PB-219 753

ORGANOPHOSPHATE PESTICIDES FOR USE AS GRAIN PROTECTANTS IN INDIA: DEGRADATION OF THEIR RESIDUES DURING MILLING AND COOKING OF CERAL GRAINS

Leonard Merrick Lockwood

Kansas State University

Prepared for:

Agency for International Development

1973

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

BIBLIOGRAPHIC DATA SHEET	1. Report No. 631.56-L817	2.	3. Recipient's Accession No. PB-219 753
4. Title and Subtitle "Organophosphate Pesticides for Use as Grain Protectants in India: Degradation of Their Residues During Milling and Cooking of Cereal Grains".			5. Report Date 1973
			6. 109
7. Author(s) Leonard Merrick Lockwood.			8. Performing Organization Rept. No.
9. Performing Organization Name and Address Kansas State Univ. Dept. of Grain Science and Industry ~~Manhattan, Kansas~~			10. Project/Task/Work Unit No.
			11. Contract/Grant No. AID-csd-1931
12. Sponsoring Organization Name and Address			13. Type of Report & Period Covered PhD thesis
			14.

15. Supplementary Notes

16. Abstracts

Samples of rice, wheat, and sorghum were treated with different concentrations of malathion, gardona, and sumithion as protection against insect infestation during storage. Bioassay tests were conducted to determine the effectiveness of treatments in protecting the grain against insect attack. The intermediate levels of treatment and high levels of treatment protected the grain over the full test period of two months. The amount of residues in the treated grain were analyzed before and after milling. Samples of milled products were cooked by traditional Indian methods. Food preparations which involved boiling or steaming resulted in complete degradation of the residues of all three insecticides. Most of India's grain harvest is retained at the farm and village level. Any program aimed at reducing waste due to insect damage during storage must provide methods which can be used for small scale storage. Unfortunately, the most effective methods involving fumigation are not easily applicable at this level and the health regulations of the Indian Government prohibit the admixture of any insecticide with grain in storage. Recommendations have been made to amend the existing regulations to allow the use of malathion as a grain protectant to be admixed with stored grain up to a limit of 8ppm. Results indicate that the breakdown of residues during milling and cooking by typical Indian methods are comparable to the breakdown observed in the cooking and milling processes studied in the U. S. and Europe. It is also advised that other alternatives to malathion be considered, such as gardona and sumithion, in cases where insects begin to develop resistance to malathion toxicity.

17. Key Words and Document Analysis. 17a. Descriptors

17b. Identifiers/Open-Ended Terms

*Pesticides, (*Grain, Storage), *Insecticides

17c. COSATI Field/Group 631

18. Availability Statement	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 104
	20. Security Class (This Page) UNCLASSIFIED	22. Price $7.25

FORM NTIS-35 (REV. 3-72) USCOMM-DC 14952-P72

CSO/1931

PB 219 753

ORGANOPHOSPHATE PESTICIDES FOR USE AS GRAIN PROTECTANTS IN INDIA: DEGRADATION OF THEIR RESIDUES DURING MILLING AND COOKING OF CEREAL GRAINS

by

LEONARD MERRICK LOCKWOOD

B.A., Oberlin College, 1963

M.A., Kansas University, 1969

A DISSERTATION

submitted in partial fulfillment of the

requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1973

Approved by:

David R. Lineback

Major Professor

Reproduced from best available copy.

TABLE OF CONTENTS

INTRODUCTION 1

The significance of losses in grain storage 1

The role of chemicals in reducing storage losses 1

The problem of residues 2

Tolerances for residues in stored products 3

Objectives of the investigation 3

LITERATURE REVIEW 6

The use of grain protectants in India 6

Protectants for stored products 7

Degradation of protectants during processing 10

1. Milling 10

2. Bread baking 11

3. Chapati making 11

4. Wet processing 11

MATERIALS 13

Grain 13

Insects 15

Protectants 16

METHODS 19

Treatment of grain with protectants 19

Bioassay studies 20

1. Periodic bioassay tests 23

2. Infestation tests 23

METHODS (cont.)

Processing methods 28

1. Milling 28

a. Machine milling of wheat and sorghum 28

b. Hand milling of wheat 33

c. Paddy milling 33

2. Cooking 33

a. Preparation of chapaties 33

b. Boiled rice 34

d. Steaming 34

Extraction and clean-up 35

1. Selection of solvent for extraction 35

2. Preparation of samples 36

3. Extraction procedure 36

4. Clean-up procedure 37

5. Tests of extraction and clean-up efficiency 37

a. Comparison using additional solvent for extraction 37

b. Comparison of column vs. batch clean-up 38

c. Extraction from firebrick 39

d. Extraction from whole grain 39

e. Extraction from flour 40

Residue analysis 41

1. Equipment and conditions 41

2. Internal standards and calibration 42

RESULTS AND DISCUSSION 43

Tests of extraction and clean-up efficiency 43

1. Comparison using more solvent for extraction 43

2. Comparison of column vs. batch clean-up 43

3. Extraction from firebrick 43

4. Extraction from whole grain 43

5. Extraction from flour 46

Internal standards and calibration 46

Bioassay studies 55

Degradation of residues during milling 63

1. Machine grinding 63

2. Hand grinding 63

3. Paddy milling 67

Degradation of residues during cooking 69

1. Chapati making 69

2. Boiling and steaming 69

CONCLUSIONS 86

ACKNOWLEDGEMENTS 88

LITERATURE CITED 89

LIST OF TABLES

TABLE 1 Moisture content of grain samples 14

TABLE 2 Protectants used in the study and their sources 17

TABLE 3 Determination of active ingredient in secondary insecticides as compared to primary insecticides 18

TABLE 4 Extraction from firebrick 44

TABLE 5 Extraction from whole wheat 45

TABLE 6 Extraction from flour 47

TABLE 7 Concentrations of solutions used to make calibration curves .. 48

TABLE 8 Bioassay tests with paddy 56

TABLE 9 Bioassay tests with wheat 57

TABLE 10 Bioassay tests with sorghum 58

TABLE 11 Infestation tests with paddy 60

TABLE 12 Infestation tests with wheat 61

TABLE 13 Infestation tests with sorghum 62

TABLE 14 Losses of insecticide during machine grinding of wheat 64

TABLE 15 Losses of insecticide during machine grinding of sorghum 65

TABLE 16 Losses of insecticide during hand grinding of wheat 66

TABLE 17 Residue levels in polished rice 68

TABLE 18 Loss of residues during the preparation of chapaties from wheat flour 70

TABLE 19 Loss of residues during the preparation of chapaties from sorghum flour 71

LIST OF FIGURES

FIGURE 1 Infestation chamber 27

FIGURE 2 Calibration curve for malathion 50

FIGURE 3 Calibration curve for gardona 52

FIGURE 4 Calibration curve for sumithion 54

FIGURE 5 Gas-liquid chromatograms showing loss of malathion 73
residues during chapati making (short cooking period)

FIGURE 6 Gas-liquid chromatograms showing loss of malathion 75
residues during chapati making (regular cooking period)

FIGURE 7 Gas-liquid chromatograms showing loss of gardona 77
residues during chapati making (regular cooking period)

FIGURE 8 Gas-liquid chromatograms showing loss of sumithion 79
residues during chapati making (regular cooking period)

FIGURE 9 Gas-liquid chromatograms showing loss of malathion 81
residues during boiling and steaming of rice

FIGURE 10 Gas-liquid chromatograms showing loss of gardona 83
residues during boiling and steaming of rice

FIGURE 11 Gas-liquid chromatograms showing loss of sumithion 85
residues during boiling and steaming of rice

LIST OF PLATES

PLATE 1 The drum mixer used in applying insecticide emulsions to grain samples. Storage tins. Hand grinder (Chakki) 22

PLATE 2 Plastic vials used in bioassay studies 25

PLATE 3 Power mill for whole grinding of grain 30

PLATE 4 Detail of grinding stones in the power mill 32

INTRODUCTION

The significance of losses in grain storage

In India grains form 63% of the average diet as compared with 21% in the United States (1). Because a larger proportion of India's per capita income is spent on food as compared to that of the U.S., the production, marketing and consumption of grain plays a role in the economy of India even more vital than in the U.S.A.

Estimates for overall losses of grain in storage in India vary widely. However, most workers feel that losses are probably in the range of 10% to 25% (2-4). Even a small reduction in the overall loss of grain during storage would effect a significant savings in capital. The immediate effect would be a reduction in the amount of grain imported from abroad to fill the deficit in production; in 1969-70 this amounted to 3.6 million tons of cereals while the country produced 87 million tons (5).

A primary cause of loss during storage of food grains is insect damage which is estimated to be 5% to 10% of the total grain production (3). In India losses due to insects are exceeded only by losses to rodents.

The role of chemicals in reducing storage losses

An important means of minimizing grain losses during storage is the control of insect pests by application of pesticides to the grain or to the storage premises or fumigation of the grain bulk. Techniques for the protection of stored grain have been developed to a high degree. However these methods generally are geared to the conditions prevailing in the western countries, namely large centralized storage facilities with experienced personnel to handle the equipment. India maintains some large grain

storage facilities, largely to serve as buffer stocks for famine relief. The State and Central Warehousing Corporations maintain large storage depots. In general these facilities have up-to-date equipment for the application of protectants and the problem of losses is not serious. However, the greatest proportion of grain is stored on the household and village level. It is estimated that 70% to 80% of all grain produced in India is stored at the farm and village level and that 20% to 30% enters the market channels (6,7). Since it is unlikely that there will be any marked change in the storage and marketing pattern in the near future in India, it is very important that recommendations regarding the use of protectants for stored grain take into consideration the limitations imposed by the need for application by unskilled workers. Unfortunately, this rules out many fumigation methods which are among the most effective control measures used in western countries.

The problem of residues

Because stored grain may be used at any time for human consumption, the level of pesticide residues must be kept at a minimum for the safety of the consumer. Since the grain could be used in some cases almost immediately after treatment, most countries ban the use of persistent control chemicals, such as the organochlorine compounds, for direct admixture with grain. Fumigants are used widely and, because of their volatile nature, residues are minimized. Some organophosphates, particularly malathion, are used because of their low mammalian toxicity and ease of degradation. Pyrethrins are effective for short term control and their toxicity to mammals is particularly low, but, because of their expense, they do not find widespread use in grain storage.

Tolerances for residues in stored products

The United States, Brazil, Canada, France,Italy and the U.K. are among the countries which allow residues of malathion in grain up to a limit of 8 ppm (8). Kenya has a tolerance of 12.5 ppm. The Joint FAO/WHO Food Standards Program, Codex Alimentarius Commission, has recommended an international tolerance of 8 ppm malathion in stored grain (9). However, the Government of India's Rules for the Prevention of Food Adulteration (P.F.A.) specify that "No insecticide shall be added to any food" with the provision for tolerances of 3 ppm for DDT, BHC, methoxychlor and malathion resulting from the treatment of storage premises. The use of fumigants is approved (10).

Objectives of the investigation

The restrictions placed upon the use of malathion as a grain protectant by the PFA regulations has meant that, because the procedure is illegal, recommendations can not be made to farmers and traders regarding the admixture of protectants directly with grain, This has, in some cases, resulted in the indiscriminate use of insecticides for grain treatment which are not suited for protection of stored products, In some cases the doses used have been so heavy or the insecticides so toxic that there were resulting deaths among those who consumed food made from the treated grain (11).

In 1967 the Indian Government set up a commission (the Thacker Committee) to study the harmful effects of pesticides. This committee recommended that "In order to avoid contamination of foodgrains with persistent organochlorine insecticides (DDT, BHC, etc.), it is suggested that malathion and pyrethrum and similar chemicals in a suitable form for use as grain protectants may be made easily available and their use be recommended after the neces-

sary research work."(12).

Small scale fumigation is complicated by the need for dosing devices, protective kits and monitoring techniques to assure the application of fumigants at the correct concentrations. These requirements, and the demand for skilled personnel to handle the equipment, make small-scale fumigation impractical for much of the storage demands of India.

The present investigation was designed to determine the degree to which malathion and two other organophosphorous compounds are degraded in the process of milling and cooking. Bioassay tests were used to determine the effectiveness of treatments in controlling insect infestation. Emphasis throughout has been placed on trying to approximate the conditions to which the grain would ordinarily be subjected as it commonly is processed in India.

This study was carried out with the support of 211(d) Grant AID/csd 1931 "Grain Utilization in India" awarded to Kansas State University by the United States Agency for International Development. Kansas State University is one of six universities in the United Stated that participated in this particular program centered on problems of Indian agriculture. The 211(d) grants to the six universities (CUSURDI institutions) enabled students to carry out the research phase of their Masters or Doctorate programs in India. The basic objectives of the grant to Kansas State University were "to train graduate students in food grain utilization in India and other developing countries and to further increase the competency of Kansas State University in these areas."

In addition to research that is done by private firms and at universities in India, there are many large research institutes which are supported by the Central Government. In general, there is one of these

government-supported research institutes for each of the major areas of scientific investigation. The Central Food Technological Research Institute (CFTRI) in Mysore City, South India is the government organization which carries out a major part of the research related to food development, processing and utilization in India. The work reported in this dissertation was done at CFTRI over a period of 22 months from 1970 to 1972 in a program planned in co-operation with Dr. H.A.B. Parpia, the Director of CFTRI, and with the Department of Grain Science and Industry at Kansas State University. The results and opinions presented in this paper do not necessarily reflect the views of the United States Agency for International Development or the institutions at which the work was performed.

LITERATURE REVIEW

The use of grain protectants in India

The Thacker Committee's report indicated that very high levels of BHC and DDT residues in market samples of grains were not uncommon; on occasion being well over 100 ppm (12). The same report noted the high levels of DDT detected in human tissue in India. This widespread misuse of insecticides for the protection of stored grain has prompted some authorities to recommend that the Prevention of Adulteration Rules should be amended to allow the use of malathion as a grain protectant (13).

Scientists at various institutions throughout India have conducted experiments to determine the suitability of malathion as a grain protectant under conditions prevailing in India. Mookerjee *et al.* (14) applied malathion to wheat and found that 24 ppm controlled *Sitophilus oryzae* for 4 months and *Tribolium castaneum* for 3 months. Udeaan and Bindra (15) reported stu[illegible]s in which wheat was treated with different levels of malathion [illegible]ion resulting in initial residue levels ranging from 11 ppm to 56 ppm. The treated grain was then stored in a variety of receptacals representative of those in common use in India. The authors concluded that the acceptable daily intake of 0.02 mg malathion/kg of body weight, established by the Joint WHO/FAO Committee on Pesticides (16), was not exceeded in the case of wheat treated at 33 ppm and used 3 months aft[illegible] treatment. Bindra and Sidhu (17) found that residues of malathion on maize, treated at 20 ppm, 30 ppm and 40 ppm, were degraded to below the 8 ppm tolerance level recommended by the Codex Alimentarius Commission in 2 months, 6 months and 10 months, respectively.

Protectants for stored products

Although malathion appears to be well suited for use as a grain protectant in most situations, there is evidence that some strains of insect pests are developing resistance to its toxic effects. Parkin (18) has studied the onset of resistance among field populations of stored-product insects. In particular, *Tribolium castaneum* nas shown some resistance to malathion (19-21). Bhatia *et al*. (22) conducted research comparing two strains of *Tribolium castaneum* for resistance to malathion. One strain had been maintained in the laboratory without exposure to insecticides. The other insects were collected from rice bags in a storage warehouse that had received repeated surface treatments with malathion. Results indicated a definite resistance to malathion of the beetles collected from the warehouse.

The prospect of malathion becoming ineffective against certain strains of stored product insects has stimulated the search for alternatives. Strong and Sbur (23) tested 36 insecticides of different types for effectiveness against stored-product insects. Later their study was extended to include another 46 insecticides (24). The authors concluded that three insecticides (all organophosphorous compounds) were more effective than malathion and a few others approached the effectiveness of malathion. Sumithion[a] was one of those found to be more effective than malathion. Lemon (25) tested 16 organophosphorous insecticides by topical application to *Tribolium confusum* and *Tribolium madens*. Of the six that exceeded malathion in insect toxicity, sumithion was found to be the

a. Fenitrothion; 0,0-dimethyl 0-(4-nitro-m-tolyl) phosphorothioate; Bayer 41831.

most effective. In subsequent work by Lemon (26), the persistence on wheat of 1 ppm of sumithion was found to be equivalent to that of 4 ppm of malathion when using Sitophilus granarius in bioassay studies. Toxicity to rats at these respective levels was similar, thus the hazard from equivalent levels of effectiveness was similar for malathion and sumithion. Tests by Green and Tyler (27) on farm-stored grain, with application being made as a water-based emulsion, showed that 2 ppm of sumithion controlled infestations of Oryzaephilus surinamensis as effectively as 10 ppm of malathion. Kane and Green (28) tested concentrations of 1 ppm, 2 ppm and 4 ppm of sumithion applied to wheat and to barley with subsequent exposure to Oryzaephilus surinamensis, Sitophilus granarius and Tribolium castaneum. Two ppm gave protection for 6 months and 4 ppm for 10 months against all three insects. In tests of sumithion and malathion under severe conditions of grain moisture and heating, Tyler and Green (29) found that sumithion at dosages of 2 ppm and malathion at 10 ppm became ineffective after 2 weeks in the moist, heating grain near the center of stored barley with a moisture content of up to 17.6%. In cooler areas near the edge, residues remained effective for 12 weeks. It was concluded that sumithion was a useful alternative to malathion, but not markedly superior.

In the screening tests conducted by Strong and Sbur (24), gardona[a] was found to be almost as effective as malathion against Sitophilus oryzae and Tribolium confusum. Because of its low mammalian toxicity (30), a considerable amount of research has been initiated to ascertain how effect-

a. 0,0-dimethyl 2-chloro-1-(2,4,5-trichlorophenyl) vinyl phosphate; Shell SD 8447.

ive gardona is in controlling pests in a variety of applications. Bry et al. (31,32) studied the effectiveness of gardona as a mothproofer when applied to woolen fabrics during drycleaning. They found that treatment levels as low as 10 ppm were effective in protecting the cloth against the black carpet beetle, Attagenus megatoma (F.). The effectiveness of gardona in controlling pests of sweet corn has been investigated by Young and Bowman (33) who concluded that gardona was as effective as DDT. Whetstone et al. (30) examined the selective toxicity of gardona. They found that it showed high toxicity to several species of insect pests but that it was remarkably safe to laboratory mammals in acute and two-week feeding trials. In a continuation of his earlier investigation of organophosphorous compounds, Lemon (34) tested 7 new compounds for effectiveness against stored-product pests using topical application. Of those, gardona alone showed promising results and it was then tested against 8 other species of insects by exposing them to samples of wheat treated with gardona. Gardona was found to be more effective than malathion against Rhyzopertha dominica (F.), Lasioderma serricorne (F.), Stegobium paniceum (L.) and Sitophilus zeamais (Motsch.) as well as against Tribolium spp. In the case of R. dominica it even equalled sumithion in toxicity. However gardona was relatively ineffective against Oryzaephilus spp. and it was less toxic than malathion or sumithion against Sitophilus granarius (L.) and Ptinus tectus (Boield.).

Because of the potential shown by gardona and sumithion as possible substitutes for malathion in cases where insect resistance to malathion has developed, these two protectants were included in the present study to ascertain the effects of processing on their residues.

Degradation of protectants during processing

Lindgren (35) has reviewed the literature pertaining to residues in raw and processed foods resulting from post harvest insecticide treatments. Work related to the effects of processing on pesticides in food has been summarized by Liska and Stadelman (36).

1. Milling. In studies carried out by Seibel and Hertel (37), malathion residues in wheat, at a level of 3 ppm, were degraded to 1 ppm during the process of milling. This is equivalent to a loss of 66%. As would be expected, relatively little malathion is carried over into white flour as compared to bran and shorts (37). However,Acton and Farouchais (38) have shown that there is a loss even with whole grinding of wheat. Schesser et al. (39) treated wheat with concentrations of malathion ranging from 2.5 ppm to 7.5 ppm. Initial cleaning had relatively little effect on reducing residue levels, presumably because of migration of malathion into the grain. Flour produced from the treated wheat contained from 0 ppm to less than 0.3 ppm of residue. In a study of the effect of rice milling on malathion residues, McGaughey (40,41) treated rough rice (paddy) at levels of 14, 21 and 28 ppm. Maximum concentrations were found in the hulls. Residue levels in bran and polished rice increased during the initial period of storage as migration of malathion into the grains of paddy exceeded the rate of breakdown. However, the maximum level of malathion residue in polished rice, milled from paddy treated at 28 ppm,was less than 0.33 ppm. This maximum in concentration occurred a little over a month after treatment.

2. Bread baking. Acton and Parouchais (38) found that no residue occurred, above the detectable limits of 0.3 ppm, in bread baked from white flour that had been milled from wheat treated with malathion at a level of 26 ppm. In the case of whole wheat flour, however, it was found that bread contained over 1 ppm malathion in cases where the whole grain had beem treated with 8 ppm. Studies by Allessandrini (42) have shown an 84% to 92% loss of malathion residue when flour is made into bread.

3. Chapati making. The loss of malathion residues during the process of chapati making has been studied by several workers in India. Godavari Bai et al. (43) and Udeaan and Bindra (15) have shown residue losses of 78% from flour and 75% from grain in the process of making chapaties from wheat. In making chapaties from maize, Bindra and Sidhu (17) found a loss of 73% residue when maize flour was converted to chapaties.

4. Wet processing. According to Crosby (44), most organophosphorous compounds are destroyed to a major extent by normal processing and canning procedures. Farrow (45) has shown that canning and juicing procedures remove more than 99% of malathion residues in the processing of tomatoes. Koivistoinen et al. (46) have indicated losses in the range of 50% for canning and cooking of selected fruits.

The effect of laundering and drycleaning on residues of gardona applied as a mothproofing agent to woolen fabric has been investigated by Bry et al. (31). Using bioassay studies they found that three launderings removed residues of gardona to the extent that there was 0% mortality in bioassay studies using the black carpet beetle. However, in a later study (32) it was found that steam pressing had no significant effect in reducing residues of gardona applied to wool fabric.

The studies of Allesandrini (42) indicated an 89% to 90% loss of malathion residues when flour was converted to pasta. Subsequent cooking of the pasta resulted in degradation of the residue beyond the point of detectability.

In India, Godavari Bai (47) found that steam processing of rice reduced the level of malathion by 95.5%.

MATERIALS

Grain

Grain used in this study was obtained locally. The Bangaru Sanna variety of paddy was purchased from the Mysore Controlled Market. Wheat was obtained commercially through the CFTRI stores and was a hard amber variety from Maharashtra. A local variety of sorghum, D 340, was purchased in Terakanambi, a village in Mysore district.

Each batch of grain was tested for residues that might have remained on the grain from treatment prior to purchase and could interfere with tests for which the grain was being used. Samples from each lot of grain were extracted with methanol and assayed on the gas chromatograph using a phosphorous detector (described under Methods) for the presence of any organophosphorous residues. To ascertain that no other residues from previous treatments remained on the grain which might interfere with bioassay studies, adult *Sitophilus oryzae* and *Tribolium castaneum* beetles were placed in samples of the grain and counts made at intervals. None of the grain samples produced a mortality rate that was significantly higher than in the regular culture medium (control).

To determine moisture content of the grain, a sample of about 10 g was ground in a Bosch blendor equipped with a coffee-grinding attachment. A portion (5 g) of the ground grain was weighed into a tared petri dish and dried in an electrically-operated air oven for 16 hr at 105°C (48,49). The petri dish was covered, allowed to cool and reweighed to determine the original moisture content. Data for three replicate tests for moisture content for each batch of grain are shown in Table 1. A second test for moisture content was made using a Protimeter moisture meter using samples

TABLE 1

Moisture content of grain samples

Grain	Replicate	Moisture content[a] %	Average %
paddy	1	12.65	
	2	12.66	12.65
	3	12.64	
sorghum	1	11.32	
	2	11.30	11.30
	3	11.28	
wheat	1	11.64	
	2	11.66	11.63
	3	11.58	

a. Determined by oven drying ground samples for 16 hours at 105°C.

obtained at the same time as those used in the oven-drying tests. The grain was checked periodically using the Protimeter moisture meter to determine if any significant change in moisture content occurred during the period of storage. There was no appreciable change in the moisture content during the period of storage, probably because the grain was stored in metal containers with tight lids.

Grain was obtained as soon as possible after harvest in order to minimize the likelihood of storage treatments by the farmer or trader and in hope that the grain would be relatively free from infestation. In spite of this, the sorghum was found to be infested with Sitophilus oryzae and soon after purchase insects were visible in the grain. In order to disinfest the grain without resorting to chemical treatment the entire quantity of grain was stored in a cold room at 0°C for over a month. At this temperature it takes a little over 2 weeks to kill all stages of S. oryzae (50) and it was found that there was no subsequent indication of the infestation. There was no indication of infestation in either the wheat or the paddy that would require refrigeration.

Insects

Cultures of Tribolium castaneum (Hbst.) (red flour beetle) in whole wheat flour and Sitophilus oryzae (L.) (rice weevil) in whole wheat were obtained from the insect culture rooms af the Discipline of Infestation Control and Pesticides (CFTRI). In order to minimize mortality due to ageing, the insects used in bioassay tests were drawn from cultures which had been cleared of adults a maximum of 7 days previously. This insured that the age of the adults was less than one week at the start of a test.

Protectants

Table 2 lists the protectants used in this investigation and gives the source for each.

Samples of the primary and secondary pesticides were assayed by gas-liquid chromatography to determine the amount of active ingredient and to check for secondary peaks resulting from breakdown products. A stock solution (1/1000 dilution of each insecticide) was made by weighing one drop of insecticide in a flask and diluting this with 1 ml of methanol per mg of insecticide. A second dilution was made by diluting 0.1 ml of each stock solution to 10 ml with additional methanol. This dilution yielded solutions of 10 ppm insecticide in methanol.

Malathion (primary) was used as an internal standard to compare primary and secondary gardona. Gardona (primary) was used as the internal standard for comparisons of primary and secondary sumithion or primary and secondary malathion. The concentration of internal standard was adjusted so that, when injected together, the peak heights for both the sample and the standard would be on scale and sufficiently large to make accurate area measurements. The results are shown in Table 3. In no case was there any indication of secondary peaks.

TABLE 2

Protectants used in the study and their sources

Protectant	Source
malathion ..... analytical grade secondary standard	American Cyanamid Co. Agricultural Division Princeton, New Jersey
sumithion ..... analytical grade technical grade	Sumitomo Chemical Co. 15, 5-Chome, Kitahama Higashi-ku, Osaka, Japan
gardona ..... analytical grade	Woodstock Agricultural Research Centre (Shell) Sittingbourne, Kent, England
gardona ..... technical grade	C.F.T.R.I. (obtained from Indian distributor)

TABLE 3

Determination of active ingredient in secondary insecticides as compared to primary insecticides

Insecticide	Standardized peak area[a]	Percent of active ingredient in secondary insecticide
malathion		
primary	274	
secondary	256	94%
gardona		
primary	196	
secondary	179	92%
sumithion		
primary	756	
secondary	680	90%

a. $\frac{\text{Area of protectant peak}}{\text{Area of standard peak}} \times 100$. Determined from g.l.c. analysis.

METHODS

Treatment of grain with protectants

Malathion and gardona were applied to grain samples at 10 ppm, 40 ppm and 80 ppm while sumithion was applied at a rate of 2 ppm, 8 ppm and 16 ppm. Following a procedure outlined by Kadoum (51), the insecticide was added to the grain as an emulsion in which the concentration of insecticide had been adjusted to give the desired level of treatment when applied to grain at the rate of 2 ml per kg.

In order to attain as much accuracy as possible in the level of insecticide applied to the grain, the emulsions were freshly prepared for each treatment using analytical grade insecticide. Initially, 0.8 g of malathion (0.8 g of gardona or 0.16 g of sumithion) was weighed into a 10 ml flask. To this was added 4 ml of benzene followed by 0.2 ml of emulsifying agent (Tween 80). A few ml of distilled water was added and, after shaking, the contents were transferred to a measuring cylinder. Using additional distilled water to rinse the flask, this procedure was repeated until the volume of emulsion in the measuring cylinder was 20 ml. Half of this was set aside and the remaining 10 ml were diluted with an equal quantity of distilled water. After shaking, half of this in turn was set aside and another 10 ml of distilled water added. This serial dilution was carried out until there were three dilutions in addition to the original concentration. The respective concentrations of these dilutions were 40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml for malathion and gardona and 8 mg/ml, 4 mg/ml, 2 mg/ml and 1 mg/ml for sumithion.

Grain was treated in 5-kg batches. When 10 ml of insecticide

emulsion containing 40 mg/ml was applied to this quantity of grain, the effective treatment level was 80 ppm. The other emulsion concentrations were used in this manner to obtained the desired levels of treatments cited previously. One lot of grain was treated with emulsion containing no insecticide and served as a control.

Application of emulsion to grain was carried out using a tumbler consisting of a metal drum (16 in. diam. and 24 in. length) which was mounted at an angle to its axis on a shaft that could be turned by hand (Plate 1). A hinged and gasketed door was opened to fill or empty the drum and was bolted shut during operation. Metal baffles on the inside of the drum assured thorough mixing of the contents. Grain to be treated was poured into the drum and 1 or 2 ml of emulsion applied using a pipette to distribute the drops over the surface of the grain. The drum was closed and rotated about 10 times and another application of emulsion was made. This procedure was repeated untill 10 ml of emulsion had been applied. Finally, the drum was rotated for an additional 5 min before being emptied into one of the metal tins used for storage (Plate 1).

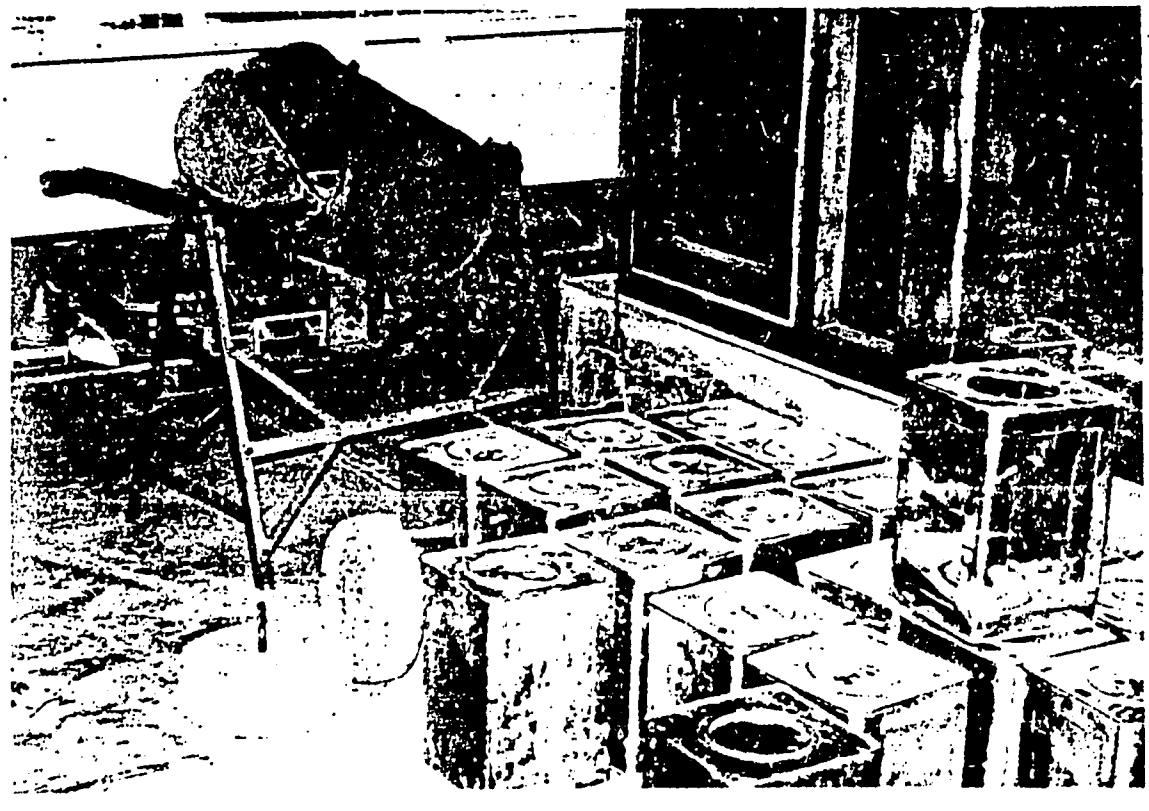
Bioassay studies

Two types of bioassay studies were conducted in order to determine the effectiveness of the different treatments in controlling stored-product insects. The first study was designed to determine the degree of protection that the insecticide residues afforded 24 hr, 1 month and 2 months after application. At these times samples were simultaneously removed for bioassay tests and for milling and cooking tests. The object of the second study was to determine the point when a particular treatment of grain became ineffective in protecting it against insect infes-

21

PLATE 1

The drum mixer used in applying insecticide emulsion to grain samples. Storage tins. Hand grinder (Chakki).



tation. In these tests samples of treated grain were exposed to populations of insects. In both studies the test insects were Sitophilus oryzae (L.) (rice weevil) and Tribolium castaneum (Hbst.) (red flour beetle).

1. Periodic bioassay tests. From each tin of grain being tested, three replicate samples of about 20 g each were removed from different locations within the grain mass. Half of each sample was ground coarsely in a coffee grinder and the mixed with the unground half. This mixture of ground and unground grain was used to fill a plastic vial (4 cm diam. x 5 cm height) half full (Plate 2). Ten adult rice weevils and ten adult flour beetles were placed in each vial which was identified with a paper label placed inside. The vial was covered with a piece of cloth mesh held in place by a rubber band. After 7 days, vials were emptied and counts made to determine the number of living insects and dead insects. Controls were run in each series using grain which had been treated with emulsion to which no insecticide had been added.

2. Infestation tests. Infestation tests were initiated at the same time as the first (24-hr) bioassay tests. The grain samples were drawn from the same, partially ground, material that was used for the bioassay tests. In this case two replicate vials were filled almost to the top. A set of 7 vials representing 2 replicates for each of the three treatment levels for a particular insecticide and one control were placed in an empty 1-kg milk powder tin. Grain infested with flour beetles and rice weevils was used to fill the space around the vials to the same level as the grain in the vials. Cloth netting was placed loosely over the grain and vials to provide access to any part of the surface for the insects (Figure 1). Observations were made twice weekly to determine if insects

24

PLATE 2

Plastic vials used in bioassay studies.

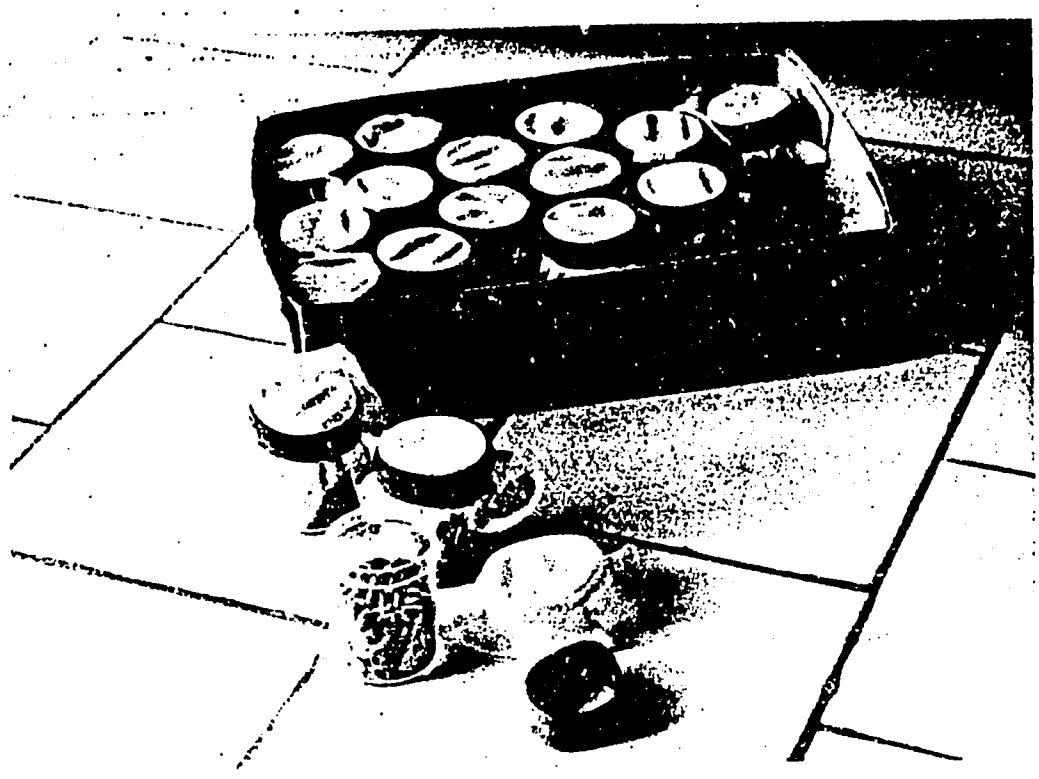
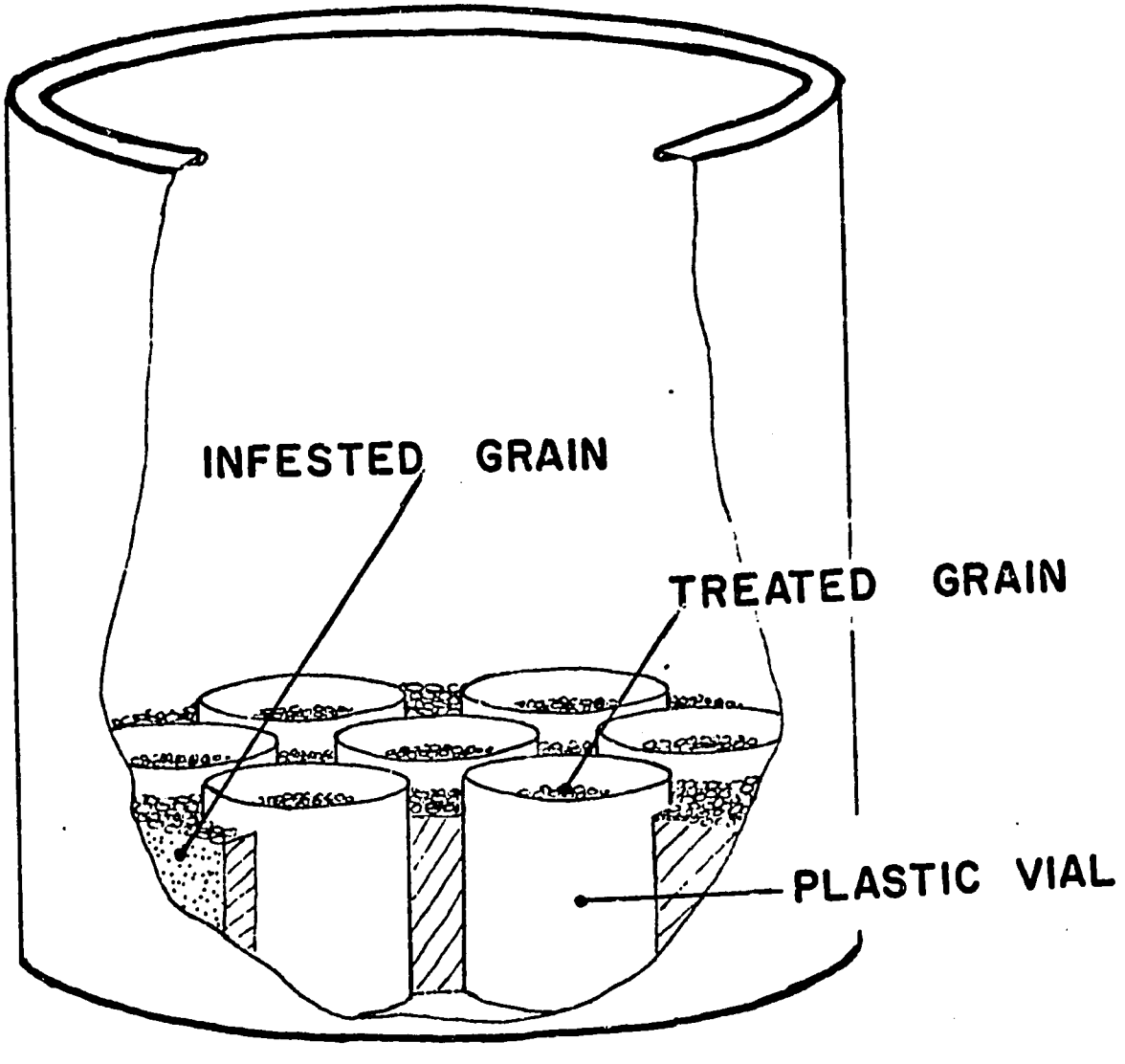


FIGURE 1

Infestation chamber
Vials containing samples of grain were kept in a tin containing grain infested with rice weevils and flour beetles.


INFESTED GRAIN
TREATED GRAIN
PLASTIC VIAL

had infested the treated samples or the controls. At this time the cloth mesh was removed and the contents of each vial were stirred to check on insects below the surface of the grain. If there were more insects in a vial than might occur from the random wanderings of the population, the vial was noted as having a light infestation. When there was a dense population of insects in a vial, accompanied by visible damage to the grain and the presence of immature forms of the insect, the vial was noted as having a heavy infestation.

Processing methods

1. Milling

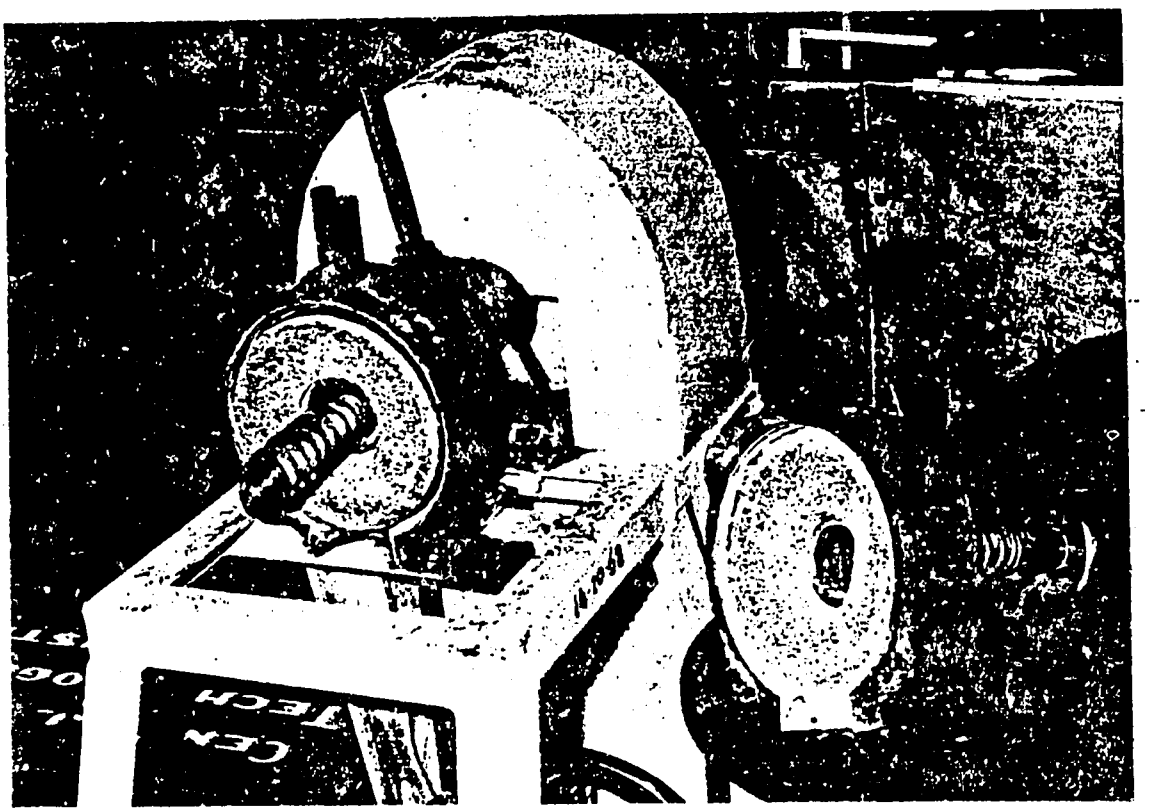
a. Machine milling of wheat and sorghum. Samples (500 g) of wheat and sorghum were milled on a Bharat Electrical Industries flour mill which was equipped with two discs of carborundum, one of which was rotated at 700 rpm by an electric motor (Plates 3 and 4). The position of the stationary disc was adjusted by a screw and by this means the coarseness of the flour could be controlled. Grain was fed from an overhead hopper by an adjustable vibrating gate. The positions of the stones were adjusted so that, with one pass through the mill, 90% or more of the flour would pass through a 32-mesh sieve. Considerable heating commonly occurs in this type of mill. Therefore, before a series of samples was milled, untreated grain was ground in order to raise the temperature to near that occurring during normal operation. In order to avoid contamination between runs, the mill was disassembled and thoroughly cleaned with a brush and compressed air.

PLATE 3

Power mill used to grind whole grain. Manufactured by Bharat Electrical Industries (Bombay, India).

PLATE 4

Detail of grinding stones of the power mill.



b. Hand milling of wheat. A hand-operated grinding stone or "chakki" was used to prepare hand-milled wheat flour. The chakki consisted of two granite stones hewn in the form of discs (11 in. diam. and 3 in. thick). Embedded in the center of the lower stone was a 5-in. long piece of ½-in. diam. iron rod (Plate 1). The upper stone had a hole in its center and fitted over the iron rod which then served as an axis around which the upper stone could be rotated by means of a wooden handle set near its edge. As with the power mill, the size of samples used with the chakki was 500 g. In order to obtain a degree of grinding similar to that obtained with the power mill, it was necessary to regrind a sample on the chakki several times. After each pass, the ground product was sifted using a 32-mesh sieve and that portion which did not pass through was returned to the grinding stone. This procedure was repeated until more than 90% of the ground product would pass through the sieve.

c. Paddy milling. One-kg samples of paddy were hulled using a McGill sheller. Unhulled kernels were removed by hand and 700 g of the brown rice were polished in a McGill No. 3 polisher. The polisher was set to run 15 seconds at 1 lb, followed by 10 sec with no weight. This was found to give a 4% polish which is considered typical for that part of India. Bran was sifted through a 32-mesh sieve to remove small pieces of hulls and broken rice.

2. Cooking

a. Preparation of chapaties. For each treatment level of wheat or sorghum that was milled, three replicate chapaties were prepared. Each chapati was made with about 30 g of flour and sufficient distilled water

to make a suitable dough. The dough was pressed into the form of a thin disc and cooked on a griddle without the use of any fat or oil.

b. Boiled rice. For each sample, about 50 g of polished rice was placed in a 400-ml beaker and distilled water added until the total volume was four times the volume of the rice. The rice was boiled over low heat so that the kernels were cooked through by the time that all the water had been absorbed into the rice.

c. Boiled sorghum. In addition to being prepared in the form of chapaties, a boiled preparation made from sorghum is commonly eaten by low income groups in the areas around Mysore. This was prepared in the laboratory by mixing sorghum flour into a dough and forming balls of about 1-in. diameter. These were boiled in water until they were cooked throughout (approximately 10 minutes).

d. Steaming. A common product made from rice is the iddli. This is prepared by grinding rice and a little black gram with water to form a thin paste. This paste is fermented overnight and then steamed in an iddli cooker. The iddli cooker resembles an egg poacher, except that it is not submerged in water but is exposed to steam in a vessel such as a pressure cooker. After steaming, the iddli has a moist fluffy texture. It is eaten with a variety of sauces and chutneys. In this study iddlies were prepared from rice alone to avoid the additional variables that would have been encountered by using black gram in the preparation. It was found that steaming for 10 minutes was sufficient to cook the iddlies thoroughly.

Extraction and clean-up

1. Selection of solvent for extraction. The manual which accompanied the alkali flame ionization detector listed the following solvents, in order of preference, for use as carriers for samples to be injected onto the column of the gas-liquid chromatograph.

Water
Methanol
Ethyl ether
Hexane (and some other hydrocarbon solvents)

To determine which solvent would be most suitable for extraction of organophosphorous compounds from grain, a comparison was made using 15 g of sorghum removed from a batch of grain that had been treated with 40 ppm of malathion. The sample of sorghum was ground in a blendor and divided into 3 equal portions. One portion was extracted with 20 ml of methanol, the second portion was extracted with ethyl ether (20 ml) and the third with hexane (20 ml). Extraction was accomplished by shaking the sample for 2 hr on a mechanical shaker. The mixtures were filtered and the solvents were passed through a clean-up column containing 1 g of Nuchar attaclay. Samples (2 µl) were injected into the gas chromatograph. In these tests, the methanol extracts produced peaks that were more than 10 times the area of the peaks produced with the other solvent extracts. Subsequently, Crisp and Tarrant (52) discussed the suitability of methanol for extraction of malathion and dichlorvos from grain for analysis with a gas chromatograph equipped with a phosphorous detector. Tests conducted by them indicated that methanol was more efficient than acetone for this purpose.

2. Preparation of samples. In the case of ground products and in the case of whole grain taken immediately after treatment, there was no need for any special preparation of the samples before extraction with methanol. With the cooked products, however, it was necessary to first dry and then grind the material in order to assure the same efficiency of extraction that would be obtained for the milled products. Drying was accomplished in an electrically-heated air oven at 50°C. The cooked sample was first cut into small pieces and then dried for a period of about 12 to 16 hr.

In order to determine if any breakdown of insecticide occurred during the drying procedure, an uncooked sample of the same material was placed in the drying oven and was subsequently extracted and analyzed together with the other samples.

After drying, the samples were ground in the coffee grinding attachment of a Bosch blendor, a procedure similar to the one used by Rowlands (53) in extracting malathion from maize and wheat. The sample was sifted through a 32-mesh sieve and the coarse material that remained was ground by hand in a mortar since the coffee grinder would not pulverize small quantities of material.

3. Extraction procedure. In the case of whole grain and most of the milled products, the size of sample used for extraction was 5 g. In the case of paddy hulls and rice bran, because of their bulk and generally higher concentration of insecticide, the sample size was 1 g.

Each sample was weighed into a 100-ml conical flask with ground glass stopper. Methanol (10 ml) was then added to each flask and the mixture agitated for two hours on a mechanical shaker. The mixture was filtered through Whatman No.1 filter paper in a Buchner funnel.

The flask was rinsed with two aliquots of methanol (5 ml each) and these were added to the original filtrate.

4. Clean-up procedure. Clean-up of the samples was accomplished by adding 1 g of Nuchar attaclay to each sample. The mixture was then agitated for 2 hr on a mechanical shaker. After agitation the samples were filtered and washed using the technique described previously. Finally, the volume was adjusted to 25 ml. This usually involved adding methanol but in a few cases the volume was greater and the solvent was evaporated at room temperature in a beaker to attain the correct volume. Crisp and Tarrant (52) found that, in most cases, when using methanol to extract malathion from grain, it was unnecessary to use a clean-up step. However, when required they used a charcoal column.

5. Tests of extraction and clean-up efficiency. In order to determine the efficiency of the techniques being used, taking into consideration limitations imposed by materials and equipment, several tests were made to determine the effect of changes in the methods used for extraction and clean-up.

a. Comparison using more solvent for extraction. This comparison was designed to show whether or not the amount of solvent used in the extraction step was a limiting factor in the overall extraction and clean-up procedure. A fresh stock solution of malathion in methanol (1/1000) was prepared by weighing one drop of malathion onto a flask using scales reading to 0.1 mg. One drop usually weighed between 10 mg and 20 mg. Methanol was added to the flask at a volume of 1 ml per mg of malathion, thus giving a one part per 1000 solution. A second dilution was made by diluting 0.4 ml of the stock solution to 20 ml with

methanol, yielding a 0.02 mg/ml solution. Ten ml of this solution were placed in each of two flasks containing 5-g samples of untreated wheat flour. Thus each flask contained 0.2 mg of malathion which is equivalent to extracting flour with 40 ppm of malathion residue.

One sample was extracted and cleaned-up in the usual way (page 30). For the second sample, an additional 10 ml of methanol was added before the 2-hr agitation period. This solvent was removed by filtration. Methanol (10 ml) was added to the residue followed by agitation for an additional hour. The solvent was removed by filtration and the two filtrates were combined. Clean-up of this sample was analagous to that of the other samples, except that two 10-ml washes were used rather than the 5-ml washes. The final volumes of both samples were adjusted to 60 ml. Finally, internal standards were added to each sample and comparison runs were made on the gas chromatograph.

b. Comparison of column vs. batch clean-up. In preliminary work, clean-up was carried out using glass columns (8 mm i.d.) containing 1 g of Nuchar attaclay. It was found that the time required to clean-up one sample was more than one day. Although this did not present a problem when running a limited number of samples in initial tests, it would have imposed serious problems when large numbers of samples had to be processed. A quicker method for clean-up was a batch process in which the adsorbent was added to the sample in a flask and agitated for a period of time. In order to determine how much loss in extraction efficiency this caused, the following test was run.

In the same manner as the previous test, two flasks were prepared containing 5 g of untreated wheat flour and 10 ml of methanol containing 0.2 mg of malathion. This was equivalent to extracting flour with

40 ppm of malathion residue. The flasks were agitated for 2 hr and filtered using the usual technique. For clean-up, the contents of one flask were passed through a column containing Nuchar attaclay followed by two 5-ml aliquots of methanol as a wash. The contents of the second flask were agitated with 1 g of Nuchar attaclay for 2 hr on a shaker. This was followed by filtering and washing the residue with two 5-ml portions of methanol. Volumes of both samples were adjusted to 25 ml, internal standard was added and the solutions were assayed on the gas chromatograph.

c. Extraction from firebrick. To test the efficiency of extraction, an inert material (40/60 mesh firebrick) was selected. Using a stock solution of 1/1000 malathion in methanol as before, 4 flasks were prepared containing 10 ml of methanol and 0.2 mg of malathion. To each of three flasks was added 5 g of firebrick, the equivalent of 40 ppm insecticide treatment. These three flasks were then extracted and cleaned-up (using the batch method) in the normal manner, adjusting the final volume to 25 ml. The 4th flask, which had not had firebrick added and had not gone through the extraction and clean-up procedure, served as a control. Its volume was also adjusted to 25 ml.

Samples from each flask were analyzed on the gas chromatograph to determine how much residue was lost during the extraction process. This same procedure was repeated for gardona and for sumithion (in this case the concentration of sumithion in the flask was 0.04 mg or the equivalent of 8 ppm treatment of 5 g of firebrick).

d. Extraction from whole grain. Five g of clean wheat were placed into each of three flasks. Into each of these flasks, plus a 4th empty flask, was added 1 ml of methanol containing 0.2 mg of malathion. The flasks

containing the wheat were agitated to distribute the solvent and then left unstoppered at room temperature to allow the solvent to evaporate and deposit the malathion on the surface of the grain. The 4th flask, with the malathion solution and no wheat, was kept sealed. After the wheat samples had thoroughly dried they were extracted and the extract cleaned-up in the usual manner. After adjusting all volumes to 25 ml, the samples were analyzed on the gas chromatograph. The percentage efficiency was calculated by comparing the amount of malathion extracted from the wheat samples compared to the control. The test was repeated for gardona and for sumithion (using 0.04 mg per flask in the case of sumithion).

e. Extraction from flour. Untreated whole wheat flour (200 g) was placed in an empty powdered milk tin (500 g size). Malathion in methanol (8 ml of 1/1000 stock solution) was applied to the flour using a glass sprayer. Small portions of the solution were applied at a time and the flour was mixed between applications. After all the solution had been applied, the tin of flour was tumbled for 4 hr on a mechanical tumbler.

Three 5-g samples were extracted and diluted to 25 ml with methanol. A control solution was made by diluting 0.2 ml of stock solution to 25 ml. All samples were analyzed on the gas chromatograph and the peak areas compared.

Residue analysis

1. Equipment and conditions. Analysis was accomplished using a Varian Aerograph model 1400 gas-liquid chromatograph equipped with an alkali flame ionization detector with cesium bromide salt tip. The pyrex column was 6 ft long (1/4 in. o.d., 2 mm i.d.) and was packed with 1.5% OV-225 on high performance Chrom W. The column oven was operated at 210°C, the injection port at 230°C and the detector at 240°C. Hydrogen was supplied from a Varian Aerograph hydrogen generator with the flow rate adjusted to 36 ml per min at the detector. Flow rates of air and nitrogen were adjusted over ranges of 200 ml to 300 ml per min and 14 ml to 40 ml per min, respectively, as required to adjust the detector for satisfactory response. A model 30 Varian Aerograph strip chart recorder with 0-1 mv range was used to record the output. Methanol was used as the solvent for all samples and 2 µl samples were injected into the chromatograph with a 5 µl Hamilton syringe. The technique used for injections was to first flush the syringe with methanol, then draw in 0.5 µl of methanol followed by 0.5 µl of air. After wiping the needle to remove excess solvent, 2 µl of the sample were drawn into the syringe and the needle wiped again. The plunger was drawn back a little to draw the sample out of the needle to avoid vaporization of the sample in the needle. When the injection was made the methanol, first drawn into the syringe, flushed out the dead space in the needle and lessened the chance of one sample being carried over and contaminating the following sample.

2. Internal standards and calibration. Malathion was used as an internal standard for samples containing gardona. Gardona was used as an internal standard for samples containing malathion or sumithion. For each combination of internal standard and unknown, a calibration curve was prepared in the following manner. An estimation was made of the range of concentrations that was likely to be encountered for a given insecticide. A concentration near the middle of this range was selected and a test solution of the insecticide prepared accordingly. Test runs were made on the gas chromatograph and the attenuation adjusted so that a 2 µl injection of the test solution would give a peak height approximately half of full scale response. By trial and error, a series of different concentrations of the internal standard were then prepared and analyzed without changing the attenuation, until a concentration was found which would produce a similar response to the sample of insecticide representing the middle of the range to be detected. The concentration of internal standard thus determined was used for all future analyses of the insecticide. A calibration curve was prepared by analyzing a series of concentrations of the insecticide containing internal standard. For each sample the ratio of the insecticide peak area to the internal standard peak area represented the concentration of the insecticide. This value was multiplied by a factor of 100 to avoid decimals and is referred to as the standardized peak area. For the calibration curves the standardized peak areas of the test solutions were plotted against the concentration of insecticide. In order to determine the concentration of insecticide extracted from a sample, the standardized peak area determined from the g.l.c. analysis was located on the calibration curve and the concentration of insecticide was determined from the other axis.

RESULTS AND DISCUSSION

Tests of extraction and clean-up efficiency

1. Comparison using more solvent for extraction. In the case of the sample extracted in the normal fashion (page 36), the standardized peak area for the malathion residue was 696. For the sample undergoing the more thorough extraction and clean-up procedure, the standardized peak area was 758. Thus the regular method of extraction and clean-up was 8% less efficient than the method using more solvent and longer extraction time. Because of limitations on the availability of large quantities of analytical grade solvent, the procedure using the smaller volume of methanol and 2-hr extraction period was used for all subsequent work.

2. Comparison of column vs. batch clean-up. The standardized peak area of the sample cleaned-up with the batch procedure was 323. For the sample which was cleaned-up by the column procedure, the standardized peak area was 342. The batch clean-up method, then, was about 5% less efficient than the column clean-up method. The batch clean-up method was used throughout the remainder of the work due to its rapidity for large numbers of samples.

3. Extraction from firebrick. Table 4 shows the results obtained from g.l.c. analysis of samples extracted from firebrick. The average extraction efficiency for malathion was 95%. The efficiencies for gardona and sumithion were 94% and 93%, respectively.

4. Extraction from whole grain. Table 5 shows results obtained with similar tests using whole grain instead of firebrick. The extraction efficiencies for malathion, gardona and sumithion were 85%, 82% and 84%, respectively.

TABLE 4

Efficiency of extraction and clean-up procedures using 5-gram samples of firebrick treated with 40 ppm of malathion, 40 ppm of gardona or 8 ppm of sumithion.

Insecticide	Treatment level	Replicate	Std. peak area[a] Wheat extract	Std. peak area[a] Control	Extraction[b] %	Avg. %	Std.[c] dev. %
malathion	40 ppm	a	420	442	95		
		b	416	442	94	95	1.0
		c	425	442	96		
gardona	40 ppm	a	229	248	92		
		b	234	248	94	94	1.5
		c	236	248	95		
sumithion	8 ppm	a	172	186	92		
		b	176	186	95	93	2.1
		c	170	186	91		

a. $\frac{\text{Area of protectant peak}}{\text{Area of standard peak}} \times 100$. Determined from g.l.c. analysis.

b. % Extraction = $\frac{\text{std. peak area of firebrick extract}}{\text{std. peak area of control}} \times 100$

c. Standard deviation.

TABLE 5

Efficiency of extraction and clean-up procedures using 5-gram samples of whole wheat treated with 40 ppm of malathion, 40 ppm of gardona or 8 ppm of sumithion.

Insecticide	Treatment level	Replicate	Std. peak area[a] Wheat extract	Std. peak area[a] Control	Extraction[b] %	Avg. %	Std.[c] dev. %
malathion	40 ppm	a	161	192	84		
		b	161	192	84	85	1.2
		c	166	192	86		
gardona	40 ppm	a	192	243	79		
		b	203	243	84	82	2.6
		c	201	243	83		
sumithion	8 ppm	a	217	263	83		
		b	220	263	84	84	1.5
		c	225	263	86		

a. $\frac{\text{Area of protectant peak}}{\text{Area of standard peak}} \times 100$. Determined from g.l.c. analysis.

b. $\% \text{ Extraction} = \frac{\text{std. peak area of firebrick extract}}{\text{std. peak area of control}} \times 100$

c. Standard deviation.

5. Extraction from flour. Results for the extraction of residues from wheat flour as compared with un-extracted controls are shown in Table 6. The efficiency of extraction of malathion from flour was found to be 83%. The efficiencies of extraction for gardona and sumithion were found to be 80% and 82%, respectively.

Internal standards and calibration

The solutions of insecticides and internal standards used to prepare the calibration curves are shown in Table 7. The calibration curves derived from these samples are shown in Figures 2, 3 and 4 for malathion, gardona and sumithion, respectively. Because the range of concentrations investigated increased in a geometric progression, the calibration curves were plotted on log/log axes.

TABLE 6

Efficiency of extraction and clean-up procedures using 5-gram samples of wheat flour treated with 40 ppm of malathion, 40 ppm of gardona or 8 ppm of sumithion.

Insecticide	Treatment level	Replicate	Std. peak area[a] Wheat extract	Std. peak area[a] Control	Extraction[b] %	Avg. %	Std.[c] dev. %
malathion	40 ppm	a	220	274	80		
		b	229	274	84	83	2.3
		c	230	274	84		
gardona	40 ppm	a	124	154	81		
		b	120	154	78	80	2.1
		c	127	154	82		
sumithion	8 ppm	a	159	189	84		
		b	155	189	82	82	2.5
		c	150	189	79		

a. $\frac{\text{Area of protectant peak}}{\text{Area of standard peak}} \times 100$. Determined from g.l.c. analysis.

b. % Extraction = $\frac{\text{std. peak area of flour extract}}{\text{std. peak area of control}} \times 100$

c. Standard deviation.

TABLE 7

Concentrations of insecticides and internal standards used in preparing calibration curves.

Insecticide	Concentration of insecticide	Concentration of internal standard
malathion	16 ppm	3 ppm[a]
	4 ppm	3 ppm
	2 ppm	3 ppm
	1 ppm	3 ppm
	0.2 ppm	3 ppm
	0.08 ppm	3 ppm
gardona	8 ppm	0.3 ppm[b]
	5 ppm	0.3 ppm
	2 ppm	0.3 ppm
	1 ppm	0.3 ppm
	0.5 ppm	0.3 ppm
	0.1 ppm	0.3 ppm
sumithion	3.2 ppm	1 ppm[a]
	2 ppm	1 ppm
	1 ppm	1 ppm
	0.2 ppm	1 ppm
	0.1 ppm	1 ppm
	0.02 ppm	1 ppm

a. gardona b. malathion

FIGURE 2

Calibration curve for calculating concentrations of malathion in samples analyzed by gas-liquid chromatography.

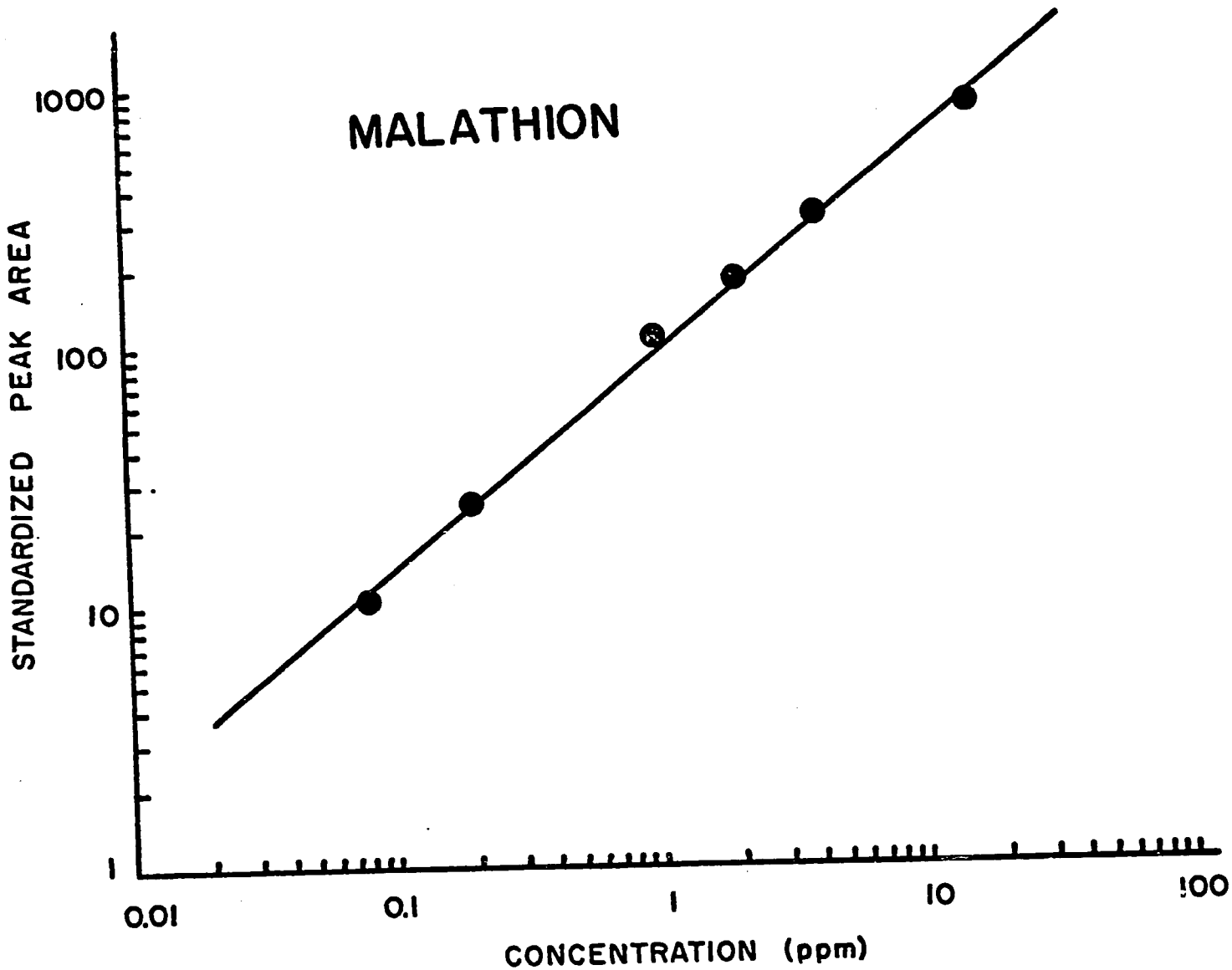

MALATHION
STANDARDIZED PEAK AREA
1000
100
10
1
0.01
0.1
1
10
100
CONCENTRATION (ppm)

FIGURE 3

Calibration curve for calculating concentrations of gardona in samples analyzed by gas-liquid chromatography.

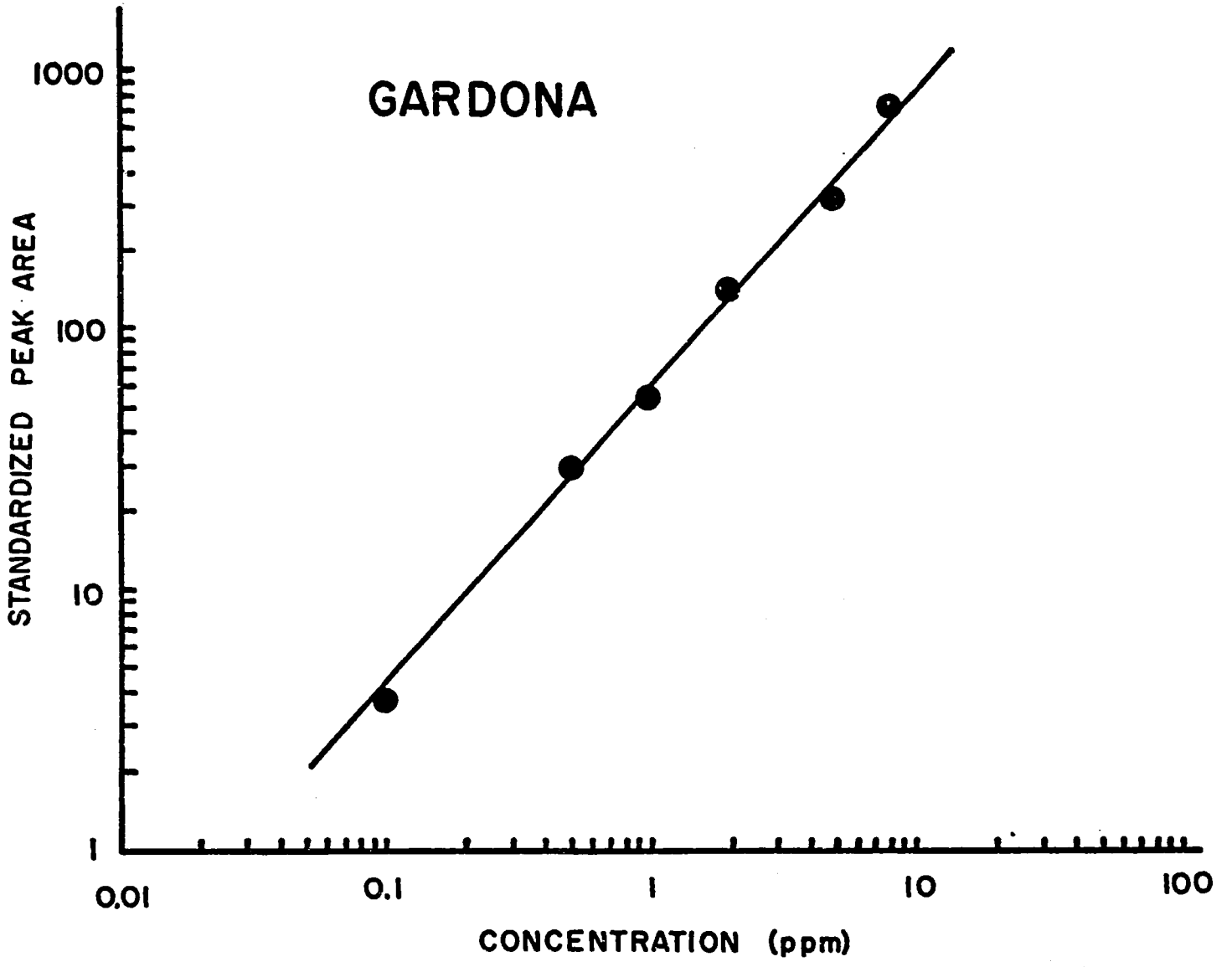
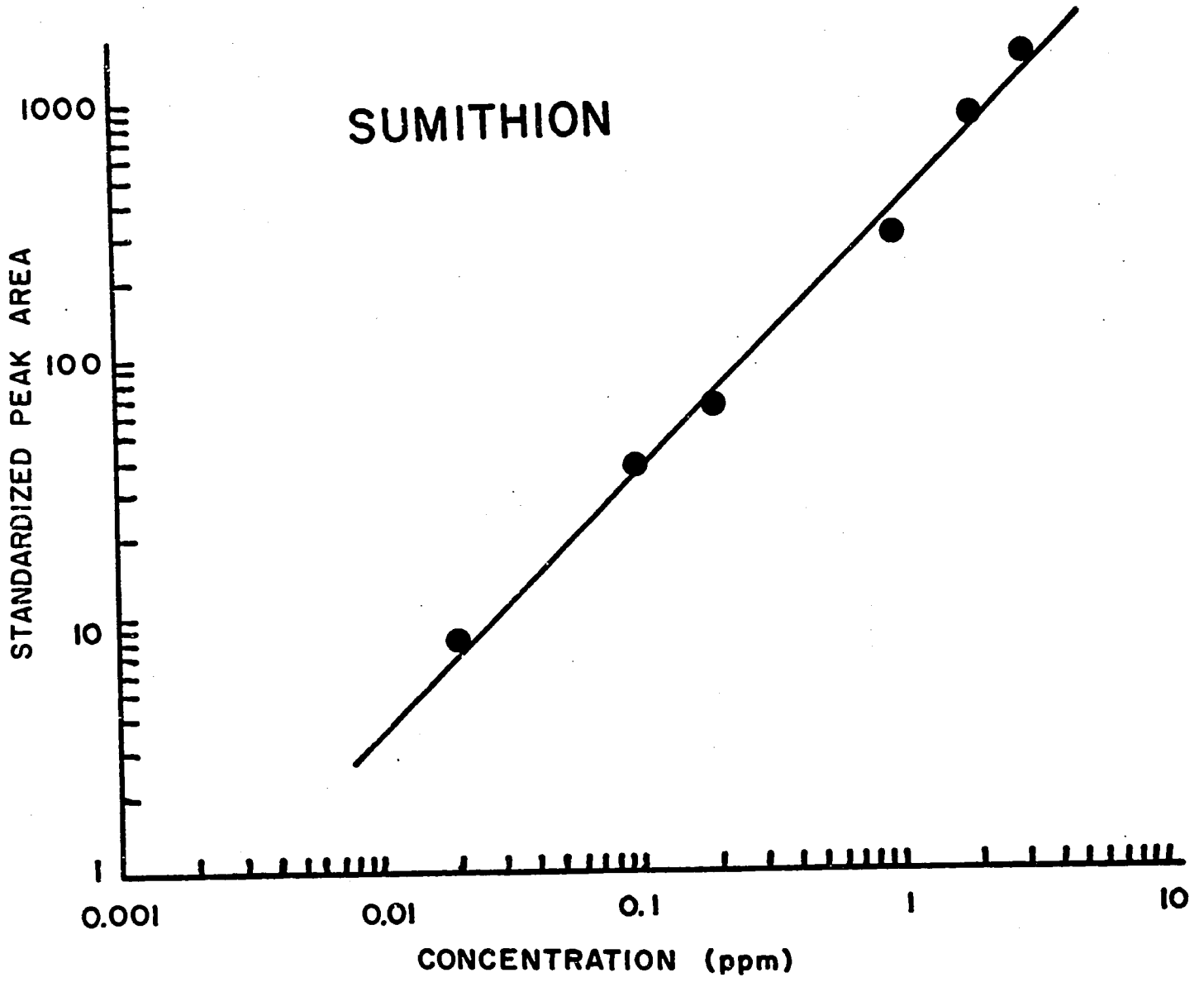

GARDONA
STANDARDIZED PEAK AREA
1000
100
10
1
0.01
0.1
1
10
100
CONCENTRATION (ppm)

FIGURE 4

Calibration curve for calculating concentrations of sumithion in samples analyzed by gas-liquid chromatography.


SUMITHION
STANDARDIZED PEAK AREA
1000
100
10
1
0.001
0.01
0.1
1
10
CONCENTRATION (ppm)

Bioassay studies

Table 8 gives the percent mortality at 0 months, 1 month and 2 months for *Sitophilus oryzae* (rice weevils) and *Tribolium castaneum* (red flour beetles) exposed to paddy treated with each of the three levels of the three test insecticides. The same information for wheat and for sorghum is given in Tables 9 and 10, respectively. The highest concentrations investigated represented 8 times the lowest concentration used. The lowest concentration of each insecticide used was determined (in a preliminary 2-month study) as the lowest effective concentration for protection of grain against insect damage. In this investigation the highest concentration was found to give 100% kill over the test period of two months. The intermediate concentration of all three insecticides was completely effective in sorghum and wheat. However, the sumithion treatment of paddy at 8 ppm had begun to lose effectiveness at the end of the 2-month test period. In the case of treatment with the lowest concentration, sumithion was relatively ineffective in controlling infestation in paddy at all time periods. By the end of the test periods, it was also losing effectiveness with the other grains. At this lowest concentration, malathion and gardona were beginning to lose effectiveness at the end of the test periods. In general, the treatments of paddy were less effective than the equivalent treatments of the other two grains. This was due in part to the method of application and mixing. The rough nature of the paddy hull results in considerably more material being worn off in the form of dust as the grain is tumbled. Application of the insecticide involved tumbling to distribute it throughout the grain and it was found that considerably more dust resulted from mixing of the paddy than for the other grains. It is likely that some of the

TABLE 8

Periodic bioassay tests for the effectiveness of insecticide treatments on control of *Tribolium castaneum* and *Sitophilus oryzae* in paddy.

Insecticide	Treatment level	Test insect	% mortality at:[c]		
			0 month	1 month	2 months
malathion	10 ppm	RFB[a]	97	90	10
		RW[b]	100	100	97
	40 ppm	RFB	100	100	97
		RW	100	100	100
	80 ppm	RFB	100	100	100
		RW	100	100	100
gardona	10 ppm	RFB	100	97	73
		RW	100	100	97
	40 ppm	RFB	100	100	100
		RW	100	100	100
	80 ppm	RFB	100	100	100
		RW	100	100	100
sumithion	2 ppm	RFB	27	10	3
		RW	97	53	13
	8 ppm	RFB	100	100	23
		RW	100	100	90
	16 ppm	RFB	100	100	100
		RW	100	100	100

a. Red flour beetles (*Tribolium castaneum*)

b. Rice weevils (*Sitophilus oryzae*)

c. 0% mortality in control vials

TABLE 9

Periodic bioassay tests for the effectiveness of insecticide treatments on control of *Tribolium castaneum* and *Sitophilus oryzae* in wheat.

Insecticide	Treatment level	Test insect	% mortality at:[c]		
			0 month	1 month	2 months
malathion	10 ppm	RFB[a]	100	97	70
		RW[b]	100	100	100
	40 ppm	RFB	100	100	100
		RW	100	100	100
	80 ppm	RFB	100	100	100
		RW	100	100	100
gardona	10 ppm	RFB	100	100	90
		RW	100	100	100
	40 ppm	RFB	100	100	100
		RW	100	100	100
	80 ppm	RFB	100	100	100
		RW	100	100	100
sumithion	2 ppm	RFB	60	7	3
		RW	100	40	7
	8 ppm	RFB	100	100	100
		RW	100	100	100
	16 ppm	RFB	100	100	100
		RW	100	100	100

a. Red flour beetles (*Tribolium castaneum*)

b. Rice weevils (*Sitophilus oryzae*)

c. 0% mortality in control vials

TABLE 10

Periodic bioassay tests for the effectiveness of insecticide treatments on control of *Tribolium castaneum* and *Sitophilus oryzae* in sorghum.

Insecticide	Treatment level	Test insect	% mortality at:[c] 0 month	1 month	2 months
malathion	10 ppm	RFB[a]	100	93	
		RW[b]	100	100	
	40 ppm	RFB	100	100	
		RW	100	100	n.d.[d]
	80 ppm	RFB	100	100	
		RW	100	100	
gardona	10 ppm	RFB	100	97	
		RW	100	100	
	40 ppm	RFB	100	100	
		RW	100	100	n.d.
	80 ppm	RFB	100	100	
		RW	100	100	
sumithion	2 ppm	RFB	97	13	
		RW	100	60	
	8 ppm	RFB	100	100	
		RW	100	100	n.d.
	16 ppm	RFB	100	100	
		RW	100	100	

a. Red flour beetles (*Tribolium castaneum*)

b. Rice weevils (*Sitophilus oryzae*)

c. 0% mortality in control vials

d. Not determined due to time limitations of the study.

insecticide that was applied to the paddy was lost in this form. As a result the actual level of insecticide residue remaining on the grains might have been less than planned. This was validated by measurements of the residues in the grains. This type of loss would not be so pronounced when the insecticide is applied to a grain stream, since there would not be as much grain movement, as in the case of tumbling, and the subsequent production of dust would be lessened.

The results from infestation tests shown in Tables 11, 12 and 13 indicate that, in the case of malathion and gardona, all treatments were effective in preventing the infestation of the grains during the 8½-week test period with the exception of the 10 ppm malathion treatment of paddy in which a light infestation of flour beetles was recorded after 6½ weeks. In the case of sumithion, the lowest level (2 ppm) failed to provide complete protection after a period of from 1½ weeks to 3 weeks in all the grains. In general the two higher concentrations of sumithion (8 and 16 ppm) were effective for the full period of 8½ weeks with only a light infestation of flour beetles, beginning at the end of the period, in paddy treated with 8 ppm of sumithion.

Reproduced from best available copy.

TABLE 11

Development of insect infestation in samples of treated paddy exposed to populations of rice weevils and flour beetles.

Insecticide	Treatment level	Test insect	Weeks[a] 0	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½
malathion	control	RFB[b]	o[d]	o	o	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
		RW[c]	o	o	o	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	10 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	-	-	-	-	-
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	40 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	80 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
gardona	control	RFB	o	o	o	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
		RW	o	o	o	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	10 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	40 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	80 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
sumithion	control	RFB	o	o	o	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
		RW	o	o	o	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	2 ppm	RFB	o	o	o	o	-	-	-	-	-	-	-	+	+	+	+	+	+	+
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	-	-	-	-	-
	8 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	-	-	-
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	16 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

a. Weeks after treatment
b. Red flour beetles (Tribolium castaneum)
c. Rice weevils (Sitophilus oryzae)
d. o = no infestation, - = light infestation, + = heavy infestation

TABLE 12

Development of insect infestation in samples of treated wheat exposed to populations of rice weevils and flour beetles.

Insecticide	Treatment level	Test insect	Weeks[a] 0	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½
malathion	control	RFB[b]	o[d]	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
		RW[c]	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
	10 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	40 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	80 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
gardona	control	RFB	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
		RW	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
	10 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	40 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	80 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
sumithion	control	RFB	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
		RW	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
	2 ppm	RFB	o	o	o	o	o	-	-	-	-	-	+	+	+	+	+	+	+	+
		RW	o	o	o	o	o	o	o	o	o	o	o	-	-	-	-	-	+	+
	8 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	16 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

a. Weeks after treatment
b. Red flour beetles (Tribolium castaneum)
c. Rice weevils (Sitophilus oryzae)
d. o = no infestation, - = light infestation, + = heavy infestation

TABLE 13

Development of insect infestation in samples of treated sorghum exposed to populations of rice weevils and flour beetles.

Insecticide	Treatment level	Test insect	Weeks[a] 0	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½
malathion	control	RFB[b]	o[d]	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
		RW[c]	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
	10 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	40 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	80 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
gardona	control	RFB	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
		RW	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
	10 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	40 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	80 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
sumithion	control	RFB	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
		RW	o	o	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
	2 ppm	RFB	o	o	o	o	o	o	o	-	-	-	-	-	-	-	-	-	-	-
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	8 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	16 ppm	RFB	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
		RW	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

a. Weeks after treatment
b. Red flour beetles (Tribolium castaneum)
c. Rice weevils (Sitophilus oryzae)
d. o = no infestation, - = light infestation, + = heavy infestation

Degradation of residues during milling

1. Machine grinding. Tables 14 and 15 show the levels of residues detected in whole grain (wheat and sorghum) after treatment and after machine grinding. The last columns give the loss of residue in ppm, the percentage reduction from the original level, the average loss for a particular insecticide and the standard deviation. The average losses for wheat and sorghum during grinding were:

Wheat treated with	malathion	71%
	gardona	30%
	sumithion	62%
Sorghum treated with	malathion	40%
	gardona	39%
	sumithion	28%

It was found that minor adjustments in the clearance of the grinding stones made a noticeable difference in the heating of the grain during grinding. It is possible that some variation in residue breakdown could have resulted from this, although the mill was always warmed-up by milling clean grain before the samples were milled. Sorghum, being softer than wheat, tended not to heat to the same extent. Some indication of this is noticeable in the greater breakdown of residues in the wheat samples as compared to sorghum samples.

2. Hand grinding. Table 16 shows the levels of residues detected in whole wheat and in hand-ground wheat. As in the previous two tables, the last columns give losses in ppm, as a percentage of the original level, the average loss for an insecticide and the standard deviation. The average losses were:

Loss in wheat treated with:	malathion	35%
	gardona	25%
	sumithion	21%

TABLE 14

Losses of insecticide residues during machine grinding of wheat.

Insecticide	Treatment level	Insecticide residue (ppm) recovered from: Whole grain	Machine-ground grain	Loss of residue ppm	%	Avg. %	Std. dev. %
malathion	10 ppm	5.8	2.2	3.6	62		
	40 ppm	31.9	9.3	22.6	71	71	8.5
	80 ppm	77.1	16.5	60.6	79		
gardona	10 ppm	18	13.5	5.5	31		
	40 ppm	39	27.5	11.5	29	30	1.2
	80 ppm	65	45	20	31		
sumithion	2 ppm	2	1	1	50		
	8 ppm	8.3	3	5.25	64	62	11.6
	16 ppm	15.5	4.2	11.3	73		

TABLE 15

Losses of insecticide residues during machine grinding of sorghum.

Insecticide	Treatment level	Insecticide residue (ppm) recovered from: Whole grain	Machine-ground grain	Loss of residue ppm	%	Avg. %	Std. dev. %
malathion	10 ppm	5.76	3.4	2.23	41		
	40 ppm	22.4	13.5	8.9	40	40	1.5
	80 ppm	53.3	33	20.3	38		
gardona	10 ppm	6.5	2.75	3.75	58		
	40 ppm	32.5	25.5	7	22	39	17.5
	80 ppm	85	53	32	38		
sumithion	2 ppm	2.5	1.8	0.7	28		
	8 ppm	9.25	6.5	2.75	30	28	2.0
	16 ppm	15.5	11.5	4	26		

TABLE 16

Losses of insecticide residue during hand grinding of wheat.

Insecticide	Treatment level	Insecticide residue (ppm) recovered from: Whole grain	Hand-ground grain	Loss of residue ppm	%	Avg. %	Std. dev. %
malathion	10 ppm	5.8	3.3	2.5	43		
	40 ppm	31.9	20	11.9	37	35	9.2
	80 ppm	77.1	57.5	19.6	25		
gardona	10 ppm	18	14.5	3.5	19		
	40 ppm	39	28.5	10.5	27	25	5.3
	80 ppm	65	46	19	29		
sumithion	2 ppm	2	1.9	0.1	5		
	8 ppm	8.3	5.9	2.35	28	21	13.9
	16 ppm	15.5	11	4.5	29		

It can be seen by comparing these figures with those for machine grinding that there is less breakdown during hand grinding. Since there is considerably less heating during hand grinding it is likely that this factor accounts, at least in part, for this difference.

3. Paddy milling. The first process in paddy milling is the hulling of the grain. The resulting brown rice is then polished to remove the bran,producing the polished, or white rice, which is the form most commonly used for consumption in India and abroad. Soon after treatment most of the insecticide is found in the hulls. Older samples show increasing amounts of residue in the bran and polished rice due to migration of residues into the grain. However, even at its highest level relatively little residue is found in polished rice compared to the amount applied. Table 17 shows the treatment levels of paddy for malathion, gardona and sumithion and the residues carried over into the polished rice 0, 1 and 2 months after application of the insecticide. It can be seen from the Table that, although there is some increase in residue in the polished rice as it ages, it never amounts to very much and, even in the case of the 80 ppm treatment of malathion, the level of residue in the rice is less than 50% of the admissable value (8 ppm) authorized in many countries (8).

Organophosphate insecticides on stored grain undergo both enzymic and chemical hydrolysis. The resulting compounds are generally of low toxicity to mammals and do not pose a serious threat to the consumer.

TABLE 17

Residues of malathion, gardona and sumithion detected in polished rice 0, 1 and 2 months after application.

Insecticide	Treatment level	Residue (ppm) in paddy at 0 months	Residue (ppm) detected in polished rice at: 0 month	1 month	2 months
malathion	10 ppm	3.5	b.d.[a]	0.16	1.55
	40 ppm	9.5	0.19	n.d.[b]	3.0
	80 ppm	37.5	0.36	0.75	3.24
gardona	10 ppm	6	b.d.	b.d.	b.d.
	40 ppm	26.5	0.8	1	b.d.
	80 ppm	52.5	2.3	1.6	1.65
sumithion	2 ppm	1.2	b.d.	b.d.	b.d.
	8 ppm	3.4	0.22	b.d.	0.21
	16 ppm	9.6	0.48	0.4	0.26

a. b.d. = below detectability
b. n.d. = not determined

Reproduced from best available copy.

Degradation of residues during cooking

1. Chapati making. Tables 18 and 19 show the losses of insecticide residues during the preparation of chapaties from wheat and sorghum, respectively. The average losses vary from 43% to 75%. However it can be seen from the data that there is considerable variability in the degree of breakdown of residues during the preparation of chapaties. The large standard deviations give another indication of the variation encountered here. Part of the variation is due to differences in the normal cooking methods for making chapaties. Even within one area of India one may find considerable variation in the type of chapati prepared in the home. An illustration of the difference that can occur is given in Figures 5 and 6. Figure 5 is composed of chromatograms showing the residues detected from flour and chapaties in the case where the chapati was cooked less than usual, as some people prefer them. It can be seen that the size of the malathion peak in relation to the standard peak is not reduced a great deal during the cooking process. In contrast with this, Figure 6 shows a marked reduction in the peak size (and thus residue level) of the insecticide in the chapati samples as compared to the flour samples. The cooking in this case was considered average. Figures 7 and 8 show chromatograms which illustrate a similar breakdown for gardona and sumithion. These results are reflected in the information given in Tables 18 and 19.

2. Boiling and steaming. Figures 9, 10 and 11 show chromatograms of samples extracted from polished rice, boiled rice and iddlies for malathion, gardona and sumithion,respectively. It can be seen that,within the detectable limits of the methods,there is a complete breakdown of the residues of all three insecticides by these wet-cooking methods. A similar loss was observed in the case of boiling sorghum balls.

TABLE 18

Loss of insecticide residues during the preparation of chapaties from wheat flour.

Insecticide	Treatment level	Months after treatment	Insecticide residue (ppm) recovered from: Machine-ground grain	Chapati	Loss during cooking ppm	%	Avg. %	Std. dev. %
malathion	10 ppm	0	2.2	1.5	0.7	32		
		1	0.8	0.23	0.57	71		
		2	0.53	0.23	0.3	57		
	40 ppm	0	9.3	5.8	3.5	38		
		1	3.5	1.3	2.2	63	51	24.0
		2	1.85	0.95	0.9	49		
	80 ppm	0	16.5	16	0.5	3		
		1	12	3.5	8.5	71		
		2	7.5	1.6	5.9	79		
gardona	10 ppm	0	13.5	b.d.[a]	13.5	100		
		1	5	2.85	2.15	43		
		2	2.35	0.65	1.70	72		
	40 ppm	0	27.5	1.7	25.8	94		
		1	27.5	7.5	20	73	75	18.3
		2	8	3	5	63		
	80 ppm	0	45	3	42	93		
		1	50	12.5	37.5	75		
		2	18	6.5	11.5	64		
sumithion	2 ppm	0	1	0.53	0.47	47		
		1	0.2	b.d.	0.2	100		
		2	0.13	0.13	0	0		
	8 ppm	0	3	1.5	1.5	50		
		1	1.1	0.09	1.01	92	52	35.1
		2	0.58	0.38	0.2	34		
	16 ppm	0	4.2	3.6	0.6	14		
		1	1.9	0.23	1.67	88		
		2	1.08	0.65	0.43	40		

a. b.d. = below detectability

TABLE 19

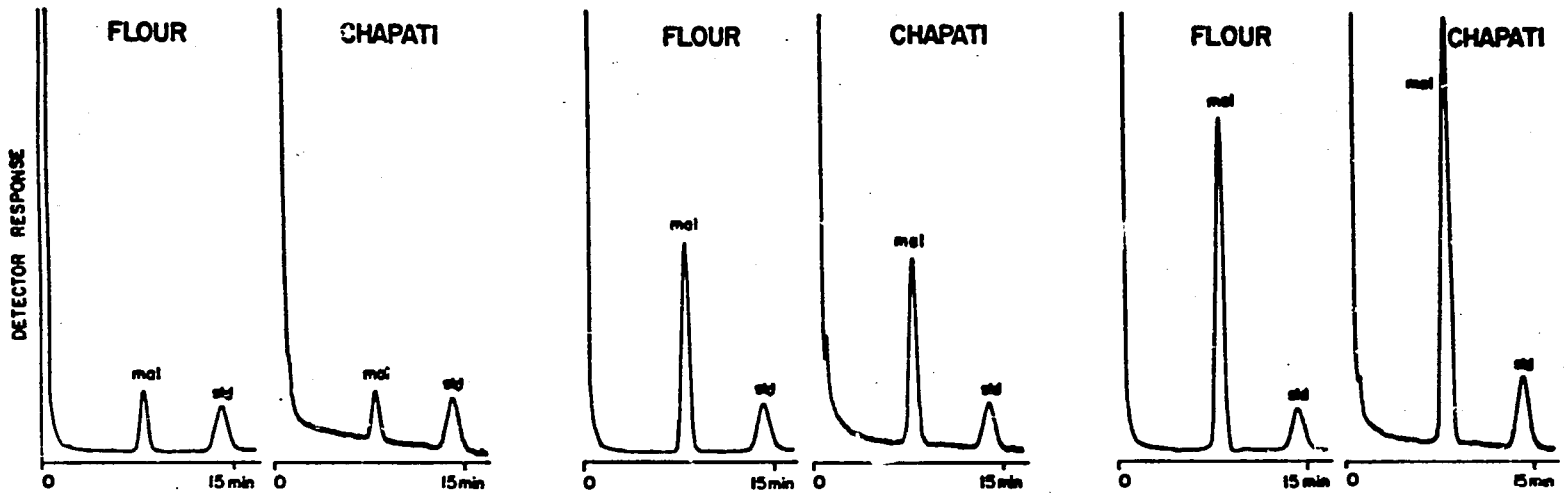
Loss of insecticide residues during the preparation of chapaties from sorghum flour.

Insecticide	Treatment level	Months after treatment[a]	Insecticide residue (ppm) recovered from: Machine-ground grain	Chapati	Loss during cooking ppm	%	Avg. %	Std. dev. %
malathion	10 ppm	0	3.4	0.7	2.7	79		
		1	1.9	0.63	1.27	67		
	40 ppm	0	13.5	3.6	9.9	73		
		1	8	2.3	5.7	71	74	4.5
	80 ppm	0	33	9	24	73		
		1	25	5.5	19.5	78		
gardona	10 ppm	0	2.75	2	0.75	27		
		1	3.25	1.2	2.05	63		
	40 ppm	0	25.5	9.5	16	63		
		1	17	6.5	10.5	62	59	15.1
	80 ppm	0	53	15	38	72		
		1	50	18	32	64		
sumithion	2 ppm	0	1.8	1.1	0.7	39		
		1	1	0.65	0.35	35		
	8 ppm	0	6.5	3.75	2.75	42		
		1	3.75	1.9	1.85	49	43	6.8
	16 ppm	0	11.5	5.65	5.85	51		
		1	5.5	3.25	2.25	41		

a. Residues not determined at 2-month period due to time limitations of the study.

FIGURE 5

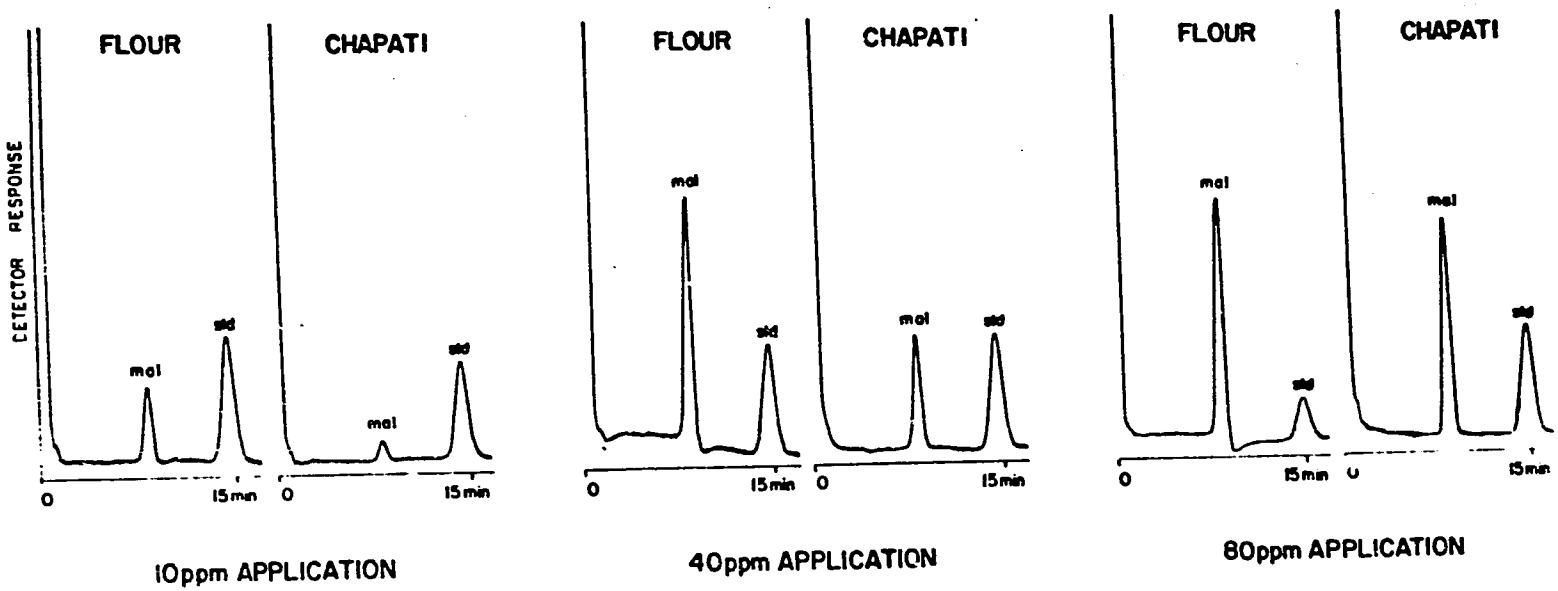
Gas-liquid chromatograms showing loss of malathion residue during chapati making (short cooking period).



MALATHION RESIDUES

FIGURE 6

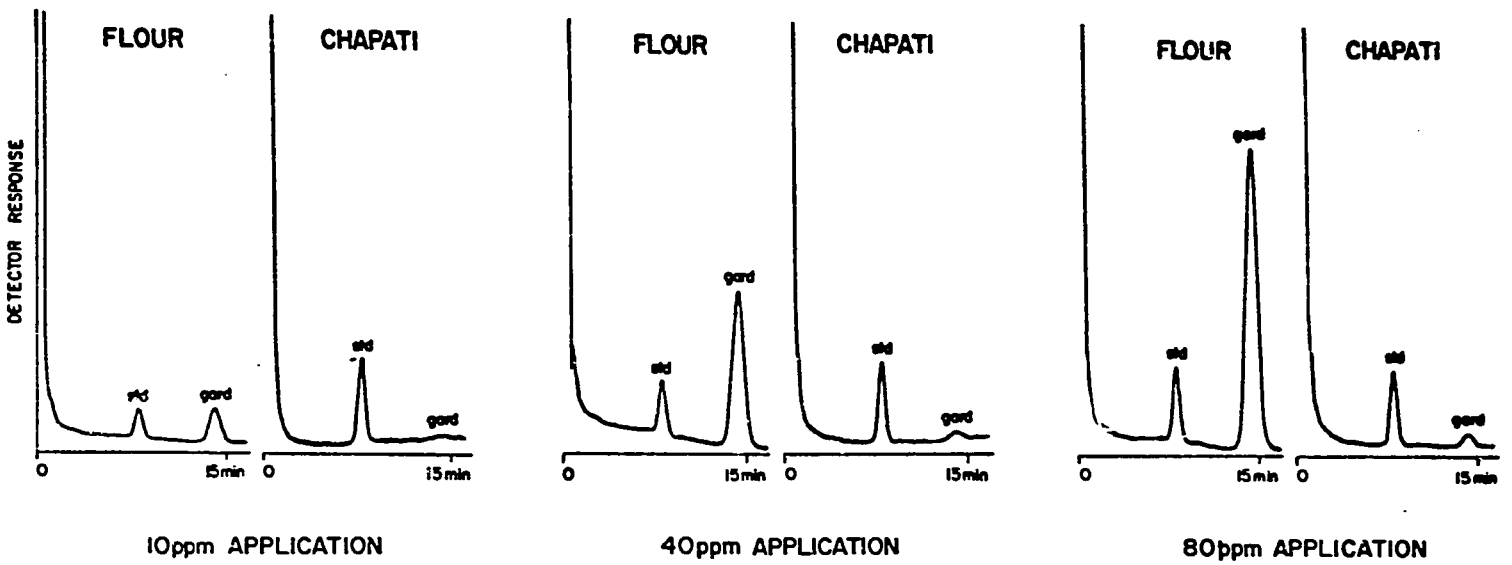
Gas-liquid chromatograms showing loss of malathion residue during chapati making. (regular cooking period)



MALATHION RESIDUES

FIGURE 7

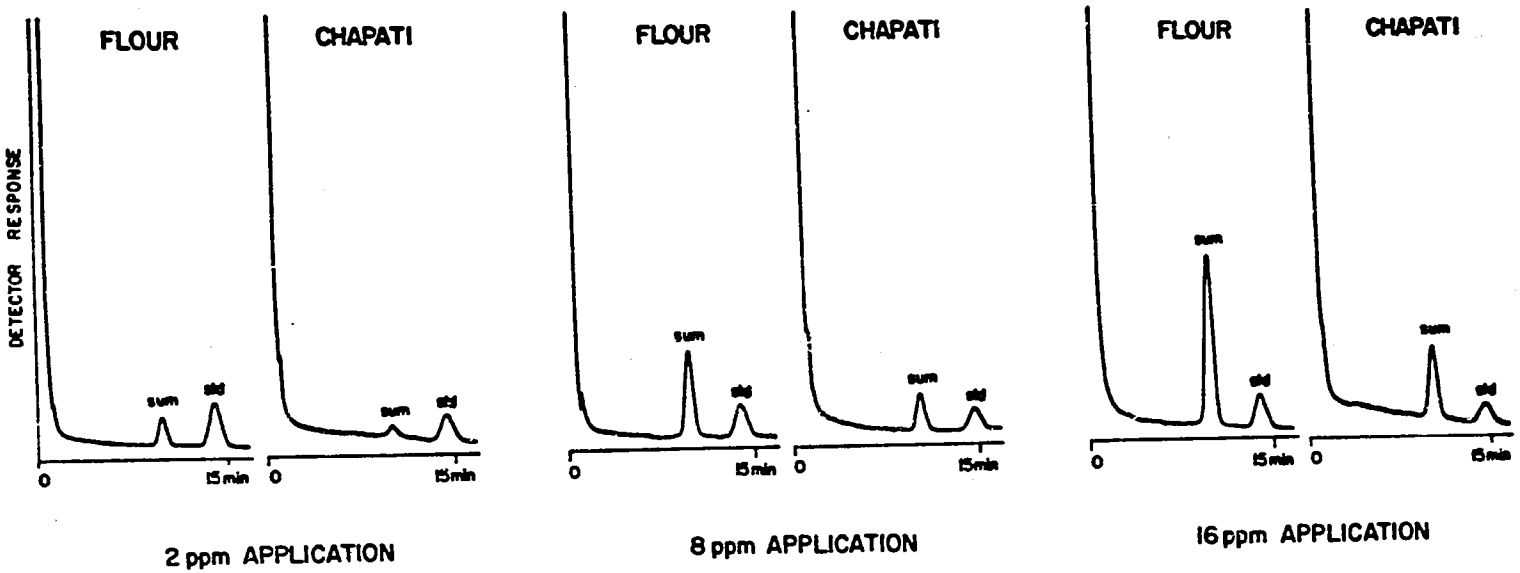
Gas-liquid chromatograms showing loss of gardona residue during chapati making (regular cooking period).



GARDONA RESIDUES

FIGURE 8

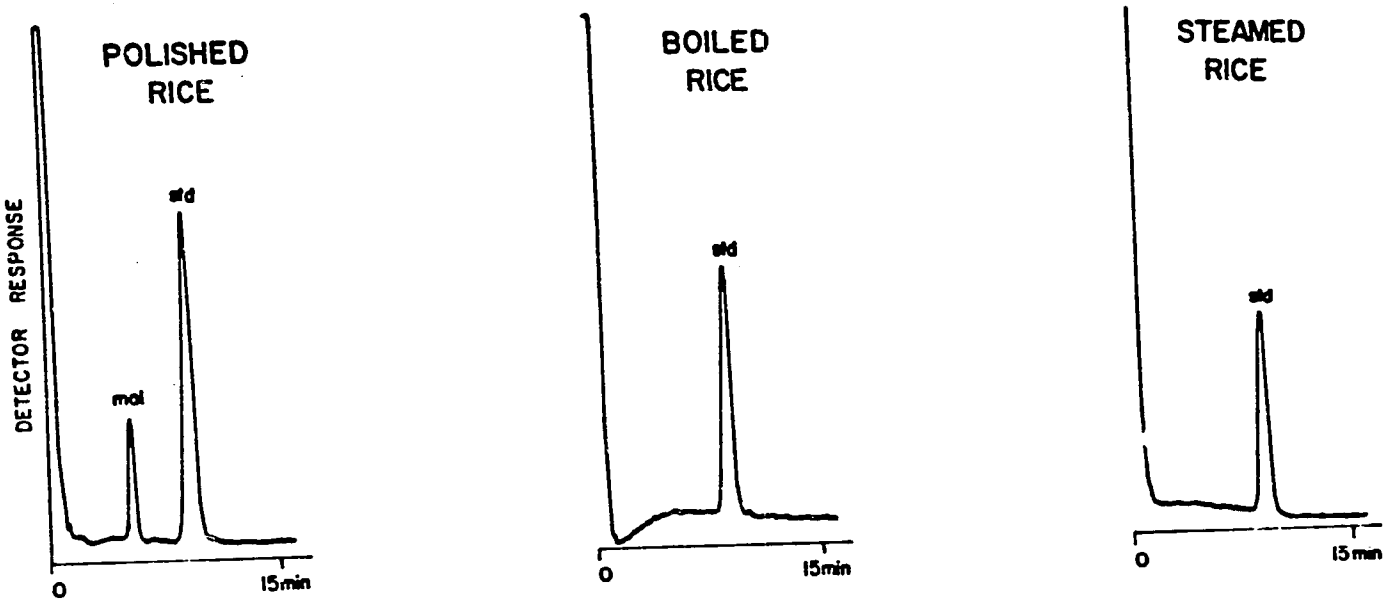
Gas-liquid chromatograms showing loss of sumithion residues during chapati making (regular cooking period).



SUMITHION RESIDUES

FIGURE 9

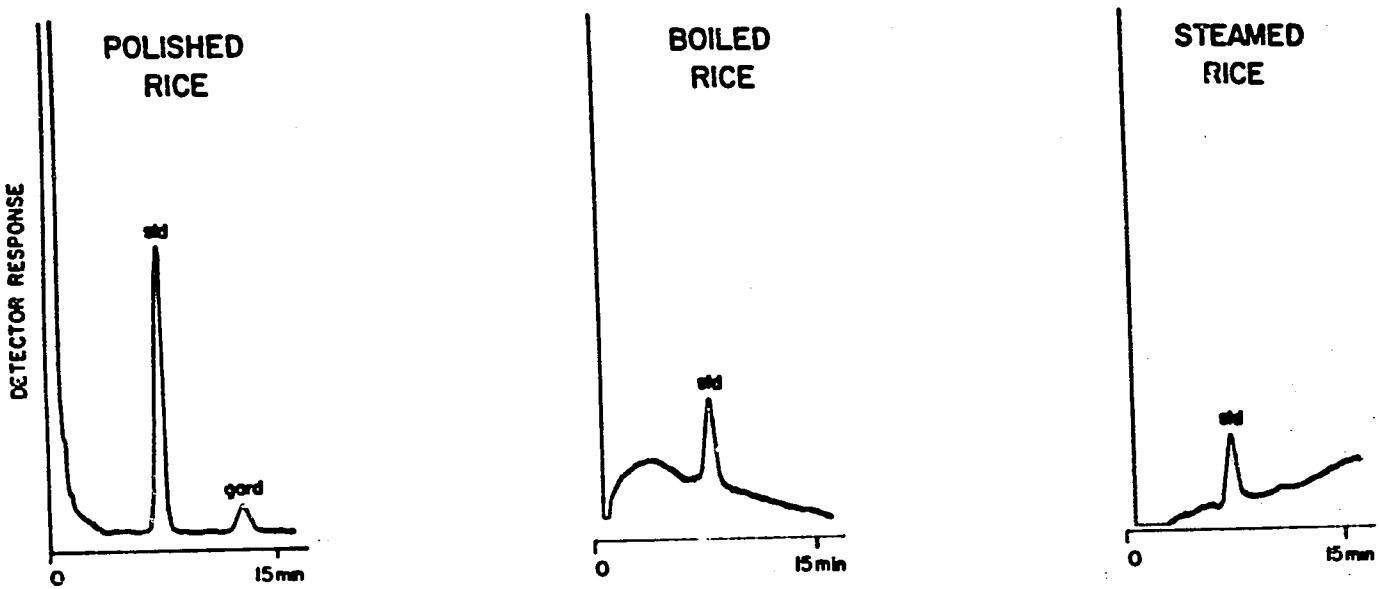
Gas-liquid chromatograms showing loss of malathion residues during boiling and steaming of rice.



MALATHION RESIDUES

FIGURE 10

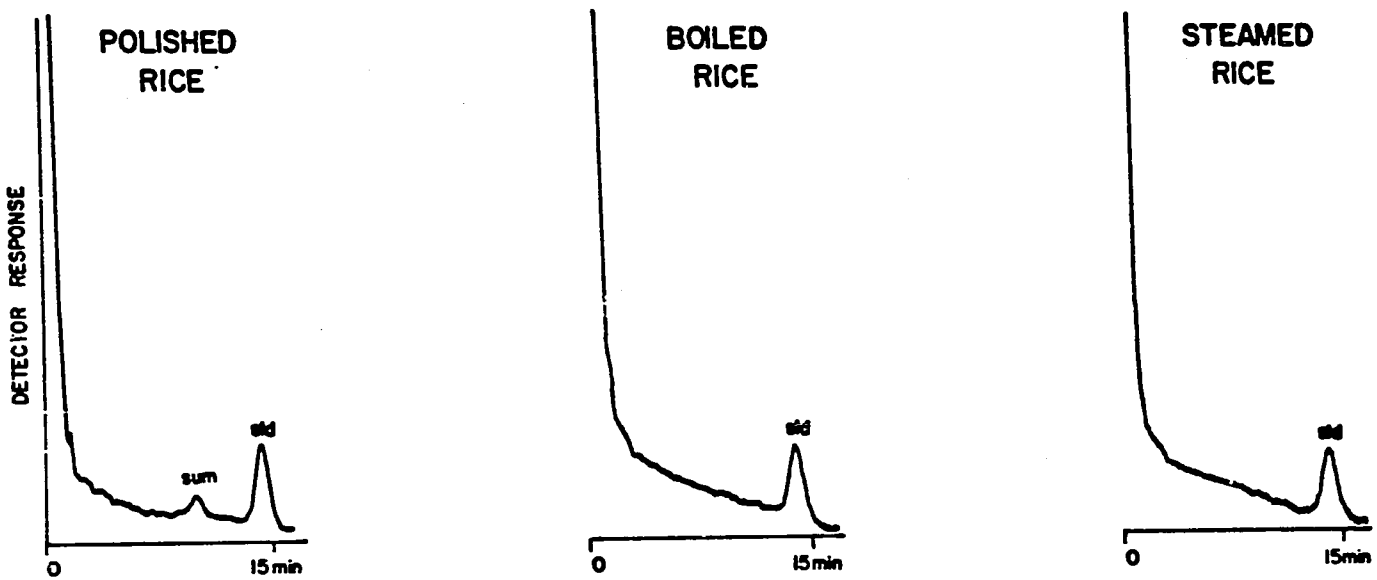
Gas-liquid chromatograms showing loss of gardona residues during boiling and steaming of rice.



GARDONA RESIDUES

FIGURE 11

Gas-liquid chromatograms showing loss of sumithion residues during boiling and steaming of rice.



SUMITHION RESIDUES

CONCLUSIONS

In evaluating the suitability of malathion, gardona and sumithion as protectants for use with stored grain in India it is important to know the extent to which these compounds are degraded during milling and cooking when they are used in concentrations that are effective in controlling insect populations.

The bioassay tests in this investigation indicate that treatments with 10 ppm of malathion and gardona and 2 ppm of sumithion are effective in disinfesting grain at the time of application and in controlling insects for a period afterwards. Malathion and gardona at 10 ppm appear to control infestation for 2 months as a minimum. Sumithion at 2 ppm is effective in disinfesting the grain, but by the end of two months the effectiveness is wearing off. For longer periods of control sumithion should be applied at a higher level, possibly 4 ppm.

Data presented here indicates that power milling reduces residues by 30% to 70% in the case of all three protectants. Before consumption of wheat in the form of chapaties, a further loss of 40% to 75% takes place in the cooking process. This means that a total loss of 59% to 93% of the insecticide residue occurs during the total processing of chapaties from whole wheat. In the case of rice, because of the wet cooking methods used, it appears that there is complete breakdown of the three insecticides.

It would appear that there is a good case for amending the Prevention of Food Adulteration rules to allow the use of malathion, and possibly other agents such as gardona and sumithion, as protectants to be directly admixed with grain in storage. This is particularly true when one considers

the hazard posed at the present time by misuse of other pesticides for control of infestation in stored grain. Part of this misuse is most likely due to lack of adequate information. Once malathion is approved, appropriate steps could be taken to acquaint farmers and traders with its safe use.

In India it is particularly difficult to try to estimate the breakdown that is likely to occur during cooking because there is such a wide variation of food types and methods of preparation. In the case of rice, however, the greatest part of the grain is boiled and would thus be subject to complete breakdown of the insecticide residues that have been noted in this study.

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. D.R. Lineback for his guidance and support during the period that I was in India and on my return to Kansas State University to complete the writing of this dissertation. I am also grateful to Dr. J.A. Shellenberger who was responsible for the initiation of my program of studies at Kansas State University.

The cooperation of Dr. H.A.B. Parpia, the Director, and the personnel at the Central Food Technological Research Institute was particularly appreciated. Every effort was made to provide any assistance or material that was required. Special gratitude is due to Dr. S.K. Majumder, Head of the Discipline of Infestation Control and Pesticides, who served as my advisor at C.F.T.R.I. He not only extended a warm welcome on behalf of the Institute, but also helped in innumerable personal ways as my family and I settled into life in Mysore.

Finally I must express my deep appreciation to my wife, Sara Ann, who provided encouragement when needed and whose happy adjustment to life in India helped to make our stay there a most pleasant one.

LITERATURE CITED

1. The United States Dapartment of Agriculture The world food budget 1960. Econ. Res. Ser., U.S.D.A., Washington, D.C. (Oct. 1964).

2. Pingale, S.V. Storage of food grains in India. Trop. Stored Prod. Inf. No. 11, 415 (1965).

3. Sarid, J.N., and Krishnamurthy, K. Post-harvest care of grain - an effective tool for immediate solution of the food problem. Bull. Grain Tech. 3:105 (1965).

4. Raizada, R.N. Preservation of foodgrain. Bull. Grain Tech. 4:31 (1965).

5. Government of India. India a Reference Annual 1971-72. Ministry of Information and Broadcasting (1971).

6. Jain, D.C. Storage of foodgrains by traders. Bull. Grain Tech. 3:109 (1965).

7. Ramasivam, T., Krishnamurthy, K., and Pingale, S.V. Studies on preservation of foodgrains in rural storage Part I: Rural storage of foodgrains. Bull. Grain Tech. 4:177 (1966).

8. FAO/WHO Evaluation of some pesticide residues in food. FAO, PL: CP/15; WHO/Food Add. 67.32. (1969).

9. FAO/WHO Recommended international tolerances for pesticide residues. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, CAC/RS 2-1969. (1969).

10. Trivedi, R.G. (ed.) A.P. Mathur's Commentaries on the Prevention of Food Adulteration Act 1954 (6th ed.) Eastern Book Co., Lucknow, India (1970).

11. Personal communication, CFTRI, Infestation Control and Pesticides (1972).

12. I.C.A.R. Report of the special committee on harmful effects of pesticides. Indian Council of Agricultural Research (August 1967).

13. Bindra, O.S. Grain protectants, a need for a revision of Govt. of India's policy regarding their use. Dept. of Entomol., Punjab Agric. Univ., Ludhiana (1972).

14. Mookerjee, P.B., Beri, Y.P., Sharma, G.C., and Dewan, R.S. Preliminary studies on the efficacy of malathion against Sitophilus oryzae and Tribolium castaneum and its persistence in stored wheat. Indian J. Entomol. 27:476 (1966).

15. Udeaan, A.S., and Bindra, O.S. Malathion residues in wheat grains in relation to dosage applied, storage period and receptacles used for storage. Bull. Grain Tech. 9:13 (1971).

16. Fitzhugh, O.G. Response of mammalian species to pesticides. In: Research in pesticides, ed. by C.O. Chichester, p.63 Academic Press, New York (1965).

17. Bindra, O.S., and Sidhu, T.S. Dissipation of malathion residues on maize grain in relation to dosage, storage conditions and baking. J. Fd. Sci. Tech. 9:29 (1972).

18. Parkin, E.A. The onset of insecticide resistance among field populations of stored product insects. J. Stored Prod. Res. 1:3 (1965).

19. Speirs, R.D., Redlinger, L.M., and Boles, H.P. Malathion resistance in the red flour beetle. J. Econ. Entomol. 60:1373 (1967).

20. Dyte, C.E., and Blackman, D.G. The spread of insecticide resistance in Tribolium castaneum (Herbst.)(Coleoptera, Tenebrionidae). J. Stored Prod. Res. 6:225 (1970).

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

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

23. Strong, R.G., and Sbur, D.E. Evaluation of insecticides as protectants against pests of stored grain and seeds I. J. Econ. Entomol. 54:235 (1961).

24. Strong, R.G., and Sbur, D.E. Evaluation of insecticides as protectants against pests of stored grain and seeds II. J. Econ. Entomol. 58:18 (1965).

25. Lemon, R.W. Laboratory evaluation of some organophosphorous insecticides against Tribolium confusum (Duv.) and Tribolium madens (Hbst.)(Coleoptera, Tenebrionidae). J. Stored Prod. Res. 1:247 (1966).

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

27. Green, A.A., and Tyler, P.S. A field comparison of malathion, dichlorvos and fenitrothion for the control of Oryzaephilus surinamensis (L.)(Coleoptera, Silvanidae) infesting stored barley. J. Stored Prod. Res. 1:273 (1966).

28. Kane, J., and Green, A.A. The protection of bagged grain from insect infestation using fenitrothion. J. Stored Prod. Res. 4:59 (1968).

29. Tyler, P.S., and Green, A.A. The effectiveness of fenitrothion and malathion as grain protectants under severe practical conditions. J. Stored Prod. Res. 4:119 (1968).

30. Whetstone, R.R., Phillips, D.D., Sun, Y.P., and Shellenberger, T.E. 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate a new insecticide with low toxicity to mammals. J. Agric. Food Chem. 14:352 (1966).

31. Bry, R.E., Lang, J.H., and Dennis, N.M. Gardona as a mothproofer of woolen fabric. J. Econ. Entomol. 62:868 (1969).

32. Bry, R.E., Dennis, M.M., and Land, J.H. Gardona applied to woolen fabric as a mothproofer during drycleaning. J. Econ. Entomol. 64:530 (1971).

33. Young, J.R., and Bowman, M.C. Evaluation of Shell SD-8447 for control of two sweet corn insects. J. Econ. Entomol. 59:170 (1966).

34. Lemon, R.W. Laboratory evaluation of some additional organophosphorous insecticides against stored-product beetles. J. Stored Prod. Res. 3:283 (1967).

35. Lindgren, D.L., Sinclair, W.B., and Vincent, L.E. Residues in raw and processed foods resulting from post-harvest insecticidal treatments. Residue Reviews 21:1 (1968).

36. Liska, B.J., and Stadelman, W.J. Effects of processing on pesticides in foods. Residue Reviews 29:61 (1969).

37. Seibel, W., and Hertel, W. Schädlingsbekämpfung und Müllereitechnik. Die Muhle 42:731 (1969).

38. Acton, F.E., and Parouchais, C. Malathion levels in wheat and wheat products. Fd. Technol. Aust. 18:77 (1966).

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

40. McGaughey, W.H. Malathion residues on rice. Rice J. 72(8):3 (1969).

41. McGaughey, W.H. Malathion on milling fractions of three varieties of rough rice: Duration of protection and residue degradation. J. Econ. Entomol. 64:1200 (1971).

42. Allessandrini, M.E. Determination of the persistence and fate of various insecticides in or on wheat during storage, milling and during baking or cooking of the products made from the treated wheat. Final Technical Report, U.S.D.A. Project No. E15-AMS-8(a) (1965).

43. Godavari Bai, S., Krishnamurthy, K., and Majumder, S.K. Studies on malathion for stored grain product insect control. Pest Technol. 2(7):12 (1960).

44. Crosby, D.G. The intentional removal of pesticide residues. In:Research in esticides, ed. by C.O. Chichester, p.213, Academic Press, New York (1965).

45. Farrow, R.P., Lamb, F.C., Cook, R.W., Kimball, J.R., and Elkins, E.R. Removal of DDT, malathion and carbaryl from tomatoes by commercial and home preparative methods. J. Agr. Food Chem. 16:65 (1968).

46. Koivistoinen, P., Kononen, M., Karinpaa, A., and Roine, P. Stability of malathion residues in food processing and storage. J. Agr. Food Chem 12:557 (1964).

47. Godavari Bai, S. Persistence of malathion residues on treated foodgrains under different conditions of storage and processing. In: Pesticides, ed. by S.K. Majumder, Academy of Pest Control Sciences (India) (1968).

48. Pixton, S.W. Moisture content - its significance and measurement in stored products. J. Stored Prod. Res. 3:35 (1967).

49. Mackay, P.J. The measurement of moisture content. Trop. Stored Prod. Inf. No.14:21 (1967).

50. Cotton, R.T. Pests of Stored Grain and Grain Products. Burgess Publishing Company, Minneapolis (1963).

51. Kadoum, A.M., and La Hue, D.W. Effect of hybrid, moisture content, foreign material, and storage temperature on the degradation of malathion residue in grain sorghum. J. Econ. Entomol. 62:1161 (1969).

52. Crisp, S., and Tarrant, K.R. The determination of residues of dichlorvos and malathion in wheat grain by gas-liquid chromatography. Analyst 96:310 (1971).

53. Rowlands, D.G. The degradation of malathion on stored maize and wheat grains. J. Sci. Fd. Agric. 15:824 (1964).

ORGANOPHOSPHATE PESTICIDES FOR USE AS GRAIN PROTECTANTS IN INDIA: DEGRADATION OF THEIR RESIDUES DURING MILLING AND COOKING OF CEREAL GRAINS

by

LEONARD MERRICK LOCKWOOD

B.A., Oberlin College, 1963

M.A., Kansas University, 1969

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the

requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry

Kansas State University

Manhattan, Kansas

1973

Samples of paddy (rough rice), wheat and sorghum were treated with different concentrations of malathion, gardona and sumithion as protection against insect infestation during storage.

Bioassay tests, using Sitophilus oryzae (rice weevil) and Tribolium castaneum (red flour beetle), were conducted to determine the effectiveness of the treatments in protecting the grain against insect attack. Results of the bioassay tests indicated that the lowest levels of treatments used (10 ppm of malathion or gardona; 2 ppm of sumithion) were sufficient to eliminate any existing infestation of these insects. In the case of malathion and gardona, protection was afforded for 6 to 8 weeks. For sumithion to provide equivalent protection it would be necessary to apply concentrations higher than 2 ppm. The intermediate levels of treatment (40 ppm for malathion or gardona; 8 ppm for sumithion) and the high levels of treatment (80 ppm of malathion or gardona, 16 ppm of sumithion) protected the grain over the full test period of two months.

The amount of residues in the treated grain were analyzed by means of gas-liquid chromatography before and after milling. There was a significant loss of residues from wheat and sorghum during machine milling. On the average, machine milling resulted in 30% to 70% loss in residues for all three protectants. Less heating of the flour took place when sorghum was milled, as compared to wheat. This might explain the observation that less residue was lost during machine grinding of sorghum as compared to machine grinding of wheat. Wheat ground by hand showed up to 40% less breakdown of insecticides as compared to machine grinding. This also may be the result of less heating during hand grinding.

With paddy there is a tendency for residues to be concentrated in the hull and bran fractions. As a result, polished rice retained relatively little residue from protectants applied to the whole grain. Even with treatments of paddy at 80 ppm of malathion, residues on the polished rice were never above 4 ppm.

Samples of milled products were cooked by traditional Indian methods. Rice was boiled and iddlies were prepared by grinding rice with water, fermenting the paste and steaming it. Wheat and sorghum flour were made into chapaties. In the case of sorghum a second preparation was made in which balls of dough were boiled in water. In chapati preparation the amount of residue breakdown was closely related to the amount of cooking. Average losses during the preparation of chapaties ranged from 43% to 75% though individual samples varied over a wider range. Preparations involving boiling or steaming resulted in complete degradation of the residues of all three insecticides, so far as detectable with the methods used (less than 0.1 ppm).

Most of India's grain harvest is retained at the farm and village level. Any program aimed at reducing waste due to insect damage during storage must provide methods which can be used for small scale storage. Unfortunately, the most effective methods involving fumigation are not easily applicable at this level and the health regulations of the Indian Government prohibit the admixture of any insecticide with grain in storage. Recomendations have been made to amend the existing regulations to allow the use of malathion as a grain protectant to be admixed with stored grain up to a limit of 8 ppm. The results of this investigation indicate that the breakdown of residues during milling and during cooking by

typical Indian methods are comparable to the breakdown observed in the cooking and milling processes studied in the United States and Europe. It would appear that there is considerable justification for the recomendation to allow the use of malathion as a protectant for stored grain in India. From this investigation it would seem advisable to consider other alternatives in addition to malathion, such as gardona and sumithion. These protectants would be useful in cases where insects begin to develop resistance to the toxicity of malathion.