

Effect of Insecticide Distribution and Storage Time on the Degradation of [¹⁴C]Malathion in Stored Wheat¹

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ABSTRACT The degradation of ¹⁴C-malathion ([1,2-¹⁴C] ethyl label) in stored wheat in which 5 or 100% of the kernels were treated with the same overall amount of insecticide was investigated over a 12-month period. There was no difference in the degradation of the insecticide in either treatment. Over the 1-year storage period, quantities of chloroform-soluble radiocarbon decreased, whereas those of water-soluble and unextractable residues increased. Quantities of volatile radiocarbon reached a peak after 6 months of incubation. *Tribolium castaneum* (Herbst) developed more slowly in the presence of unextractable residues than in their absence, although these bound residues were not acutely toxic to adult beetles and did not affect the number of progeny.

It has generally been advised that insecticides used as stored-grain protectants be applied as evenly as possible. However, several reports have indicated that nonuniform insecticide application protects grain as well as conventional, uniform application. Green et al. (1970) examined the distribution of residues in a wheat sample which contained enough bromophos to control *Oryzaephilus surinamensis* (L.) (100% mortality). Bioassays and chemical analyses of single grains chosen at random showed that only 4% of the kernels contained a lethal dose of bromophos. Minett and Williams (1971) conducted an experiment in which adults of three stored-grain insect pests were exposed to wheat samples in which a varying percentage of the grains were treated with malathion and then mixed with untreated grain to give the same total concentration of insecticide (usually 10 ppm). Applying malathion to 1 to 2% of the total wheat bulk was as effective as applying it to all kernels, although control was inferior when only 0.1 to 0.2% of the kernels were treated. They also reported that malathion persisted longer when a low rather than a high percentage of wheat was treated. This effect was mainly observed when 0.1 to 1% rather than higher percentages of the kernels were treated. In a subsequent field study, Minett and Williams (1976) reported that malathion was more persistent and was more toxic to *Sitophilus oryzae* (L.) when 1% of the wheat bulk was treated than when the wheat was treated uniformly with the same total amount of insecticide. Rowlands (1975), however, found that when a small portion of grain was treated with ¹⁴C-malathion at 200 ppm and mixed with untreated grain to give a final concentration of 10 ppm, extensive redistribution of radiocarbon occurred throughout the entire bulk. Rowlands and Bramhall (1977) also found that when wheat (10.6% moisture content) was exposed to the vapors of ¹⁴C-malathion at 25 or 37°C for 7 days, some kernels had an enhanced affinity for malathion not related to the weight of the grain. Therefore, natural

processes and the inherent difficulty in obtaining uniform coverage of the grain would result in variations in residues on individual kernels even after uniform insecticide treatment.

We conducted this laboratory study to investigate the degradation of ¹⁴C-malathion in wheat in which 5% (nonuniform treatment) or 100% (uniform treatment) of the kernels were treated with the same total amount of insecticide. The degradation of ¹⁴C-malathion by stored wheat over a 1-year storage period at 26°C, 60% relative humidity (RH), was also investigated, as well as the biological activity of unextractable (bound) residues.

Materials and Methods

Chemicals

¹⁴C-Malathion ([1,2-¹⁴C] ethyl label), with a specific activity of 15 mCi/mmol, was purchased from Amer-sham Corp. The insecticide was at least 99% pure, as determined by thin-layer chromatography (TLC), autoradiography, and liquid scintillation counting (LSC). The ¹⁴C-malathion was diluted with nonradioactive malathion of 98% purity to a specific activity of 0.48 mCi/mmol before use. Nonradioactive malathion, malaaxon, malathion monocarboxylic acid, malathion dicarboxylic acid, and *O*-demethyl malathion (potassium salt) were obtained from American Cyanamid Co. Diethyl 2-mercaptopuccinate was purchased from Aldrich Chemical Co., whereas thiomalic acid was purchased from Tridom Chemical Co. Acetone, benzene, and hexane were redistilled before use. Acetonitrile, chloroform, toluene, diethyl ether, methylene chloride, and 2-methoxyethanol were analytical reagent grade.

Wheat Treatment

Hard red winter wheat, harvested in 1980, which had not been treated with insecticides or fungicides, was purchased locally. A Carter Dockage Tester was used to remove dust, chaff, and other foreign material from the grain. The moisture content of the wheat was adjusted from 11.2 to 12.5%, as determined by oven drying 10 g of whole grain for 18 h at 130°C. Wheat was allowed to equilibrate at this moisture at 26°C, 60%

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RH, in glass jars with screw-top lids for 1 week before treatment with ^{14}C -malathion.

^{14}C -Malathion was applied to wheat as described by Anderegg and Madisen (1983). An acetone solution of ^{14}C -malathion was mixed with 100% of the wheat (uniform treatment) to yield a dry-weight concentration of 11.4 ppm (0.019 $\mu\text{Ci/g}$, dry weight). In the nonuniform treatment, 5% of the wheat was treated with a final dry-weight concentration of 228 ppm of ^{14}C -malathion (0.377 $\mu\text{Ci/g}$, dry weight). To ensure efficacious treatment of the small quantity of grain, acetone solutions of ^{14}C -malathion were applied to 5% of the wheat in eight 0.25-ml portions. The grain was mixed thoroughly, and the acetone was evaporated between insecticide applications. Aliquots of this ^{14}C -malathion-treated wheat were weighed into individual untreated grain samples and mixed thoroughly to ensure that each replicate had the proper ratio of treated to untreated wheat. Three replicates of each treatment (uniform and nonuniform) at each of the incubation periods (1, 6, and 12 months) were prepared. Two additional replicates of each treatment were extracted immediately to determine initial insecticide concentration. Subsequent data were expressed in percentage of this initial insecticide concentration. Each 15-g (dry weight) replicate was placed into an 475-ml incubation jar designed to trap ^{14}C -volatile compounds and $^{14}\text{CO}_2$ derived from ^{14}C -malathion (Anderegg and Madisen 1983). The wheat was incubated in the dark at 26°C, 60% RH, for 1, 6, or 12 months. After incubation, air was purged sequentially through the incubation jars, the polyurethane trap, and the $^{14}\text{CO}_2$ trap for 8.5 h at a flow rate of 100 ml/min. The various components of the system were then dismantled and analyzed as described below.

Extraction and Analysis

^{14}C -Malathion and its degradation products were extracted from the wheat by using a modification of the procedure described by Cook and Moore (1976). Each 15-g (dry weight) equivalent of wheat was extracted with 250 ml of acidified acetonitrile and 25 ml of dis-

tilled water for 5 min at a high speed in a Waring blender. Extracts were vacuum filtered, concentrated on a rotary evaporator at 35°C, mixed with 50 ml of 2% (aq) Na_2SO_4 and adjusted to pH 8.5 with 5% (aq) Na_2CO_3 . Samples incubated for 1 month were partitioned successively with two 25-ml portions each of ether and methylene chloride. The methylene chloride phases were discarded since they did not contain radiocarbon. The ether phases were purified by mixing with acid-washed, activated carbon as described by Lichtenstein et al. (1973), transferred into chloroform, and dried over acidified Na_2SO_4 . The extraction procedure was simplified for the analysis of samples incubated for 6 or 12 months. These samples were each partitioned with two 35-ml portions of chloroform which were dried over acidified Na_2SO_4 . Water extraction phases resulting from the partitions were mixed with 5% NaCl (wt/vol), adjusted to pH 2 with 6 N HCl and partitioned with 50 ml of chloroform. All chloroform phases for each sample were pooled, concentrated, and adjusted to a 10-ml volume. ^{14}C -Volatile compounds were removed from the polyurethane traps by washing with three 100-ml portions of acetone.

Aliquots of organic solvent and water extraction phases were mixed with 10 ml of a scintillator containing 12 g of Preblend 2a7O (Research Products Intl. Corp.), 1,500 ml of toluene, and 1,500 ml of 2-methoxyethanol. Aliquots of NaOH from the $^{14}\text{CO}_2$ traps were counted in the same scintillator containing 3.5% Cab-O-Sil to prevent precipitation. Unextractable residues were quantified by pelleting extracted grain and combusting it in a modified Schöniger apparatus (Buyske et al. 1963). Samples were analyzed in a Searle/Isocap 300 model 6872 liquid scintillation counter. Data were corrected for background, counter efficiency, and dilutions. TLC of the chloroform extraction phases was done using E. Merck silica gel 60 plates developed in benzene, then redeveloped in the same direction in hexane-benzene-acetic acid (3:1:1). ^{14}C -Malathion and its degradation products were quantified by autoradiography and LSC (Lichtenstein et al. 1978).

The identity of malathion was confirmed by gas-liquid chromatography, and that of malathion monocarboxylic

Table 1. Effect of insecticide distribution and storage time on the metabolism of ^{14}C -malathion in stored wheat^a

Time after treatment	% Of grain treated with ^{14}C -malathion	Radiocarbon recovered (% of applied ^{14}C -malathion)				Total
		Chloroform soluble	Water soluble	Bound ^b	Volatile ^c	
1 mo	100	65.0 ± 0.4a ^d	7.8 ± 0.8a	9.9 ± 0.2a	7.7 ± 1.2ab	90.4 ± 1.8a
	5	67.0 ± 3.5a	6.2 ± 0.7a	8.2 ± 1.6a	7.8 ± 0.4ab	89.2 ± 4.6a
6 mo	100	48.7 ± 1.1b	22.2 ± 0.9b	13.1 ± 0.3b	8.5 ± 0.9bd	92.5 ± 0.8a
	5	50.9 ± 1.6b	19.4 ± 3.1b	10.0 ± 1.6ab	10.2 ± 1.3d	90.5 ± 4.6a
12 mo	100	35.5 ± 1.5c	31.5 ± 1.3c	21.2 ± 1.9c	4.7 ± 0.4e	92.9 ± 1.6a
	5	40.5 ± 4.9d	35.1 ± 3.0c	23.0 ± 2.7c	6.6 ± 0.3a	105.2 ± 7.9b

^aValues represent means ± SD of triplicate tests. ^{14}C -Malathion was applied to 100% of the wheat (12.5% moisture content) at 11.4 ppm (0.019 $\mu\text{Ci/g}$, dry weight), or to 5% of the wheat at 228 ppm (0.377 $\mu\text{Ci/g}$, dry weight).

^bUnextractable ^{14}C -residues determined by combustion to $^{14}\text{CO}_2$.

^c ^{14}C Trapped in polyurethane.

^dMeans in each column followed by the same letter are not significantly different ($P < 0.05$, Duncan's multiple range test).

Table 2. Effect of insecticide distribution and storage time on the production of chloroform-soluble ¹⁴C-malathion metabolites^a

Time after treatment	% Of grain treated with ¹⁴ C-malathion	Recovered (% of total chloroform-soluble radiocarbon) ^b			
		Malathion (R _f = 0.81)	Malathion monocarboxylic acid (R _f = 0.70)	Unknown I (R _f = 0.76)	Unknown II (R _f = 0.16)
1 mo	100	94.5 ± 1.5a ^c	2.6 ± 1.0a	2.9 ± 0.7ab	ND ^d
	5	97.0 ± 0.5a	1.8 ± 0.7a	1.2 ± 0.2a	ND
6 mo	100	87.1 ± 2.2b	8.1 ± 0.5b	4.8 ± 2.1b	ND
	5	87.2 ± 2.3b	8.4 ± 0.5b	4.4 ± 2.5ab	ND
12 mo	100	62.5 ± 0.2c	11.3 ± 0.6c	12.4 ± 1.3c	13.8 ± 2.0a
	5	60.4 ± 3.5c	10.9 ± 1.8c	11.3 ± 2.4c	17.4 ± 3.1a

^aSee footnote a to Table 1.

^bData for total chloroform-soluble radiocarbon are presented in the first column of Table 1. ¹⁴C-Malathion and its metabolites were separated by TLC and analyzed by autoradiography and LSC.

^cSee footnote d to Table 1.

^dND, Nondetectable.

acid was confirmed by high-performance liquid chromatography as described by Anderegg and Madisen (1983).

Bioassay

The biological activity of the bound residues was investigated using the red flour beetle, *Tribolium castaneum* (Herbst), as a test insect. Grain of 13.5% moisture content was treated with unlabeled malathion at 20 ppm and incubated at 26°C, 60% RH, for 3 months. The malathion-treated wheat or untreated wheat (control) was ground finely and extracted with acidified acetonitrile-distilled water (10:1) at a solvent to grain ratio of 3:1. After removing the solvent by vacuum filtration, the extracted grain was rinsed well with acetonitrile and allowed to air dry. The bioassay procedure used was similar to that described by Quinlan et al. (1979) in which three 96-g replicates of each treatment (extracted wheat with or without unextractable residues) were each infested with fifty adult beetles and incubated at 26°C, 60% RH. Mortality counts were made after a 1-week exposure period. Progeny, including adults, pupae, and larvae, were counted after an additional 56 days.

To determine the approximate quantities of unextractable residues on grain treated with unlabeled malathion, aliquots of wheat were treated with ¹⁴C-malathion at 20

ppm, incubated for 3 months, extracted, and analyzed as previously described.

Results and Discussion

No significant difference was found in the degradation of ¹⁴C-malathion in wheat samples in which the same total amount of insecticide was applied to either 5 or 100% of the total wheat bulk (Table 1). The chloroform extraction phase contained malathion and its apolar degradation products. Detoxified, water-soluble degradation products were found in the water extraction phase. Bound residues are often formed under conditions favoring insecticide degradation (Lichtenstein et al. 1977). The exact nature of these residues is usually unknown, since they are altered by the drastic treatments required to extract them from the grain. Similar quantities of chloroform-soluble, water-soluble, bound, and volatile radiocarbon were recovered from both treatments at the end of each incubation period. The one exception to this was that, after 12 months, grain which was treated non-uniformly contained higher quantities of chloroform-soluble and volatile radiocarbon than that which was treated uniformly. However, when the data were calculated in percentage of total recovered radiocarbon, there was no difference in the quantities of chloroform-soluble or volatile residues recovered from either treatment. Furthermore, there was no significant difference in the quantities

Table 3. Effect of unextractable residues on the number and developmental rate of red flour beetle progeny^a

Treatment ^b	Progeny				
	Adults (A)	Larvae (L)	Pupae (P)	Subtotal (L + P)	Total (A + L + P)
Control	415 ± 128	43 ± 11	189 ± 41	232 ± 52	647 ± 111
Bound	63 ± 4 ^c	156 ± 83	239 ± 48	395 ± 67 ^d	458 ± 64

^aValues represent means ± SD of triplicate tests.

^bBound = bound residues remaining in wheat after incubation with ¹⁴C-malathion for 3 months and extraction: control = insecticide-free wheat extracted as described in the text.

^cResults are significantly different from those of controls at the 1% level (Student's *t* test).

^dResults are significantly different from those of controls at the 5% level (Student's *t* test).

of ^{14}C -malathion or its degradation products recovered from wheat receiving a uniform or nonuniform insecticide treatment (Table 2). Data presented in Tables 1 and 2 indicate that the degradation of ^{14}C -malathion was not affected by the uniformity of its application. Our results do not provide an explanation for the superior persistence and effectiveness of uneven insecticide distribution reported by Minett and Williams (1971, 1976). One important difference between our study and theirs is that the non-uniform insecticide treatment in their experiments contained a smaller percentage of insecticide-treated grain (1 to 2%) than that in our experiment.

Over the 1-year storage period, the quantities of chloroform-soluble residues steadily decreased, whereas the amounts of water-soluble and unextractable residues increased (Table 1). Larger quantities of ^{14}C -volatile compounds were recovered after 6 months than after either 1 or 12 months. The relatively low quantities of ^{14}C -volatile compounds recovered after 12 months may be related to the fact that much of the ^{14}C -malathion was degraded to water-soluble and unextractable residues and was thus unavailable for volatilization. The data further indicate that ^{14}C -volatile radiocarbon may be reabsorbed by the grain, although it is possible that the low quantities of ^{14}C -volatile compounds recovered after 12 months resulted from leakage from the incubation jars. No $^{14}\text{CO}_2$ was recovered at the end of 1, 6, or 12 months of incubation. The relatively large quantities of water-soluble and unextractable radiocarbon recovered in 12-month samples indicate extensive ^{14}C -malathion degradation.

^{14}C -Malathion was the major compound recovered in the wheat chloroform extracts at each of the incubation periods tested (Table 2). The total quantity of malathion remaining in the wheat after the 12-month storage period was calculated from the data presented in Tables 1 and 2. In the uniform treatment, for example, 35.5% of the total applied radiocarbon was chloroform-soluble (Table 1), and of that 62.5% was malathion (Table 2). Thus, this wheat contained 22.2% of the originally applied malathion 12 months after treatment. Similarly, Kadoum and LaHue (1979) found 21% of applied malathion in wheat of 12% moisture after 12 months.

Malathion monocarboxylic acid and two unidentified compounds were the principle degradation products recovered. Although ^{14}C -malathion monocarboxylic acid was the major degradation product recovered after 6 months, the amounts of both unknown I and unknown II recovered were equal to or greater than that of malathion monocarboxylic acid after 12 months. There was a decrease in the amount of ^{14}C -malathion and a concurrent increase in the quantities of the three ^{14}C -degradation products over the 12-month storage period. TLC analyses indicated that unknown compounds I and II did not correspond to malathion, malaoxon, malathion carboxylic acids, diethyl mercaptosuccinate, or the alkyl phosphate metabolites. Furthermore, unknown compound I did not correspond to malaoxon carboxylic acids, demethyl malathion, or thiomalic acid. Malathion dicarboxylic acid was not directly detectable in our system because it did not contain the radiocarbon label.

The biological activity of unextractable insecticide residues was investigated as described previously, using a red flour beetle bioassay. After 3 months, an insecticide equivalent of 2.2 ppm or $11.2 \pm 1.0\%$ of applied ^{14}C -malathion was recovered as unextractable radiocarbon in the ^{14}C -malathion-treated samples. The mortality of adult red flour beetles incubated on wheat containing unextractable residues was not significantly different from that of beetles incubated on control wheat. The mortality of beetles on wheat with and without unextractable residues was 11 ± 4 and $16 \pm 2\%$, respectively. Similar results were obtained by Lichtenstein et al. (1977), who examined the effect of unextractable residues derived from ^{14}C -fonofos- or ^{14}C -methylparathion-treated soils on fruit flies. No mortality of fruit flies was observed during a 24-h exposure period to the soil, and only slight mortalities occurred during an additional 48-h exposure period. Although the adult red flour beetle mortalities in our study were not affected by the presence of unextractable residues, beetles did develop more slowly in wheat containing bound residues than in control wheat (Table 3). Wheat containing unextractable residues had fewer adults, more larvae, and more pupae than did the control. Thus, although the unextractable residues were not directly toxic to adult red flour beetles, they did retain biological activity.

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REFERENCES CITED

- Anderegg, B. N., and L. J. Madisen. 1983. Effect of dockage on the degradation of ^{14}C -malathion in stored wheat. *J. Agric. Food Chem.* (in press)
- Buyske, D. A., R. Kelly, J. Florini, S. Gordon, and E. Peets. 1963. Determination of tritium and ^{14}C in biological samples by rapid combustion techniques, pp. 185-191. *In* S. Rothchild [ed.], *Advances in tracer methodology*. Plenum Press, New York. 332 pp.
- Cook, G. H., and J. C. Moore. 1976. Determination of malathion, malaoxon, and mono- and dicarboxylic acids of malathion in fish, oyster, and shrimp tissue. *J. Agric. Food Chem.* 24: 631-634.
- Green, A. A., P. S. Tyler, J. Kane, and D. G. Rowlands. 1970. An assessment of bromophos for the protection of wheat and barley. *J. Stored Prod. Res.* 6: 217-228.
- Kadoum, A. M., and D. W. LaHue. 1979. Degradation of malathion on wheat and corn of various moisture contents. *J. Econ. Entomol.* 72: 228-229.
- Lichtenstein, E. P., T. W. Fuhremann, K. R. Schulz, and T. T. Liang. 1973. Effects of field application methods on the persistence and metabolism of phorate in soils and its translocation into crops. *Ibid.* 66: 863-866.
- Lichtenstein, E. P., J. Katan, and B. N. Anderegg. 1977. Binding of "persistent" and "nonpersistent" ^{14}C -labeled insecticides in an agricultural soil. *J. Agric. Food Chem.* 25: 43-47.
- Lichtenstein, E. P., T. T. Liang, and T. W. Fuhremann. 1978. A compartmentalized microcosm for studying the fate of chemicals in the environment. *Ibid.* 26: 948-953.

Minett, W., and P. Williams. 1971. Influence of malathion distribution on the protection of wheat grain against insect infestation. *J. Stored Prod. Res.* 7: 233-242.

1976. Assessment of non-uniform malathion distribution for insect control in a commercial wheat silo. *Ibid.* 12: 27-33.

Rowlands, D. G. 1975. The metabolism of contact insecticides in stored grains. III. 1970-1974. *Residue Rev.* 58: 113-155.

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

Quinlan, J. K., G. White, J. L. Wilson, L. I. Davidson, and L. H. Hendricks. 1979. Effectiveness of chlorpyrifos-methyl and malathion as protectants for high moisture stored wheat. *J. Econ. Entomol.* 72: 90-93.