown #8, which is possibly malaoxon monoacid, was detected in both plant GRO and near ultraviolet light exposed samples.

The predominant hydrolytic malathion degradative reaction was the formation of malathion monoacid. Production of O,O-dimethyl phosphorodithioate and O,O-dimethyl phosphorothioate by cleavage of the phosphorothiolo linkage appeared to be a much less important degradative reaction than was noted with the photodegradation of malathion induced by far ultraviolet light. This observation was noted in samples exposed to both plant GRO and near ultraviolet light. Malathion monoacid was detected at 1-2 microgram levels in samples exposed to near ultraviolet light 15 or more days. Slightly greater quantities of malathion monoacid were detected in plant GRO exposed samples after 10 days of exposure. Only traces of malathion diacid were detected in samples exposed to both light. Generally the diacid was detected 5-10 days after the first appearance of malathion monoacid.

Although very low levels of O,O-dimethyl phosphorodithioate and O,O-

dimethyl phosphorothioate were detected in plant GRO and near ultraviolet light exposed samples, the presence of unknowns #9 and #10 indicated that the dithioate and thioate were further degraded. Levels of these two unknown compounds were still lower than those present in far ultraviolet light exposed samples. No phosphate esters such as dimethyl phosphate, monomethyl phosphate, or inorganic orthophosphate were detected in any of the samples exposed to plant GRO or near ultraviolet light.

Table 3. Degradation products of malathion applied to glass beads, sorghum and wheat grains exposed to infrared light (119±2 C). Estimated micrograms of degradation product per 100 micrograms of original malathion deposit indicated. Compounds not detected indicated by (-).

Compound 1/		0	1	5	10	15	20	25	30
	glass	-	1	1-2	1	<1	-	-	-
Unknown #1	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	-	1-2	1	2	-	-	-	-
Unknown #2	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	-	2	1-2	2	<1	-	-	-
Unknown #3	sorghum	-	1	1	1-2	<1	-	-	-
	wheat	-	1	1	1	-	-	-	-
	glass	-	<1	<1	<1	-	-	-	-
Unknown #4	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	-	-	-	1	1-2	2	-	-
Unknown #5	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	100	10	1-2	<1	-	-	-	-
Malathion	sorghum	100	20	5	<1	-	-	-	-
	wheat	100	25	8	2	<1	-	-	-
	glass	-	15	10	5	1-2	<1	-	-
Malaoxon	sorghum	-	10	5	5	2	-	-	-
	wheat	-	10	6	2	1	-	-	-
	glass	-	2	1	<1	<1	-	-	-
Malathion	sorghum	-	1	1	<1	<1	<1	-	-
monoacid	wheat	-	1	1	<1	<1	-	-	-
	glass	-	5	5	2	1	-	-	-
Unknown #7	sorghum	-	2	4	1	<1	-	-	-
	wheat	-	2-3	3	1	<1	-	-	-
	glass	-	-	-	1	1	-	-	-
Malathion	sorghum	-	-	-	<1	<1	<1	-	-
diacid	wheat	-	-	-	-	<1	-	-	-

Table 3. continued.

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
	glass	-	1-2	1-2	1	-	-	-	-
Unknown #8	sorghum	-	1	1	2	<1	-	-	-
	wheat	-	1	<1	1-2	<1	-	-	-
O,O-dimethyl	glass	-	2	1-2	<1	<1	-	-	-
phosphorodi-	sorghum	-	1	1	<1	-	-	-	-
thioate	wheat	-	-	1	<1	<1	-	-	-
O,O-dimethyl	glass	-	1-2	1	<1	<1	-	-	-
phosphoro-	sorghum	-	1	1	<1	-	-	-	-
thioate	wheat	-	1-2	1	<1	<1	-	-	-
	glass	-	<1	<1	4-5	2-3	<1	<1	<1
Unknown #9	sorghum	-	<1	<1	<1	1-2	1-2	1	<1
	wheat	-	-	1	1-2	2	1	<1	<1
	glass	-	1-2	1	<1	-	-	-	-
O-demethyl	sorghum	-	1-2	1	<1	<1	<1	<1	-
malathion	wheat	-	<1	<1	<1	-	-	-	-
	glass	-	1-2	2	1	1	1	-	-
Unknown #10	sorghum	-	1-2	1	1	1	<1	<1	-
	wheat	-	1	1	1	1	1	1	-
	glass	-	2	1-2	1	2	1-2	1	1
Unknown #11	sorghum	-	1	1	1-2	1	1-2	1	1
	wheat	-	1	1	1-2	1	1	-	<1
	glass	-	-	-	-	-	-	<1	<1
Unknown #12	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	-	<1	1	1-2	2	3	4-5	5
Unknown #13	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	-	-	-	-	-	-	-	-
Dimethyl	sorghum	-	-	-	-	-	-	-	-
phosphate	wheat	-	-	-	-	-	-	-	-
	glass	-	-	-	-	-	-	<1	<1
Phosphoric	sorghum	-	-	-	-	-	-	<1	<1
acid	wheat	-	-	-	-	-	-	-	<1

Table 4. Degradation products of malathion applied to glass beads, sorghum and wheat grains exposed to far ultraviolet light (36±1 C). Estimated micrograms of degradation product per 100 micrograms of original malathion deposit indicated. Compounds not detected indicated by (-).

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unknown #1	glass	-	1	1	1	<1	<1	-	<1
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #2	glass	-	2	5	5	2	2	4	1
	sorghum	-	1	2-3	2	<1	1	1	1-2
	wheat	-	2	-	2	1	1	-	<1
Unknown #3	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #4	glass	-	2-3	5	3	2	1-2	1	1-2
	sorghum	-	1-2	2	3	1-2	2	1-2	1
	wheat	-	-	2	2	<1	1	1	<1
Unknown #5	glass	-	2-3	1-2	1	1	1-2	3	1
	sorghum	-	-	<1	-	1	-	-	-
	wheat	-	-	1	<1	-	<1	-	-
Malathion	glass	100	55	40	30	20	20	11	5-6
	sorghum	100	60	55	50	40	35	35	20
	wheat	100	70	55	60	50	30	25	20
Malaoxon	glass	-	4	4	3	3	5	5	5-6
	sorghum	-	1-2	2	5	5	2	1-2	1
	wheat	-	1	1-2	2	2	<1	2-3	2
Malathion monoacid	glass	-	<1	3	2-3	<1	<1	-	-
	sorghum	-	1-2	4-5	4	2-3	1	1	1-2
	wheat	-	2	2	1-2	1	1	<1	1-2
Unknown #7	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion diacid	glass	-	<1	1	<1	<1	<1	2	1
	sorghum	-	-	<1	1-2	2	2	1-2	2
	wheat	-	-	1	1-2	2	<1	<1	<1

Table 4. continued.

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
	glass	-	<1	1	<1	<1	<1	1	<1
Unknown #8	sorghum	-	-	<1	1-2	3	2	1-2	2
	wheat	-	-	1-2	2	2	1	2	1-2
O,O-dimethyl	glass	-	2	2	1	3	3-4	2-3	4
phosphorodi-	sorghum	-	2	1	1-2	1-2	2	1	1
thioate	wheat	-	1	1	-	-	1-2	1	1
O,O-dimethyl	glass	-	1-2	1-2	1	1	<1	1	1
phosphoro-	sorghum	-	1	1	1	-	1	1	1
thioate	wheat	-	1	1	-	1	1-2	1	<1
	glass	-	1-2	1-2	1	1	1	<1	<1
Unknown #9	sorghum	-	1	1	1	-	1	<1	1
	wheat	-	1	<1	-	<1	1-2	1	1
	glass	-	<1	<1	<1	1	1	1-2	<1
O-demethyl	sorghum	-	<1	1	1	1-2	1	1	1-2
malathion	wheat	-	<1	<1	<1	1	1	1	<1
	glass	-	2	2	1-2	1-2	1-2	1	1
Unknown #10	sorghum	-	1	1	<1	<1	<1	1	<1
	wheat	-	1	1	1-2	1-2	2	<1	<1
	glass	-	-	-	-	-	-	-	-
Unknown #11	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	-	-	<1	<1	<1	1	1-2	1-2
Unknown #12	sorghum	-	-	-	<1	<1	<1	1	1
	wheat	-	-	-	<1	<1	<1	<1	<1
	glass	-	-	<1	<1	<1	1	1-2	1-2
Unknown #13	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
	glass	-	-	-	-	-	-	5	-
Dimethyl	sorghum	-	-	-	-	-	-	-	-
phosphate	wheat	-	-	-	-	-	-	-	-
	glass	-	-	<1	<1	<1	1-2	1	1-2
Phosphoric	sorghum	-	-	<1	<1	<1	<1	1-2	1-2
Acid	wheat	-	-	-	<1	<1	<1	<1	1-2

Table 5. Degradation products of malathion applied to glass beads, sorghum and wheat grains exposed to plant GRO light (35±1 C). Estimated micrograms of degradation product per 100 micrograms of original malathion deposit indicated. Compounds not detected indicated by (-).

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unknown #1	glass	-	<1	<1	<1	<1	<1	<1	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #2	glass	-	-	<1	<1	<1	-	<1	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #3	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #4	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #5	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion	glass	100	80	70	60	55	50	50	40
	sorghum	100	90	80	65	50	50	45	40
	wheat	100	80	85	70	65	60	50	50
Malaoxon	glass	-	<1	1	3-4	2	2-3	2	1
	sorghum	-	-	<1	1	3	4	2-3	3-4
	wheat	-	-	-	<1	1	4	2	3-4
Malathion monoacid	glass	-	-	<1	1	3	2	2	2
	sorghum	-	-	<1	<1	-	1	1	1
	wheat	-	-	<1	<1	<1	2	1	1
Unknown #7	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion diacid	glass	-	-	-	-	<1	<1	<1	<1
	sorghum	-	-	-	-	-	<1	<1	<1
	wheat	-	-	-	-	<1	-	<1	<1

Table 5. continued.

compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unknown #8	glass	-	<1	<1	1	1-2	1	1	<1
	sorghum	-	-	<1	<1	<1	<1	1	<1
	wheat	-	-	-	<1	<1	<1	<1	1
O,O-dimethyl phosphorodithioate	glass	-	<1	1	1	1	1	<1	<1
	sorghum	-	-	<1	<1	<1	1	<1	<1
	wheat	-	<1	<1	1	1-2	<1	<1	1
O,O-dimethyl phosphorothioate	glass	-	<1	1	1	1	1	1	1
	sorghum	-	-	1	1	1	1	1	1
	wheat	-	1	-	1	1	1	1	1
Unknown #9	glass	-	<1	1	1	2	2	1	1
	sorghum	-	-	1	-	1	1-2	1	-
	wheat	-	-	1	1	1	2	1	1
O-demethyl malathion	glass	-	<1	1	1	<1	1	-	<1
	sorghum	-	-	<1	<1	<1	-	<1	<1
	wheat	-	<1	-	<1	<1	<1	<1	<1
Unknown #10	glass	-	1	1	1	1	1	2	1-2
	sorghum	-	-	<1	<1	1	-	1	1
	wheat	-	<1	<1	<1	<1	1	2	1
Unknown #11	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #12	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #13	glass	-	-	-	-	-	<1	1	1-2
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Dimethyl phosphate	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Phosphoric Acid	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-

Table 6. Degradation products of malation applied to glass beads, sorghum and wheat grains exposed to near ultraviolet light (35±1 C). Estimated micrograms of degradation product per 100 micrograms of original malathion deposit indicated. Compounds not detected indicated by (-).

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unknown #1	glass	-	<1	<1	<1	-	<1	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #2	glass	-	<1	-	<1	1	<1	1	<1
	sorghum	-	-	-	<1	<1	1	1	<1
	wheat	-	-	-	-	-	<1	-	<1
Unknown #3	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #4	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #5	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion	glass	100	80	75	65	60	60	50	40
	sorghum	100	75	60	40	50	50	45	40
	wheat	100	85	80	70	65	60	50	50
Malaoxon	glass	-	-	-	-	2	1-2	1-2	3
	sorghum	-	-	-	-	1	1	1	2
	wheat	-	-	-	-	1	1	2	1
Malathion monoacid	glass	-	-	-	<1	1	2	2	1
	sorghum	-	-	<1	<1	1	1	<1	1
	wheat	-	-	-	<1	<1	-	-	<1
Unknown #7	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion diacid	glass	-	-	-	-	-	<1	<1	-
	sorghum	-	-	-	-	<1	<1	-	<1
	wheat	-	-	-	-	-	<1	-	-
Unknown #8	glass	-	-	-	<1	1-2	1	1	1
	sorghum	-	-	-	-	-	<1	-	<1
	wheat	-	-	-	-	-	<1	<1	<1

Table 6. continued.

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
O,O-dimethyl phosphorodithioate	glass	-	<1	1	1	1-2	1	1	<1
	sorghum	-	-	1	1	-	<1	1	1
	wheat	-	-	-	1	1	<1	-	1
O,O-dimethyl phosphorothioate	glass	-	1	1	1	1	<1	1	1-2
	sorghum	-	<1	<1	<1	1	1	<1	1
	wheat	-	<1	<1	<1	1	-	-	1
Unknown #9	glass	-	1-2	1-2	1	1-2	2	1	1-2
	sorghum	-	-	1	1	1	1	<1	<1
	wheat	-	-	<1	1	1	1	<1	<1
O-demethyl malathion	glass	-	-	-	<1	1-2	1	1-2	<1
	sorghum	-	-	-	<1	<1	<1	<1	1
	wheat	-	-	-	-	<1	<1	<1	<1
Unknown #10	glass	-	<1	<1	<1	1	1-2	1-2	<1
	sorghum	-	-	-	1	<1	<1	1	1
	wheat	-	-	-	-	<1	<1	1	<1
Unknown #11	glass	-	-	-	-	<1	1-2	1	1
	sorghum	-	-	-	-	-	-	<1	<1
	wheat	-	-	-	-	-	-	-	<1
Unknown #12	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #13	glass	-	-	-	-	-	<1	<1	<1
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Dimethyl phosphate	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Phosphoric acid	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-

Table 7. Degradation products of malathion applied to glass beads, sorghum and wheat grains kept in darkness at 119±2 C. Estimated micrograms of degradation product per 100 micrograms of original malathion deposit indicated. Compounds not detected indicated by (-).

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unknown #1	glass	-	<1	<1	1	<1	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #2	glass	-	1	1	<1	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #3	glass	-	2	1	1	<1	-	-	-
	sorghum	-	1	1-2	1	-	-	-	-
	wheat	-	1	<1	1	-	-	-	-
Unknown #4	glass	-	1	1	2	1	-	-	-
	sorghum	-	-	<1	1	-	-	-	-
	wheat	-	-	-	<1	-	-	-	-
Unknown #5	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion	glass	100	25	10	<1	-	-	-	-
	sorghum	100	20	15	2	-	-	-	-
	wheat	100	30	10	1-2	-	-	-	-
Malaoxon	glass	-	10	10	2	1	-	-	-
	sorghum	-	15	5	1	<1	-	-	-
	wheat	-	10	3	<1	<1	-	-	-
Malathion monoacid	glass	-	-	1	1	<1	<1	-	-
	sorghum	-	1	1	1	<1	<1	<1	-
	wheat	-	-	<1	1	<1	-	<1	-
Unknown #7	glass	-	5	5	1-2	<1	-	-	-
	sorghum	-	4	5	2	-	-	-	-
	wheat	-	2	4	1	-	-	-	-
Malathion diacid	glass	-	-	-	-	1	<1	-	-
	sorghum	-	-	-	-	1	1	<1	-
	wheat	-	-	-	-	-	<1	<1	-

Table 7. continued.

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unknown #8	glass	-	1-2	1	2	2	1	-	-
	sorghum	-	-	1	2	1	<1	<1	-
	wheat	-	-	1	1	<1	-	<1	-
O,O-dimethyl phosphorodithioate	glass	-	1	<1	<1	-	-	-	-
	sorghum	-	1	<1	-	<1	-	-	-
	wheat	-	1	<1	-	-	-	-	-
O,O-dimethyl phosphorothioate	glass	-	1	<1	<1	-	-	-	-
	sorghum	-	1	<1	<1	-	-	-	-
	wheat	-	1	<1	-	-	-	-	-
Unknown #9	glass	-	-	<1	1-2	1	1	1-2	1
	sorghum	-	-	-	1	1	1	<1	<1
	wheat	-	-	<1	1	1	1	1	<1
O-demethyl malathion	glass	-	1	<1	1	<1	-	-	-
	sorghum	-	-	<1	<1	<1	-	-	-
	wheat	-	<1	<1	1	-	-	-	-
Unknown #10	glass	-	1	1-2	1	1	1-2	-	-
	sorghum	-	<1	<1	1-2	1	1	<1	-
	wheat	-	<1	<1	<1	1	1	<1	-
Unknown #11	glass	-	1	1-2	1	1	1	1-2	1
	sorghum	-	1	1	<1	<1	1	1	<1
	wheat	-	<1	1	1	1	1	1	<1
Unknown #12	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #13	glass	-	<1	1	1	2	2-3	4	5
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Dimethyl phosphate	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Phosphoric acid	glass	-	-	-	-	-	-	<1	<1
	sorghum	-	-	-	-	-	-	-	<1
	wheat	-	-	-	-	-	-	-	<1

Table 8. Degradation products of malathion applied to glass beads, sorghum and wheat grains kept in darkness at 35±1 C. Estimated micrograms of degradation product per 100 micrograms of original malathion deposit indicated. Compounds not detected indicated by (-).

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unkown #1	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #2	glass	-	-	-	-	-	<1	<1	1
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #3	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #4	glass	-	-	-	-	-	-	<1	<1
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #5	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion	glass	100	90	90	85	80	75	70	60
	sorghum	100	85	75	70	65	60	60	50
	wheat	100	80-85	80	70	55	60	50	50
Malaoxon	glass	-	-	-	-	1	1-2	1	1-2
	sorghum	-	-	-	-	-	-	1	1
	wheat	-	-	-	-	-	-	1	1
Malathion monoacid	glass	-	-	-	-	<1	1	2	1-2
	sorghum	-	-	-	<1	<1	1	1	<1
	wheat	-	-	-	-	<1	<1	1	1
Unknown #7	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Malathion diacid	glass	-	-	-	-	<1	<1	1	1
	sorghum	-	-	-	-	-	<1	<1	<1
	wheat	-	-	-	-	-	<1	<1	<1

Table 8. continued.

Compound 1/		Days Exposure 0	1	5	10	15	20	25	30
Unknown #8	glass	-	-	-	-	-	-	<1	<1
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	<1
O,O-dimethyl phosphorodithioate	glass	-	-	-	<1	<1	1-2	1	1
	sorghum	-	-	-	-	1	1	<1	1
	wheat	-	-	-	-	<1	1	1	1-2
O,O-dimethyl phosphorothioate	glass	-	-	-	<1	1	1	1	1-2
	sorghum	-	-	-	-	<1	<1	<1	<1
	wheat	-	-	-	<1	<1	1	<1	<1
Unknown #9	glass	-	-	-	<1	<1	<1	1	1
	sorghum	-	-	-	<1	<1	<1	-	<1
	wheat	-	-	-	<1	<1	-	<1	<1
O-demethyl malathion	glass	-	-	-	-	-	-	<1	<1
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #10	glass	-	-	-	-	<1	1	1-2	1-2
	sorghum	-	-	-	-	-	<1	<1	<1
	wheat	-	-	-	-	-	<1	<1	<1
Unknown #11	glass	-	-	-	-	-	-	<1	<1
	sorghum	-	-	-	-	-	-	-	<1
	wheat	-	-	-	-	-	-	-	-
Unknown #12	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Unknown #13	glass	-	-	-	-	-	<1	<1	<1
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Dimethyl phosphate	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-
Phosphoric acid	glass	-	-	-	-	-	-	-	-
	sorghum	-	-	-	-	-	-	-	-
	wheat	-	-	-	-	-	-	-	-

SUMMARY AND CONCLUSIONS

Several degradation reactions of malathion and secondary degradation reactions of malathion degradation products were noted in this study. Although several of these reactions may occurr in the absence of light under various climatic conditions and in biological systems, it is evident that various regions of the electromagnetic spectrum may greatly influence the rate of malathion degradation and the route of breakdown. The primary photodegradation reactions of malathion or reactions that directly altered the malathion molecule were of three basic types: oxidation, isomerization, and hydrolysis. Secondary reactions that degraded malathion degradation products were also noted. Some of these secondary degradation reactions were hydrolytic, however, because many of the products of these reactions were not identified, it is difficult to determine the type of reaction(s) that had taken place.

Oxidation: Malaoxon, the oxygen analog of malathion, was detected in samples exposed to all four sources of light. It was the principal malathion degradation product detected in light exposed samples. Apparently malathion is more susceptible to oxidation under the experimental conditions than any other type of degradative reaction. It was evident that each of the four lights accelerated the degradation of malathion by this route. The highest concentrations of malaoxon were detected in samples exposed to infrared light. Control samples kept in darkness at the same temperature as the infrared light chamber contained only slightly lower levels of this degradation product indicating the importance of temperature as a degradative factor. Walters (1968) exposed malathion deposits to infrared light at a lower temperature (38-48°C) and showed that infrared light as well as high temperature were important contributing factors to the oxidation of malathion to malaoxon.

The other three lights showed varying degrees of photo-oxidative activity. Far ultraviolet light produced smaller quantities of malaoxon than infrared light, whereas plant GRO and near ultraviolet lights were less active than far ultraviolet light.

Isomerization: Isomerization of malathion to its S-methyl isomer is an important malathion degradation reaction under conditions of high temperature. In this study, heat produced by infrared light induced the formation of an unidentified compound (unknown #7) which could possibly be this isomer. Walters (1968) reported detecting an anti-cholinesterase compound with identical TLC properties, which he suggested was the S-methyl isomer of malathion. In this study, unknown #7 was detected only in samples exposed to infrared light and high temperature. None of the samples exposed to far ultraviolet light, near ultraviolet light or plant GRO light contained detectable quantities of this compound.

Hydrolysis: The products of a number of hydrolytic malathion degradation reactions were detected after malathion deposits were exposed to light. Some of these products were also detected in control samples kept in darkness. They were, however, at significantly lower concentrations than in light exposed samples. The light products detected indicated that three linkages in the malathion molecule are susceptible to hydrolysis after exposure to light: the phosphorothiolo linkage, the carboethoxy linkages of the side chain, and the methoxy linkages. Detection of O,O-dimethyl phosphorodithioate and O,O-dimethyl phosphorothioate indicated that the phosphorothiolo linkage was cleaved at either side of the sulfur. Far ultraviolet and infrared light exposed samples contained the highest amounts of these two malathion degradation products. Plant GRO and near ultraviolet light exposed samples

contained similar quantities of the thioate and dithioate as control samples kept in darkness. It is interesting to note that far ultraviolet light induced the formation of two compounds (unknowns #2 and #4) that have been tentatively identified as the acids of O,O-dimethyl phosphorothioate and O,O-dimethyl phosphorodithioate. Smaller quantities of these same two compounds were also detected in infrared light exposed samples. El-Refai (1960) also detected O,O-dimethyl phosphorodithioic acid and O,O-dimethyl phosphorothioic acid after exposing malathion deposits to far ultraviolet light. In addition to the attack of the phosphorothiolo linkage of malathion, further degradation of the phosphorothiolo linkages of malathion degradation products was indicated by the detection of dimethyl phosphate, orthophosphate, and an unknown tentatively identified as monomethyl phosphate. Although a few infrared light exposed samples contained trace amounts of orthophosphate, it was evident that far ultraviolet light exhibited the greatest activity for inducing the hydrolysis of malathion and some of its degradation products at the phosphorothiolo linkage. Several other unidentified compounds were detected that may have been the result of the hydrolysis of malathion degradation products.

The carboethoxy linkages of the malathion side chain were also susceptible to hydrolysis after exposure to light. Malathion monoacid was detected in many samples exposed to the four lights. Further hydrolysis of the ethyl ester of malathion monoacid to form malathion diacid was also noted. Two unknown compounds (unknowns #8 and #13) that are possibly the monoacid and diacid derivatives of malaoxon were detected.

Another hydrolytic reaction of malathion (and possibly malaoxon) is O-demethylation to form O-demethyl malathion. Low levels of O-demethyl

malathion were detected after exposure to all four lights. No previous mention of this malathion degradation product has been made by researchers who studied malathion photodegradation. Walters (1968) and El-Refai (1960) apparently were unable to separate and detect this malathion degradation product from other malathion degradation products. Unknown #11, which is a cholinesterase inhibitor, showed a similar tendency to migrate on silica gel TLC plates as O-demethyl malathion and is possibly O-demethyl malaoxon.

Although several photodegradation products of malathion were identified in this study, positive identification of 13 unknown compounds detected on thin-layer chromatograms was not accomplished. Further study including isolation and purification, determination of physical properties, infrared spectroscopy, and electromagnetic resonance spectroscopy should be carried out in order to identify these unknown compounds. Further study might also include determination of the effect of malathion penetration into grains as it effects photodegradation and the effect of grain moisture content on photodegradation.

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PHOTODEGRADATION OF MALATHION ON STORED GRAIN

by

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AN ABSTRACT OF A MASTER'S THESIS

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Malathion, a phosphorodithioate insecticide, was applied to glass beads, wheat and sorghum grains and exposed in separate light chambers to infrared, far ultraviolet, near ultraviolet and plant GRO (artificial sunlight) lights. Individual samples were analyzed by thin-layer chromatography (TLC) after light exposure intervals of 0, 1, 5, 10, 15, 20, 25 and 30 days to determine the malathion degradation products and the relative photodegradative activity of the different lights.

Use of silica gel TLC plates and several solvent systems of increasing polarity enabled separation and detection of 22 different spots corresponding to malathion and its degradation products. Although some of the spots detected probably represented acids and salts of the same compounds, it was apparent that several different linkages in the malathion molecule are susceptible to chemical change after exposure to different sources of light. The following sequence of malathion photodegradative activity was determined: infrared > far ultraviolet > plant GRO > near ultraviolet light. The same order of activity was noted on all three types of samples, although the disappearance of malathion occurred more rapidly on glass beads than on grain samples.

Heat producing infrared light induced the most rapid disappearance of malathion with over 90% degradation of malathion occurring in only 5 days. Oxidation of malathion to malaoxon by infrared light was the predominant degradative reaction and was noted on glass beads, wheat and sorghum grains. Subsequent breakdown of malaoxon was indicated by the appearance of an unknown compound, which was possibly malaoxon monoacid. Isomerization of malathion to its S-methyl isomer was also thought to be a degradative reaction after exposure to infrared light. Although this isomer was not positively

identified in the infrared light exposed samples, an unknown compound which exhibited properties of the isomer was noted. Hydrolytic degradation of malathion at the phosphorothiolo linkage to form O,O-dimethyl phosphorodithioate and O,O-dimethyl phosphorothioate were other degradation reactions noted. O-demethyl malathion, and malathion monoacid and diacid were also detected but at much lower concentrations. Trace amounts of orthophosphate were detected in the 25 and 30 day infrared light exposed samples indicating that complete degradation of small quantities of malathion had occurred. After 30 days of exposure to infrared light, four unidentified compounds were detected. One of these compounds could be a degradation product of O,O-dimethyl phosphorodithioate, whereas, the others are possibly malaoxon monoacid, malaoxon diacid, and O-demethyl malaoxon.

Photodegradation of malathion induced by far ultraviolet light proceeded at a much slower rate. Malathion was detected after all exposure periods with about 80-90% degradation after 30 days. Degradation of malathion by this light was both hydrolytic and oxidative in nature. Hydrolysis of malathion at the phosphorothiolo linkage was indicated by detection of two unknown compounds tentatively identified as O,O-dimethyl phosphorodithioic acid and O,O-dimethyl phosphorothioic acid. These two compounds were detected throughout the 30 day exposure period. Hydrolysis of the ethyl esters of the side chain to form malathion monoacid and diacid was also noted, however, to a lesser degree. Far ultraviolet light induced photo-oxidation of malathion was also noted. Malaoxon was detected in all samples exposed to this light. Like infrared light exposed samples, two unknown compounds which are possibly malaoxon monoacid and malaoxon diacid were detected.

Detection of phosphate esters with the Hanes-Isherwood reagent indicated

that far ultraviolet light exhibits greater hydrolytic degradative activity toward producing secondary malathion degradation products such as dimethyl phosphate, monomethyl phosphate, and orthophosphate than the other three light sources. Orthophosphate and an unknown tentatively identified as monomethyl phosphate were detected after only 5 days exposure to far ultraviolet light. Samples exposed to the other lights contained only trace amounts of these compounds.

Plant GRO light had less malathion photodegradative activity than far ultraviolet light. Plant GRO light was also found to be only slightly more active than near ultraviolet light. Although 50-60% of the original malathion deposit had disappeared after 30 days exposure to both lights, the earlier detection of malaoxon and malathion monoacid in plant GRO light exposed samples indicated that plant GRO light was slightly more active. Both lights induced degradation of malathion by oxidation to malaoxon and hydrolysis of the carboethoxy moiety to form malathion monoacid. Small amounts of the unidentified compounds detected in far ultraviolet and infrared light exposed samples were also detected.