# STUDIES ON THE DISTRIBUTION AND MOVEMENT OF $^{14}$C-MALATHION IN STORED WHEAT

by

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Several reports have indicated that nonuniform insecticide application protects stored grain as well as uniform application. Green et al. (1970) found that a wheat sample in which only 4% of the kernels contained a lethal dose of bromophos was toxic enough to control Oryzaephilus surinamensis (L.) (100% mortality).  Minett and Williams (1971) reported that applying the same quantity of malathion to 1-2% of the total wheat sample 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.  Similarly, Minett and Williams (1976) reported that Sitophilus oryzae (L.) was controlled more effectively and for a longer period of time when the same amount of malathion was applied to 1% rather than 100% of a wheat bulk in a field situation. Several studies have indicated that kernel variability and the inherent difficulty in obtaining uniform coverage of grain would result in different quantities of residues on individual kernels even after uniform insecticide treatment.  Rowlands (1975) reported that when 5% of a grain sample was treated with $^{14}$C-malathion, extensive redistribution of radiocarbon occurred throughout the entire sample. Rowlands and Bramhall (1977) found that individual wheat kernels exposed to vapors of $^{14}$C-malathion had different affinities for the insecticide which were not related to the grain weight.

Studies were conducted to investigate the movement and the degradation of $^{14}$C-malathion in wheat in which 5 or 100% of the kernels were treated with the same total amount of insecticide (Anderegg and Madisen, 1983a).  Studies were also conducted to investigate the movement and degradation of $^{14}$C-malathion in wheat containing dockage (Anderegg and Madisen, 1983b).

Effect of insecticide distribution on the movement and degradation of $^{14}$C-malathion.  To investigate the effect of uneven insecticide application on the degradation of $^{14}$C-malathion, 100% of a wheat sample of 12.5% moisture content was treated with $^{14}$C-malathion at a dry weight concentration of 11.4 ppm.  Five percent of a similar wheat sample was treated with $^{14}$C-malathion at a dry weight concentration of 228 ppm.  Thus both treatments received the same total amount of insecticide.  The wheat was placed into sealed incubation jars fitted with polyurethane plugs to trap $^{14}$C-volatile compounds and stored at 26°C, 60% RH.  After 1, 6, and 12 months, samples were extracted and analyzed, resulting in data for organic-soluble,

water-soluble, unextractable, and volatile radiocarbon. ^{14}C-Malathion and its chloroform-soluble degradation products were quantified by thin-layer chromatography, autoradiography, and liquid scintillation counting.

Over the one year storage period, the quantities of chloroform-soluble residues steadily decreased, whereas the amounts of water-soluble and unextractable residues increased (Table 1). The formation of water-soluble and unextractable residues provide a good indication of the degree of ^{14}C-malathion degradation whereas ^{14}C-malathion and its apolar degradation products partition into the chloroform phase. There were no significant differences found in the chloroform-soluble, water-soluble, unextractable, or volatile radiocarbon in wheat treated uniformly (100%) or nonuniformly (5%) after 1, 6, or 12 months. Furthermore, the uniformity of insecticide application did not significantly affect the formation of chloroform-soluble ^{14}C-malathion degradation products (Table 2). Data presented in Tables 1 and 2 therefore indicate that the uniformity of insecticide application did not affect its subsequent metabolism. Our results do not provide an explanation for the superior persistence and effectiveness of uneven insecticide distribution reported by Minett and Williams (1971, 1976).

The maximum quantities of ^{14}C-volatile compounds were recovered after 6 months. It's possible that after 12 months, most of the ^{14}C-malathion was degraded to water-soluble and unextractable residues which were not volatile in nature. The fact that higher quantities of ^{14}C-volatile compounds were recovered after 6 months than after 12 months further indicates that the volatiles may be reabsorbed by the grain. Loss of ^{14}C-volatile compounds by leakage from the incubation jars is another possibility.

Data shown in Tables 1 and 2 may be used to calculate the total amount of ^{14}C-malathion recovered from wheat extracts. After 12 months, for example, 35.5% of the total applied radiocarbon in the uniform treatment was chloroform-soluble (Table 1) and of that 62.5% was malathion (Table 2). Thus 22.2% of the originally-applied insecticide was still present 12 months after treatment. Similar results were reported by Kadoum and LaHue (1979), who found 21% of the applied malathion in wheat of 12% moisture after 12 months.

Since ^{14}C-malathion was metabolized to the same degree whether it was applied uniformly or nonuniformly, a companion experiment was conducted to investigate the redistribution of ^{14}C-malathion residues in wheat receiving an uneven insecticide application. A uniform treatment was prepared in which 100% of a wheat sample of 13.1% moisture content was treated with ^{14}C-malathion at a dry weight concentration of 10 ppm. A nonuniform treatment was also prepared in which 5% of a similar wheat sample was treated with 200 ppm ^{14}C-malathion. In the uneven treatment, wheat kernels were dyed with ethyl violet prior to insecticide treatment for identification purposes. After treatment, the grain was stored at 26°C, 60% RH in 39-ml screw-capped vials. Kernels were oxidized 0, 1, 7 and 50 days posttreatment in a Packard TriCarb Sample Oxidizer, model 306. Each

Table 1. Effect of insecticide distribution on the metabolism of $^{14}$C-malathion in stored wheat[a].

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

a/ Values represent means ± SD of triplicate tests. $^{14}$C-Malathion was applied to 100% of the wheat (12.5% moisture content) at 11.4 ppm or to 5% of the wheat at 228 ppm.

b/ Unextractable $^{14}$C-residues determined by combustion to $^{14}CO_2$.

c/ $^{14}$C trapped in polyurethane.

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

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Table 2. Effect of insecticide distribution on the production of chloroform-soluble $^{14}C$-malathion metabolites[a].

| Months after treatment | Percent of grain treated with $^{14}C$-malathion | Recovered in % 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 MONTH | 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 MONTHS | 100% | 87.1 ± 2.2b | 8.1 ± 0.5b | 4.8 ± 2.1b | ND |
| | 5% | 87.2 ± 2.3h | 8.4 ± 0.5b | 4.4 ± 2.5ab | ND |
| 12 MONTHS | 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 |

a/ see footnote a, Table 1.

b/ Data for total chloroform-soluble radiocarbon are presented in the first column of Table 1. $^{14}C$-Malathion and its metabolites were separated by thin-layer chromatography and analyzed by autoradiography and LSC.

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

d/ ND = nondetectable.

of the treated kernels of the nonuniform treatment was oxidized individually. Thirty pairs of kernels from the uniform treatment and 30 pairs of untreated kernels from the nonuniform treatment, chosen at random, were used to estimate the radiocarbon distribution in each respective sample.

Results presented in Table 3 show that in the nonuniform treatment, the levels of ^{14}C-malathion and its breakdown products in the treated kernels steadily decreased, while those in the untreated kernels steadily increased over the 50-day period. However, the levels of ^{14}C-residues found in the treated kernels were still over 9 times higher than those found in the untreated kernels. Apparently, this nonuniform distribution of ^{14}C-malathion residues (Table 3) did not affect the extent of insecticide degradation (Tables 1 and 2).

Effect of dockage on the movement and degradation of ^{14}C-malathion in stored wheat. The effect of dockage concentration on the movement of ^{14}C-malathion in stored wheat was studied by adding dockage at concentrations of 2.5%, 5.0%, and 10.0% (w/w) to whole grain of 12.5% moisture content. Wheat in which all of the dockage was removed by sieving was used as a control. The term dockage is generally used to describe the small particles of broken grain, weed seeds, dust, and other foreign material which is often found in stored grain. However, the dockage in these experiments was composed totally of ground grain. This dockage was prepared by grinding the wheat in a Hobart coffee grinder to yield a product in which 20% of the total dockage weight was 1700-2000 μm in diameter, 73% was 600-1700 μm in diameter, and 7% was less than 600 μm in diameter as determined by screening and weighing the grain. Acetone solutions of ^{14}C-malathion were applied to each treatment to yield a dry-weight concentration of 10 ppm. The wheat was then placed in incubation jars fitted with polyurethane plugs to trap ^{14}C-volatile compounds and stored at 26°C, 60% relative humidity for 2 months. Prior to analysis, the dockage was screened from the grain and analyzed as a separate sample. At least 95% of the added dockage was recovered after the 2-month storage period.

As the proportion of dockage in the grain increased, the total quantity of ^{14}C-malathion residues recovered in the dockage fraction also increased significantly (Table 4). The large surface area of the cracked wheat relative to that of whole grain may be one reason why ^{14}C-malathion residues accumulate in the dockage fraction. The exposure of tissues normally covered by the pericarp may be another reason. Similar results were found by Quinlan (1982) who treated wheat containing 2.0% cracked wheat with malathion and found that the concentration of malathion per unit weight of wheat particle increased as the particle size decreased. Although the quantity of radiocarbon in the dockage increased as the levels of dockage in the grain increased, the evolution of volatile radioactive compounds was inversely related to the proportion of dockage in the grain. The relationship of ^{14}C-volatiles to the toxicity of ^{14}C-malathion remains to be clearly elucidated. Although malathion is generally considered to be a contact insecticide, its vapor toxicity has been reported (Matsumura, 1975). Storey (1972) showed that malathion or a toxic

Table 3. Effect of uneven insecticide application on the distribution of $^{14}$C-malathion residues in wheat[a]

| Treatment | DPM per kernel | | | |
|---|---|---|---|---|
| Days Posttreatment | 0 | 1 | 7 | 50 |
| 100% kernels treated | | | | |
| treated kernels[b] | 429 ± 70 | 399 ± 64 | 418 ± 70 | 402 ± 118 |
| 5% kernels treated | | | | |
| treated kernels[c] | 7,717 ± 1,467 | 5,660 ± 1,458 | 3,666 ± 1,281 | 3,246 ± 1,188 |
| untreated kernels[b] | 34 ± 8 | 182 ± 61 | 272 ± 125 | 345 ± 247 |

a/ $^{14}$C-Malathion was applied to 100% of the wheat (13.1% moisture content) at 10 ppm or to 5% of the wheat at 200 ppm.

b/ Values represent means ± SD of 60 kernels analyzed in 30 pairs.

c/ Values represent means ± SD of 26 kernels analyzed individually.

Table 4. Movement and degradation of $^{14}$C-malathion in stored wheat containing different amounts of dockage and stored for 2 months[a].

| Recovered from | Radiocarbon recovered, % of applied $^{14}$C-malathion | | | |
|---|---|---|---|---|
| | No dockage | 2.5% dockage[b] | 5.0% dockage | 10.0% dockage |
| Grain | 88.2 ± 1.7[d] | 73.2 ± 1.4 | 67.4 ± 3.2 | 53.3 ± 2.8 |
| Dockage | ------ | 17.7 ± 1.5 | 27.4 ± 1.6 | 43.0 ± 3.4 |
| Volatile[c] | 10.9 ± 0.3 | 7.4 ± 1.0 | 5.7 ± 0.3 | 4.0 ± 0.4 |
| Total | 99.1 ± 1.9a | 98.3 ± 1.2a | 100.5 ± 4.0a | 100.3 ± 0.8a |

a/ Values represent means ± SD of triplicate tests. [$^{14}$C]Malathion was applied at 10 ppm to wheat kernels of 12.5% moisture content. Treated grain was incubated at 26°C, 60% relative humidity.

b/ Grams of dockage per gram fresh weight of sample. Dockage consisted of ground grain.

c/ See footnote c, Table 1.

d/ "Grain", "dockage", "volatile", or "total" means on each line followed by the same letter are not significantly different (P<0.05, Duncan's multiple range test).

malathion degradation product could be removed from the wheat via aeration. On the other hand, Desmarchelier et al. (1976) reported that when Sitophilus oryzae (L.) or Tribolium castaneum (Herbst) were caged and exposed to vapors from grain freshly treated with 10 ppm malathion, there was no mortality.

The effect of dockage on the degradation of ^{14}C-malathion over a period of time was also investigated. Dockage was prepared as previously described and added at a concentration of 2.5% (w/w) to whole grain. A batch of grain containing dockage and a batch of grain without dockage were each treated with ^{14}C-malathion at a dry weight concentration of 10 ppm and then stored in 475-ml jars at 26°C, 60% RH. After 2 or 6 months, triplicate samples from each batch were extracted and analyzed, resulting in data for organic-soluble, water-soluble, and unextractable residues. ^{14}C-Volatile compounds were not determined in this study.

Over the 6-month storage period, the quantity of radiocarbon recovered in the dockage fraction steadily increased (Table 5). The movement of ^{14}C-malathion residues into the dockage fraction was also seen when the data were calculated in percent of total recovered radiocarbon. After 0, 2, and 6 months, $7.7 \pm 0.3\%$, $12.8 \pm 1.4\%$, and $18.5 \pm 2.2\%$ of the total recovered radiocarbon, respectively, was recovered from the dockage fraction.

Although grain both with and without dockage contained lower quantities of chloroform-soluble radiocarbon after 6 months than after 2 months, this trend was more pronounced in the treatment containing dockage than in that without dockage. Furthermore, grain plus dockage always contained higher quantities of unextractable residues than clean grain. Of the bound residues found in grain plus dockage, approximately half were found in the whole grain and half in the dockage. A similar pattern was seen when the data were calculated on a per gram fresh weight basis. For example, after 6 months, the whole grain fraction contained $0.8 \pm 0.1\%$ of applied ^{14}C per gram fresh weight as bound residues while the dockage fraction contained $27.4 \pm 2.7\%$. The dockage or cracked wheat portion of a wheat bulk provides a good environment for the degradation of stored grain protectants for several reasons. Insecticides absorbed by ground wheat may have greater access to tissues containing hydrolytic enzymes. Ground grain is also an excellent breeding ground for stored grain fungi which may have a role in insecticide metabolism (Anderegg and Madisen, 1983c).

In summary, there was no difference in the metabolism of ^{14}C-malathion applied to either 5 or 100% of a wheat sample after 1, 6, or 12 months of storage. Results from a companion experiment indicated that after 50 days, the treated kernels contained over 9 times the quantity of ^{14}C-residues found in the untreated kernels. Therefore, the degradation of ^{14}C-malathion did not seem to be affected by relatively large differences in the residues found on individual kernels. In wheat containing dockage, the total quantity of ^{14}C-malathion residues recovered in the dockage fraction increased significantly both as the ratio of dockage to whole grain

Table 5. Effect of dockage on the movement and degradation of $^{14}C$-malathion in stored wheat incubated for 2 or 6 months[a].

| Recovered from | Radiocarbon recovered, % of applied $^{14}C$-malathion | | | |
| --- | --- | --- | --- | --- |
| | Chloroform-soluble | Water-soluble | Bound[b] | Total |
| **Two Months** | | | | |
| No Dockage | | | | |
| TOTAL | 67.7 ± 2.8[d] | 10.3 ± 2.6[a] | 7.6 ± 1.0 | 85.6 ± 4.4[a] |
| 2.5% Dockage[c] | | | | |
| Grain | 60.1 ± 2.5[a] | 8.5 ± 1.6 | 7.6 ± 1.5[b] | 76.2 ± 3.0[b] |
| Dockage | 3.3 ± 0.5[a] | 0.4 ± 0.1[c] | 7.4 ± 0.8[c] | 11.1 ± 1.1[c] |
| TOTAL | 63.4 ± 2.3 | 8.9 ± 1.7[a] | 15.0 ± 1.8[a] | 87.3 ± 2.3[a] |
| **Six Months** | | | | |
| No Dockage | | | | |
| TOTAL | 48.7 ± 0.4 | 23.0 ± 0.3[b] | 17.9 ± 1.2[a] | 89.6 ± 1.6[a] |
| 2.5% Dockage[c] | | | | |
| Grain | 38.3 ± 0.9 | 18.4 ± 1.0 | 12.7 ± 2.1[b] | 69.4 ± 2.8[b] |
| Dockage | 3.6 ± 1.3[a] | 1.7 ± 0.6[c] | 10.6 ± 1.2[c] | 15.9 ± 3.0[c] |
| TOTAL | 41.9 ± 1.8 | 20.1 ± 1.5[b] | 23.3 ± 3.1 | 85.3 ± 5.8[a] |

a/ See footnote a, Table 4.

b/ See footnote b, Table 1.

c/ See footnote b, Table 4.

d/ "Grain", "dockage", or "total" means in each column followed by the same letter are not significantly different (P<0.05, Duncan's multiple range test).

increased and as the storage time increased. The large quantities of unextractable residues recovered from the dockage fraction may indicate that the insecticide is more rapidly degraded in dockage than in whole grain. As the proportion of dockage in the grain increased, the recovery of $^{14}C$-volatile compounds decreased. These volatile compounds may contribute to the toxicity of the insecticide.

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