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Residues of Pesticides in Grains Locally Grown in Saudi Arabia

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In Saudi Arabia, the rapid development of agriculture necessitated the use of a wide number of pesticides on crops. Soliman studied the pattern of pesticides used in Saudi Arabia (personal communication). He estimated that the total value of pesticides imported to Saudi Arabia in 1976 was 46 million Saudi Riyals (US $ 12,267,666), which had increased to 331,400 million Saudi Riyals (US $ 8,837,333) in 1990. The estimated number of tons used had increased from 4999 tons in 1976 to more than 20 000 tons of pesticides in 1990. From the same report, the total agricultural land area increased from half a million hectare in 1976 to a million hectare between the eighties and nineties. For example, the total production of wheat grown locally rose from 350 thousand tons in 1982 to about 4 million tons in 1991. In general, wheat grain is of great preference among Saudis. Apart from the studies by Kadous (1984) and Barakat *et al.* (1985), there have been no published reports from Saudi Arabia addressing the nature and extent of pesticide use.

The objective of this work is to analyze pesticide residues in wheat grain samples locally grown in different parts of Saudi Arabia. The technique presented in this paper involves the use of Gas chromatography/Mass Spectrometry (GC/MS) as a primary screening tool for multiresidual pesticides in Saudi wheat. The purpose of this study is to obtain basic information about what pesticide residues were present on domestically grown wheat offered for sale, with special attention to those pesticide residues applied such as dimethoate, malathion and triademefon which have been widely used for the protection against insects feeding on wheat in Saudi Arabia. Organophosphorus insecticides such as dimethoate and malathion are widely used in the Kingdom. These compounds inhibit acetylcholinesterase, an enzyme essential for the normal functioning of both the central and nervous systems (Lopez-Carillo and Lopez-Cervantes 1993). On the other hand, triadimefon is a systematic fungicide used to control powdery mildews, rusts, and other fungal pests that feed on cereals, fruits, vegetables, turf, shrubs, and trees. Animal studies have indicated that triadimefon produces a neurotoxic syndrome characterized by increased motor activity, stereotyped behaviour, and altered monamine metabolism (Walker and Mailman 1996; Crofton 1996; lkaiddi *et al.* 1997).

MATERIALS AND METHODS

Fifty samples of wheat grains were collected from seven areas in Saudi Arabia through the Saudi Grain Silos Flour Mills Organization for investigation.

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For extraction and clean-up, we used a modified method described by Hong *et al.* (1993) which seemed to be suitable for the analysis of various pesticides in fatty crops such as wheat. A composite sample of 25 g of wheat was ground and extracted with a mixed solvent of 100 ml acetone and 50 ml of methanol in a blender jar for 10 minutes at medium speed. Triphenyl phosphate (0.025 μg/g) and d-fluorene (0.05 μg/g) were added to each wheat sample, working standards and fortified samples as an internal standard. The extract was filtered through 12 cm Buchner funnel fitted with shark skin filter paper and into a 500 ml flask. After reducing the volume of the extract to 100 ml using vacuum rotary evaporator with water bath (37°C), 30 ml saturated sodium chloride solution, 50 ml water and 100 ml methylene chloride were added. The funnel was shaken vigorously for 30 sec. The organic layer was collected into a 500 ml round-bottom flask. The aqueous layer were re-extracted with an additional 50 ml methylene chloride and combined with the organic layer. The combined extracts were evaporated to dryness using vacuum rotary evaporator with water bath (37°C). The residue was dissolved with 5 ml 1 :l (methylene chloride: cyclohexane) and cleaned up using the Bio-Bead S-X3 procedure to remove the interferences from the fatty matrix components of wheat. A 30 g of 200-400 mesh Bio-Beads S-X3 (Bio-Rad Laboratories, Richmond, CA, USA) was soaked overnight in 200 ml I:1 methylene chloride:cyclohexane. The slurry of Bio-beads was used to fill a 1 cm glass column equipped with a Teflon stopcock. The column was partially filled with methylene chloride:cyclohexane, the bio-beads added, and allowed to settle. The column was topped with methylene chloride:cyclohexane. The sample extract was added and the column eluted with 20 ml of methylene chloride: cyclohexane (1 :I). The eluate was collected in two fractions: the first fraction (11 mi) containing lipids was discarded, whereas the second fraction (12 ml) was collected and evaporated to dryness under a nitrogen stream. The dried residue was dissolved with 2 ml hexane. All reagents and chemicals used were of pesticide grade. Concentrations were expressed per weight as μg/g.

Analyses of dimethoate, malathion and triadimefon in wheat samples were done using Hewlett Packard gas chromatograph Model 5890 series II with automatic injector and a 7673 autosampler, coupled with a Hewlett Packard quadrupole mass spectrometer (detector Model 5972 series MSD was used). This system was controlled by a Hewlett Packard Vectra 466/33N and a Hewlett Packard MS Chemstation software. A cross-linked methyl silicone HP5-MS capillary column (30 m x 0.25mm id. x 0.25 μm thickness) was used with a column head pressure of 11 psi in splitless injection mode. Ultra high purity (99.9999%) helium was used as a carrier gas. The GC temperature program was as follows: initial oven temperature was 80°C held for 1.2 min, increased by 30°C/min to 170°C, and held for 4 min. Then, heated to 225°C at 2.5°C/min, held for 2 min., the temperature increased to 275°C/min at 10" C/min for 5 minutes. Injector temperature was 250°C and injection volume was 1 μl. The Mass spectrometer was operated at 70 eV in the electron impact (El) ionization mode. All samples were injected in duplicate, allowing analysis in both scan and selected ion monitoring (SIM) data acquisition modes.

Ion source temperature was 195°C. Scan mass range was 50-450 amu at 1.8 scan/sec. The characteristic ions for d-fluorene, dimethoate, malathion, triadimefon and triphenyl phosphate using SIM mode are shown in Table 1.

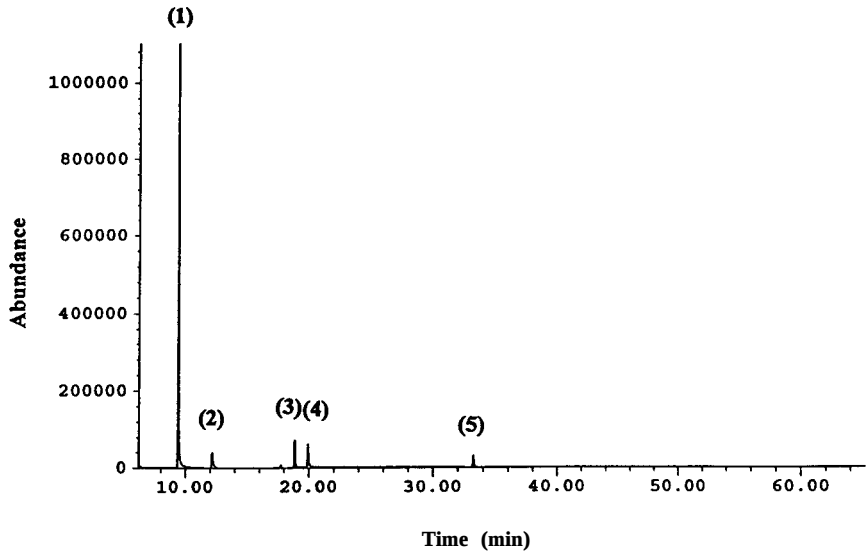

Figure 1. GC chromatogram of working standard mixture of the tested pesticides and internal standards: (1) dimethoate (1.5 μg); (2) malathion (1.5 μg); d-fluorene (1.25 μg); (4) triadimefon (0.5 μg); (5) triphenyl phosphate (0.625 μg).

Table 1. Selection of characteristic ions for d-fluorene, dimethoate, malathion, triadimefon and triphenyl phosphate using SIM mode.

Retention time (min)	Relative retention time (min)*	Characteristic ions	Pesticides	Dwell time (milliseconds)
10.519	1.0	166, 165, 139	d-Fluorene**	100
13.53	1.286 0.388	87, 125, 229	Dimethoate	100
20.61	1.959 0.591	173, 125, 127	Malathion	100
21.709	2.064 0.623	208, 181, 128	Triademfon	100
34.854	1.0	326, 215, 169	Triphenyl** phosphate	100

* Relative retention time: ratio of pesticide retention time to the retention time of the internal standard.
** Internal standard.

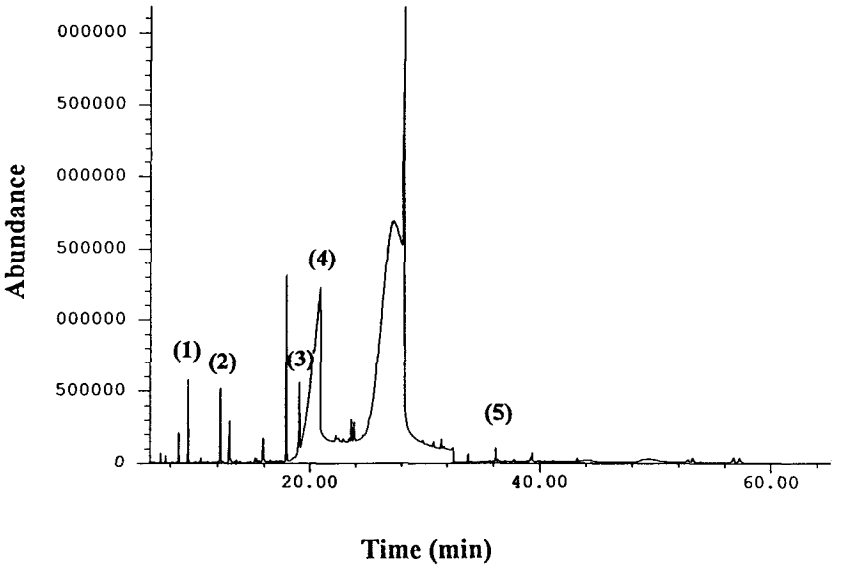

Figure 2. Total ion chromatogram of extracted wheat sample fortified with the tested pesticides and internal standards: (1) dimethoate (0.08 μs/g); (2) malathion (0.08 μg/g); (3) d-fluorene (0.05 μg/g); (4) triadimefon (0.032 μg/g); (5) triphenyl phosphate (0.025 μg/g).

Table 2. Concentration of tested pesticides (μg) in working standards

Tested pesticides	Level 1	Level 2	Level 3
Dimethoate	1.5	2.5	5.0
Malathion	1.5	2.5	5.0
Triademfon	0.5	1.25	5.0
d-Fluorene*	1.25	1.25	1.25
Triphenyl* phosphate	0.625	0.625	0.625

* Internal standard.

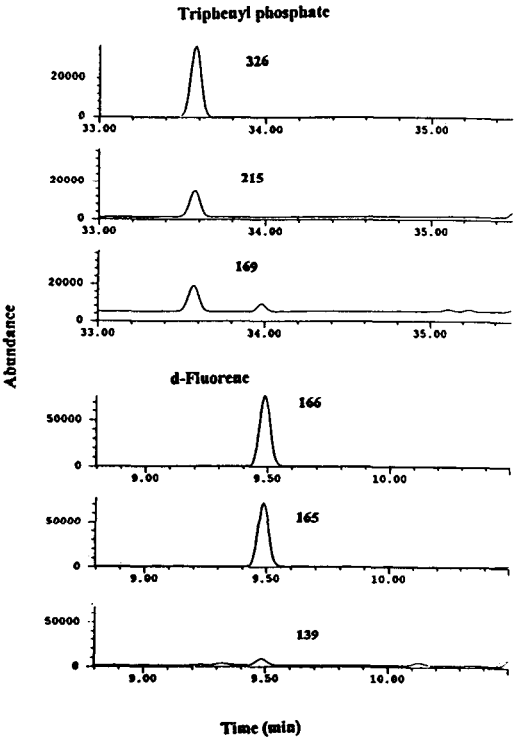

Figure 3. Example of extracted ion chromatogram of the most characteristic ions of the internal standards d-fluorene and triphenyl phosphate in wheat sample.

Table 3. Fortified sample % recoveries

Level (µg/g)	Recovery %
Dimethoate	
0.08	80
0.12	72
0.16	90
Malathion	
0.08	99.6
0.12	97.6
0.16	96.4
Triademfon	
0.032	106.7
0.10	80.4
0.16	84.5

n = 4

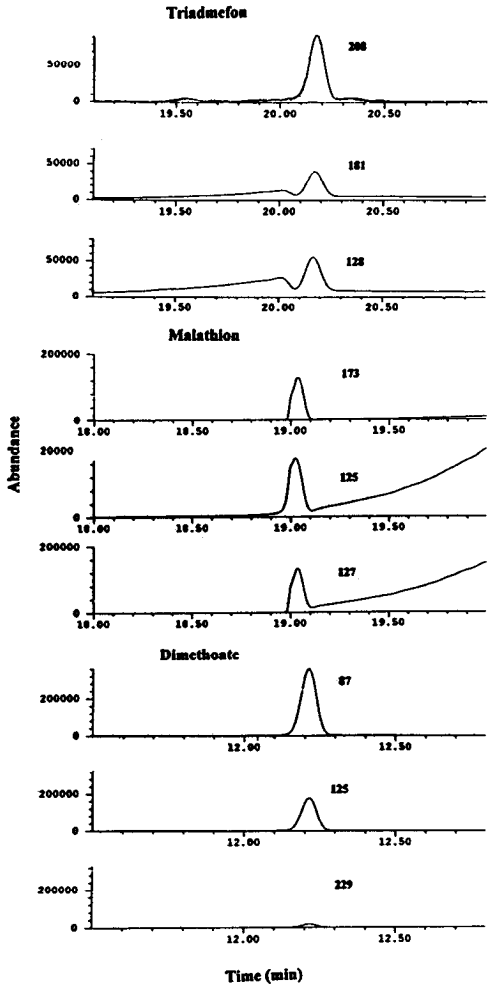

Figure 4. Extracted ion chromatograms of tested pesticides and internal standards: (1) dimethoate (0.12 μg/g); (2) malathion (0.12 μg/g); (3) d-fluorene (0.05 μg/g); (4) triadimefon (0.1 μg/g); (5) triphenyl phosphate (0.025 μg/g).

The determinations of dimethoate, malathion and triadimefon in wheat samples were achieved by GC/MS using SIM. Three-point quantitative calibration was made for each of tested pesticides as listed in Table 2. Chromatogram of a mixture of the reference solutions is shown in Figure 1.

The efficiency of the method was evaluated by fortifying wheat samples with dimethoate, malathion and triadimefon at various concentration levels. The recovery of these targeted pesticides is listed in Table 3. Figure 2 presents an example of a total ion chromatogram of fortified wheat samples with dimethoate, malathion and triadimefon.

RESULTS AND DISCUSSION

Pesticide residues in the sample extracts were identified by matching their retention time and characteristic mass spectra with those of the standards, An extracted ion chromatogram of the most characteristic ions of the internal standards d-fluorene and triphenyl phosphate) and tested pesticide residues dimethoate, malathion and triademfon in extracted wheat sample is shown in Figures 3 and 4 respectively. Table 4 shows the concentrations of the tested pesticide residues in extracted wheat samples and are compared with the MRL -Maximum Residue Limit (FAO/WHO 1989; Codex Alimentarius Commission 1996).

Table 4. Means ± SD (μg/g), ranges, and number of positive wheat samples with the tested pesticide residue in wheat.

Residue	No. of positives	Mean ± SD	Range	MRL* (mg/kg)
Dimethoate	20/50	0.00215 ± 0.00629	0-0.0224	0.05
Malathion	9/50	0.0064 ± 0.00635	0-0.016	8.0
Triadimefon	25/50	0.00579 ± 0.01604	0-0.072	0.5

*Maximum residue limit.

Triadimefon was found in 50% of the tested wheat samples, while dimethoate and malathion were found in 40% and 18% of the samples respectively. All residue values were well below the MRLs. If we assume that Saudis can consume 1 Kg of wheat per day, this would expose them to 2.15, 6.4 and 5.79 μg/kg per day of dimethoate, malathion and triadimefon, respectively. When the residues of all tested pesticides are added together, it gives a combined mean residue of 0.004545 ± 0.01184 μg/g in the range of 0 to 0.072 μg/g. Regional differences in the single and combined mean residue of dimethoate, malathion and triadimefon in wheat samples are indicated in Table 5. The total residue in wheat samples from Riyadh and Qassim regions had higher combined pesticide residues than other regions. However, none had residues above the MRLs.

Table 5. Average (μg/g) of tested pesticide residues in wheat samples collected from different locations in Saudi Arabia.

Residue	Riyadh (n=5)	Wadi Douasir (n=8)	Hail (n=6)	Tabouk (n=2)	Qassim (n=13)	Jouf (n=2)	Eastern (n=14)
Dimethoate	0.0184	0.00821	ND	ND	ND	0.0044	ND
Malathion	0.0052	0.008	ND	ND	0.4	ND	0.0068
Triadimefon	0.0048	0.0018	0.0003	ND	0.0224	0.004	0.0003
Total	0.0284	0.01801	0.0003	0	0.0228	0.0084	0.0070

*ND: not detected.

Detectable amounts of other pesticides such as aminocarb, bendiocarb, butoxycarboxim, Chlorflurecol-methyl, desmethyl norflurazon and simazine were also found in the tested wheat samples. Table 6 summarizes the retention time and characteristics of the mass spectra. The use of these pesticides is legal in Saudi Arabia. Animal studies have indicated strong association between the presence of these pesticide residues and their cytogenic, immune and estrogenic effects (Bernier *et al.* 1995; Balaguer *et al.* 1996; Conner *et al.* 1996; Klotz *et al.* 1997). Pest resistant plants readily metabolize pesticides to produce possibly mutagenic byproducts (Plewa

1978). Atrazine-desethyl-desisopropyl and atrazine-desethyl-desisopropyl-2-hydroxy are the most common degradation products of simazine. Our extraction procedure was not appropriate to detect these metabolites in our screened wheat samples because of their polar nature. Herbicide degradates can have similar acute and chronic toxicity as their parent compounds (Kolpin et al 1998). Such highly toxic pesticides should receive special attention and further investigation in future monitoring programs to determine the actual level, source, and the extent of contamination.

Table 6. Pesticide residues in wheat samples as identified by GC/MS using scan mode.

Residue	Retention time (min.)	Characteristic ions	Frequency of positive samples (%)	Pesticide Class
Aminocarb	50.33	150, 150, 136	92	Methlycarbamate insecticide
Bendiocarb	46.72	151, 166, 126	26	Methylcarbamate insecticide
Butoxycarboxim	43.94	85, 55, 81	8	Carbamoyloxime Insecticide
Chlorflurecol-methyl	57.17	215, 152, 474	14	Herbicide
Desmethyl norflurazon	52.83	145, 289, 173	28	triazine herbicide
Simazine	35.64	201, 186, 158	40	1,3,5-triazine herbicide

The results from this investigation clearly indicate that low levels of various pesticide residues are present in some wheat samples locally grown in Saudi Arabia.

It is reassuring that the reported pesticide residue in the tested wheat samples are low. Concerns have been raised about the effects of low levels of pesticides on the nervous, immune and reproductive systems, as well as bone marrow chromosomes (Marinovich et *al.* 1994; Nehez *et al.* 1994; Nagymajtenyi *et al.* 1994; lnstitoris *et al,* 1995; lkaiddi *et al.* 1997). Therefore, it is crucial to rule out the cumulative effects of chronic toxicity especially for susceptible groups of the population such as children and women.

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