

## Persistence of <sup>14</sup>C-Malathion Residues in Stored Milled Rice

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### ABSTRAK

Kegigihan sisa <sup>14</sup>C-malation dalam beras yang disimpan selama 41 minggu telah dikaji mengikut keadaan yang biasa dilakukan di negara ini. Sisa keseluruhan, luar, terekstrak dan terikat dalam butir-butir beras ditentukan selepas penyemburan sekali dengan racun serangga tersebut. Selepas 41 minggu (~ 9 bulan) di dalam simpanan, 10% daripada jumlah kuantiti <sup>14</sup>C-malation yang disembur terdapat pada permukaan guni, manakala 5% berada di permukaan serta di dalam butir-butir beras. Pembentukan sisa terikat sangat kecil dan boleh diabaikan. Data juga menunjukkan bahawa 50-60% daripada jumlah sisa <sup>14</sup>C-malation pada beras merupakan sisa luar. Membasuh beras sebelum memasak akan menyingkirkan sisa-sisa yang terdapat di permukaan beras. Semasa memasak terdapat kehilangan 57% daripada jumlah sisa terekstrak.

### ABSTRACT

The persistence of <sup>14</sup>C-malathion residues in milled rice stored for 41 weeks was studied under conditions simulating local practice. Total, surface, extractable and bound residues in the rice grains were determined following a single spraying with the insecticide. After 41 weeks (~ 9 months) storage, 10% of the total quantity of the applied <sup>14</sup>C-malathion remained on the surface of the jute sack and 5% was present on the surface and inside the rice grains. Formation of bound residues was apparently negligible. Data showed also that 50-60% of the total <sup>14</sup>C-malathion residues in the rice were present as surface residues. Washing the rice thoroughly before cooking removed the surface residues. During cooking, 57% of the extractable residues were lost.

### INTRODUCTION

Rice is the staple food and the most important grain crop in Malaysia. It is stored on a huge scale primarily by LPN (National Padi and Rice Board) and on a smaller scale by private dealers. At the local granaries, rice is stored unmilled (padi) or milled (*beras*). Losses during storage are due mainly to spillage and pest attack. It was reported that between 3-50% of the total rice stored is lost through damage by a variety of storage pests e.g. *Tribolium castaneum* (Herbs) (red flour beetle),

*Sitophilus oryzae* (L) (rice weevil), *Corcyra cephalonica* (rice moth) and *Ephestia cautella* (tropical ware house moth) (Pingale, 1970). One of the methods employed to control insect pests in rice godowns is the use of the insecticide malathion. There have been a number of reports on breakdown of malathion in stored wheat (Rowlands 1964, Kadoum and Lahue 1974, Anderegg & Madisen 1983), stored maize (Bindra and Sindhu 1972, Kadoum and Lahue 1974) and stored Sorghum (Kadoum and Lahue 1969, 1972, 1974). In

such studies, malathion was applied directly to the grains. However, little work has been done to study the penetration and persistence of malathion applied to bagged milled rice. The aim of this study was to employ nuclear techniques to determine levels of the total  $^{14}\text{C}$ -residues of malathion (i.e. the parent compound plus  $^{14}\text{C}$  labelled degradation products) in treated milled rice stored under conditions simulating local practices.

## MATERIALS AND METHODS

### Malathion

$^{14}\text{C}$ -malathion (prepared by the condensation of diethyl [2, 3- $^{14}\text{C}$ ] maleate with O, O-dimethyl dithiophosphoric acid) was purchased from Amer-sham Cor., U K with specific activity 112 uCi/mg (37 mCi/mmol), purity 98% as determined by tlc. Non labelled malathion (99.5% pure) was obtained from Applied Science Lab.

### Application

Freshly milled rice (no previous pesticide treatment; moisture content 10.8% determined by oven drying at 130°C for 18 hours) was obtained from Tanjung Karang rice godown. 1500g of sound, whole rice grains (dockage excluded) were put in a jute sack (20 cm x 20 cm) which was placed on a wooden dunnage measuring 15 cm x 20 cm x 9.5 cm.

The whole set up was placed in a glass container in a fume cupboard. Thirty uCi of  $^{14}\text{C}$ -malathion and 200 mg of non-labelled pure malathion were dissolved in 10 ml of 1% Triton X-100 solution (specific activity = 348757 cpm/mg). The concentration of this mixture was equivalent to that used in practice. The prepared mixture was sprayed, using a tlc glass sprayer, onto the jute sack (containing 1.5 kg rice grains) and the surface of the dunnage. This approximates to field application rates of 5 litres of 2% solution per 100 m<sup>2</sup> surface area. It should be noted that spraying in the local rice godown is normally conducted using a thermal fogger or mist blower. However, since only 10 ml of the  $^{14}\text{C}$ -mixture were required and also to avoid radioactive contamination in the laboratory, the tlc glass sprayer was used instead.

Three fifty g samples of the treated rice grains were taken at 0 (within 24 hours), 2, 4, 8, 12, 24, 34 and 41 weeks. The rice was thoroughly mixed

by shaking the jute sack before sampling. The relative humidity (70–80%) and storage temperature (26–30°C) were similar to those in the rice godown.

### Residue Determination

Fig. 1 shows the flowchart for the determination of surface, extractable and bound residues.

#### a. Water-removable Surface Residues

50 ml of distilled water was added to the 50g grain sample in a beaker and stirred with a glass rod. 1 ml of the extract was mixed with 10 ml of a scintillator comprising 5.5g PPO, 0.1g POPOP, 667 ml toluene and 333 ml Triton X-100. Extract was counted for radioactivity. The washing was repeated twice and each extract was counted separately. Radioactivity in the samples were analysed in a LKB 1217 Rackbeta Liquid Scintillation Counter. Data were corrected for background, counter efficiency and dilutions.

#### b. Extractable Residues:

The washed grains were ground thoroughly in a mortar and transferred to a thimble in a Soxhlet apparatus. Methanol (residue grade, equal to 2X volume of thimble extraction chamber) was added. Extraction was made for 24 hours (8 hours per day for 3 days) and the methanolic extract was then concentrated to 50 ml using a rotary evaporator. 1.0 ml of the concentrated extract was mixed with 10 ml of the scintillator (as stated earlier) and counted for radioactivity.

#### c. Non-extractable (bound) Residues:

The grain in the thimble was then dried in an oven at 60°C for 2 hours. A portion of the dried sample (in 5 replicates) was taken for wet combustion to determine the level of the bound residues. Two procedures were attempted: a modified procedure of Smith *et al.* (1964) and the procedure of Mahin and Lofberg (1966).

#### d. Total Residues

Wet combustion procedure were used to determine the total residues in the grain samples at various intervals.

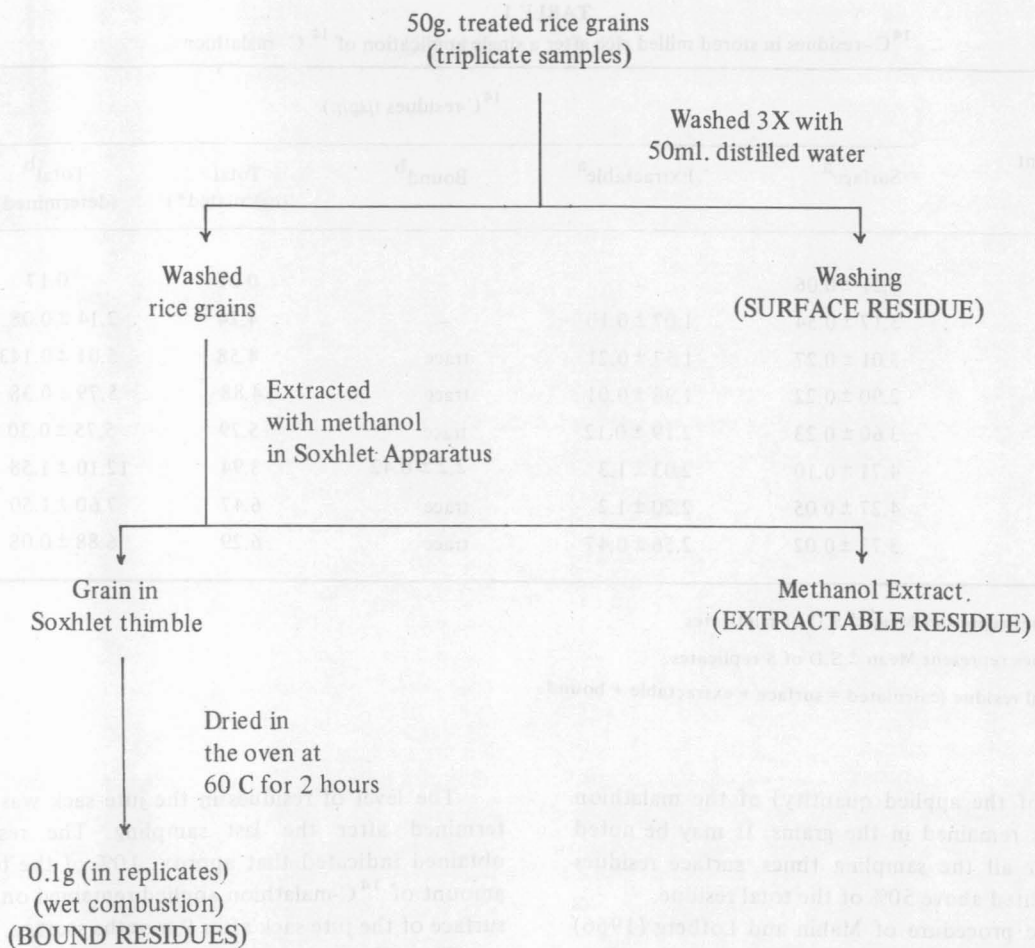


Fig. 1: Flowchart of the determination of surface, extractable and bound  $^{14}\text{C}$ -malathion residues.

e. *Determination of  $^{14}\text{C}$ -activity of the jute sack:*

After the last sampling, the jute sack was cut into 5 cm x 5 cm pieces.  $^{14}\text{C}$ -residues were extracted from the jute sack by shaking for 6 hours (using a Heidolph Rotator) with methanol. The methanolic extract was then counted to determine total activity.

*Effect of Cooking on the  $^{14}\text{C}$ -Malathion Residue*

For this particular experiment, milled rice inside the jute sack was treated 3 times (at 3 weeks interval) and stored for 12 weeks. 50 g of the rice sample (in triplicates) were washed 3 times with 50 ml distilled  $\text{H}_2\text{O}$ . The washed rice was then placed in a beaker and cooked by boiling (in the fume cupboard). Extractable residues and bound residues in the cooked rice were then determined according to the procedure described earlier.

**RESULTS**

The results in Table 1 show the level of malathion residues in the treated grains at specified time intervals. Within 24 hours of spraying, a small amount of the  $^{14}\text{C}$ -residues (0.2% of the originally applied amount) was present on the surface of the grains. After 2 weeks,  $^{14}\text{C}$ -residues in the grains increased to 4.24  $\mu\text{g/g}$  (2.1% of the applied amount), of which 25% penetrated into the grains in the form of extractable residues. The total  $^{14}\text{C}$ -residues (surface + extractable + bound) increased slightly after 8 weeks.  $^{14}\text{C}$ -residues of malathion in the treated grains sampled after 24 weeks increased to 8.9  $\mu\text{g/g}$  (6.7% of the applied amount). The concentration of the bound residues was 2.2  $\mu\text{g/g}$ . This odd high value may be related to an experimental error. After 41 weeks, 6.3  $\mu\text{g/g}$

TABLE 1  
<sup>14</sup>C-residues in stored milled rice after a single application of <sup>14</sup>C-malathion

| Period after treatment (weeks) | <sup>14</sup> C-residues (µg/g) |                          |                    |                     |                                 |
|--------------------------------|---------------------------------|--------------------------|--------------------|---------------------|---------------------------------|
|                                | Surface <sup>a</sup>            | Extractable <sup>a</sup> | Bound <sup>b</sup> | Total (calculated*) | Total <sup>b</sup> (determined) |
| 0                              | 0.21 ± 0.06                     | —                        | —                  | 0.21                | 0.17                            |
| 2                              | 3.17 ± 0.54                     | 1.07 ± 0.10              | —                  | 4.24                | 2.14 ± 0.08                     |
| 4                              | 3.01 ± 0.27                     | 1.57 ± 0.21              | trace              | 4.58                | 5.01 ± 0.143                    |
| 8                              | 2.90 ± 0.22                     | 1.98 ± 0.01              | trace              | 4.88                | 5.79 ± 0.38                     |
| 12                             | 3.60 ± 0.23                     | 2.19 ± 0.12              | trace              | 5.79                | 5.75 ± 0.30                     |
| 24                             | 4.71 ± 0.10                     | 2.03 ± 1.3               | 2.2 ± 0.42         | 8.94                | 12.10 ± 1.58                    |
| 34                             | 4.27 ± 0.05                     | 2.20 ± 1.2               | trace              | 6.47                | 7.60 ± 1.50                     |
| 41                             | 3.73 ± 0.02                     | 2.56 ± 0.47              | trace              | 6.29                | 6.88 ± 0.08                     |

a) Values represent Mean ± S.D of triplicates

b) Values represent Mean ± S.D of 5 replicates

\* Total residue (calculated = surface + extractable + bound)

(4.7% of the applied quantity) of the malathion residues remained in the grains. It may be noted that for all the sampling times, surface residues contributed above 50% of the total residue.

The procedure of Mahin and Lofberg (1966) was used for the determination of the bound residues since this procedure gave a very good recovery (almost 100%) as compared to the modified procedure of Smith *et al*, (1964), (recovery was only 30%).

The level of residues in the jute sack was determined after the last sampling. The results obtained indicated that approx. 10% of the total amount of <sup>14</sup>C-malathion applied remained on the surface of the jute sack after 9 months storage.

The effect of cooking rice which was treated 3 times (at 3 weeks interval) with the prepared <sup>14</sup>C-malathion formulation was determined. Table 2 shows that cooking reduced the amount of extractable residues by 57%.

TABLE 2  
 Effect of cooking on malathion residue level

| Sample   | <sup>14</sup> C-residues µg/g |             |       |       |
|----------|-------------------------------|-------------|-------|-------|
|          | Surface                       | Extractable | Bound | Total |
| Uncooked | 11.5 ± 0.6                    | 8.0 ± 0.5   | trace | 19.5  |
| Cooked   | 11.6 ± 0.6                    | 3.4 ± 0.3   | trace | 15.0  |

Values represent Mean ± S.D. of triplicate

## DISCUSSION

Nuclear techniques provided a useful research tool for this study particularly in the determination of bound residues in rice grains which are not generally detected by conventional methods. When the milled rice inside the jute sack was treated with a single application of <sup>14</sup>C-malathion, the total <sup>14</sup>C-residues in the grain increased gradually to reach ca. 6.9 µg/g after 41 weeks storage. This value is within the limit recommended by FAO/WHO (being 8 µg/g) and may represent the maximum possible level of malathion residues in rice grains stored in jute sacks located on the outer stack in the rice godown. It should be noted that only one application was done for this study although in the normal practice more than one spraying is carried out whenever necessary. It is also of interest to note that the formation of bound residues in the stored milled rice was apparently negligible. Anderegg and Madisen (1983) in their study on <sup>14</sup>C-malathion in stored wheat noted that over a one-year storage period, the quantity of bound residues increased amounting to ~ 20% of the applied <sup>14</sup>C-malathion after 12 months. Reports regarding formation and nature of bound malathion residues in stored grains is still lacking. A possible explanation for the observed absence of bound malathion residue in milled rice (comprised mainly of the starchy endosperm) is that it may be associated with the hull/bran and germ region of the grain which was removed during the milling process. Infact, Rowlands and Bramhall (1977) reported that malathion residues were found chiefly in the regions of high lipid content in the individual wheat grain; mainly in the germ and scutellum and seldom in the starchy endosperm.

A maximum of 6.7% of the applied quantity of malathion penetrated the sack and into the rice grains. This suggested that a large proportion of the applied <sup>14</sup>C-malathion may be present on the surface of the jute sack, dunnage and the surroundings. In fact after 41 weeks storage, the jute sack contained ~ 10% of the applied activity. Since only a small percentage of the applied malathion reached the rice grains, it appeared that the insecticide may not be effective against insect pests within the rice bags. It should be noted that the current chemical used for spraying in the local rice godowns is permethrin (Teoh, 1987). The

usage of malathion has been reduced. Spraying is normally carried out during a hot day when insects come out of the rice bags.

Washing rice prior to cooking (which is the normal practice) would have removed the surface residues amounting to ca. 50% of the total residues on/in the rice grains. During washing and cooking, approx. 80% of the residues may be lost. Lockwood *et al.*, (1974) studied the loss of malathion and fenitrothion from rough rice, wheat and sorghum, and showed that during wet cooking involving steaming or boiling, all residues were lost from the cereals.

The results obtained did not provide any information on degradation of malathion in the stored rice. Work is currently being carried out to identify and quantify malathion and its metabolites in treated stored milled rice.

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