et al., 1978; Villeneuve et al., 1979). Since mirex is known to photolyze to photomirex in the environment (Carlson et al., 1976; Ivie et al., 1974), it is possible that photomirex levels may be increasing in the environment. However, no data are currently available to dismiss or substantiate this hypothesis. Furthermore, the chemical pathway and site of conversion of mirex into photomirex into the lake ecosystem is poorly understood. Trophic level studies of mirex and photomirex in the Lake Ontario ecosystem could provide valuable additional insight into organochlorine pesticide kinetics.

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Potential Interference by Free Fatty Acids in the Gas–Liquid Chromatographic Analysis of Rice Bran for Pesticide Residues Using Electron Capture, Potassium Chloride Thermionic, and Hall Electrolytic Conductivity Detectors

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Free fatty acids were found to be potential interferences in the gas–liquid chromatographic (GLC) determinative step when rice bran was analyzed for pesticide residues using the multiresidue analytical procedure for fatty food in the Food and Drug Administration Pesticide Analytical Manual. Oil was extracted from the rice bran and cleaned up by petroleum ether–acetonitrile partitioning and Florisil column chromatography. The residues were determined by GLC. Chromatographic peaks encountered with electron capture, potassium chloride thermionic, and Hall electrolytic conductivity detectors were identified as palmitic acid, oleic acid, and linoleic acid. The oil extracted from the rice bran contained an unusually high quantity of free fatty acids, 54.4% as oleic acid. Cleanup of rice bran oil using petroleum ether–acetonitrile partitioning and Florisil column chromatography failed to separate the fatty acids from possible pesticide residues. The GLC response factor for several free fatty acids and their methyl esters was determined using electron capture, potassium chloride thermionic, and Hall electrolytic conductivity detectors.

The development of sensitive, selective detectors promoted the application of gas–liquid chromatography (GLC) to the determination of pesticide residues. The electron capture detector (ECD) (Lovelock, 1957) has been widely used for the measurement of trace amounts of organochlorine pesticide residues in complex environmental samples. The potassium chloride thermionic detector (KCl-TD) was introduced as a sensitive and selective detector for the determination of organophosphorus pesticide residues by Giuffrida (1964). The Hall electrolytic conductivity detector (HECD) is highly selective for either chlorine, bromine, iodine, sulfur, or nitrogen, depending upon the detector's mode of operation (Hall, 1976).

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These three GLC detectors make possible the quantitative determination of residues of pesticides and industrial chemicals present at trace levels in complex organic chemical matrices. In spite of the great advantages offered by the relative selectivity of these detectors for trace analysis, naturally occurring constituents extracted from food products are known to cause GLC responses that interfere with the determination of pesticidal and industrial chemical residues. Accordingly, carefully controlled cleanup of food samples is usually required before GLC to eliminate interferences and to avoid column deterioration and/or detector contamination. For example, the extracts of vegetables such as carrots and onions contain naturally occurring components which cause a response by the ECD used for pesticide determination after cleanup (Pesticide Analytical Manual (PAM I), Sec. 311. 01f, 1968). If these components are not adequately removed from the food sample extract, they will interfere with the determination of residues of pesticides or industrial chemicals with similar GLC retention times. The literature contains only a few references in which the nature of the naturally occurring interfering compound or substance has been determined. For example, sulfur, if present in environmental samples, is known to interfere in the determination of aldrin by ECD–GLC (Campbell and Sule, 1966). Treatment of the sample extract with tetrabutylammonium sulfite will eliminate the sulfur (Jensen et al., 1977).

During the analysis of rice bran for pesticide residues by a procedure described in PAM I (Sec. 211, 1971), several relatively large and asymmetric chromatographic responses were observed. The relative retention times of these responses did not correspond to any pesticide or industrial chemical known to be recovered through the method. The responses might indicate the presence of potentially toxic residues or sample matrices. Analysis of rice bran oil using several other selective GLC detectors indicated that the response might be due to free fatty acids. When the cleaned-up sample eluates were methylated with diazomethane and rechromatographed, the unknown responses were subsequently identified as fatty acid methyl esters. This paper describes the identification and GLC response factors of fatty acids not previously known to cause responses by the detectors normally used in pesticide residue analysis.

EXPERIMENTAL SECTION

Sample. Rice bran was obtained from a commercial source in New Orleans, LA.

Extraction and Cleanup. Oil was extracted from the rice bran using the fat extraction for cheese (PAM I, Sec. 211.13c, 1971; Association of Official Analytical Chemists (AOAC), Sec. 29.012(d), 1975) with two changes. First, sodium or potassium oxalate was not added. Second, because the sample was so low in water content, 60 mL of water was added to obtain two layers before partitioning the ethanol layer with ethyl ether. The extracted oil was cleaned up with petroleum ether–acetonitrile partitioning (PAM I, Sec. 211.14a, 1971; AOAC, Sec. 29.014, 1975) and Florisil column chromatography (PAM I, Sec. 211.14d, 1971; AOAC, Sec. 29.015, 1975).

Methylation. An ethereal alcoholic solution of diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (DeBoer and Backer, 1963). Methylation was performed by adding the diazomethane solution to a cleaned-up sample eluate until the solution remained pale yellow for a few minutes.

Free Fatty Acids. The free fatty acid content of the rice bran oil was determined by titration of the oil in ethanol with 0.25 M NaOH (AOAC, Sec. 28.029, 1975).

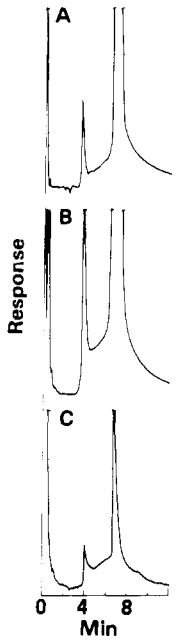

Figure 1. ^{3}H ECD gas chromatogram with 10% OV-101 column: (A) 6%, (B) 15%, and (C) 50% ethyl ether in petroleum ether eluate, each equilvalent to 0.6 mg of oil injected. RRT/A of *p,p'*-DDT was 3.03, and the response of 1 ng of heptachlor epoxide was 50% recorder deflection.

Gas Chromatography. Columns and conditions described in PAM I were used for ^{3}H ECD, KCl-TD, flame photometric (FPD) (phosphorus mode), and HECD (chlorine mode) (PAM I, Table 331A and Secs. 311.2, 331B, 313.5, and 315.5, 1975). On KCl-TD GLC, the retention time relative to parathion (RRT/P) was corrected to the retention time relative to aldrin (RRT/A) by using the following formula: RRT/A = (RRT/P)/1.04. Conditions employed for ^{63}Ni ECD–GLC analyses were as follows: 6 ft × 4 mm i.d. glass column packed with 5% OV-101 on 80–100 mesh Chromosorb W-HP; nitrogen carrier gas at 50 mL/min; and temperatures, injector 200 °C, column 200 °C, and detector 300 °C. Under the specified conditions, the RRT/A of *p,p'*-DDT was 3.04, and the response for 1 ng of heptachlor epoxide was 42% full-scale recorder deflection.

Conditions employed for flame ionization detector (FID) GLC analyses were as follows: 4 ft × 2 mm i.d. glass column packed with 2% OV-101 on 80–100 mesh Chromosorb W-HP and/or 12 ft × 2 mm i.d. glass column packed with 3% OV-225 on 80–100 mesh Chromosorb W-HP; helium carrier gas 30 mL/min, hydrogen 30 mL/min, air 240 mL/min; and temperatures injector 250 °C, column 175 °C, and detector 300 °C.

Gas Chromatography–Mass Spectrometry (GC/MS). Initial 70-eV electron impact mass spectra were obtained with a Hewlett-Packard 5992 instrument. The ion source temperature was 250 °C. The 3% OV-225 GLC column described above was used.

RESULTS AND DISCUSSION

The Florisil column eluates (6, 15, and 50% ethyl ether in petroleum ether) of a rice bran sample were analyzed by gas chromatographs equipped with ^{3}H ECD (Figure 1) and KCl-TD (Figure 2). On ^{3}H ECD every eluate had responses at RRT/A 0.97 and 1.68. Similar responses were obtained with KCl-TD, although the RRT/A values of the peaks were a little longer, 1.03 and 1.77, respectively. In addition, the 50% eluate exhibited another response at RRT/A 0.90 when analyzed by KCl-TD and FPD. This

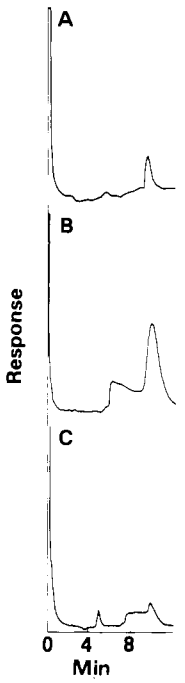

Figure 2. KCl-TD gas chromatogram with 10% OV-101 column: (A) 6% ethyl ether in petroleum ether eluate, equivalent to 0.6 mg of oil injected, (B) 15%, and (C) 50% ethyl ether in petroleum ether eluate, each equilvalent to 0.9 mg of oil injected. RRT/A of ethion was 2.46, and the response of 2 ng of parathion was 50% recorder deflection.

peak was identified as malathion (0.15 ppm). Due to the low levels present, it was not observed on the ^{3}H ECD. No correlation between the RRT/A values of the other responses to any of the chemicals listed in the PAM I Appendix (1978) of chromatographic retention data could be made. Methylation of the 15% ethyl ether in petroleum ether Florisil eluate was attempted since peak tailing suggested the existence of polar functional groups. Methylation eliminated peak tailing, and the chromatograms were considerably improved. The results of the peak changes were essentially the same with every Florisil eluate when it was methylated. On the ECD the responses at RRT/A 0.97 and 1.68 disappeared after methylation, and responses at RRT/A 0.74 and 0.85 were observed (Figure 3). These responses were negligible on other detectors (KCl-TD and FID), and they were shown to be caused by substances other than the methylated fatty acids identified below. With the KCl-TD the responses previously observed at RRT/A 1.03 and 1.77 were no longer present, but two different responses at RRT/A 0.87 and 1.58 were observed (Figure 3). Examination of the methylated 15% eluate by FID-GLC revealed two significant responses on the 2% OV-101 column (Figure 4). These responses (Figure 4B) correspond favorably with the responses observed using a 10% OV-101 column with KCl-TD (Figure 3D). These were resolved into three separate responses on the 3% OV-225 column (Figure 5). The peaks present in all three methylated Florisil eluates were identified by GC/MS as methyl palmitate, methyl oleate, and methyl linoleate and confirmed by comparison with standards. These results suggested that free fatty acids might give significant responses when they are injected into a gas chromatograph equipped with ECD or KCl-TD.

Analytical standards of several fatty acids and their methyl esters were examined under the same GLC conditions as the rice bran oil. The results of the responses of ^{3}H ECD and KCl-TD are shown in Tables I and II. The response of ^{63}Ni ECD to these compounds is given in Table III. When a sufficient quantity of fatty acids was injected, a positive response was observed on both ECD (^{3}H and ^{63}Ni) and KCl-TD. Several minor negative responses occurred on the ECD when microgram quantities of fatty acid methyl esters were examined. The negative responses could not be unequivocally attributed to these compounds since several responses occurred upon the injection of a single compound. Injection of microgram quantities of fatty acid methyl esters caused a positive response on KCl-TD.

Table I. Responses of Fatty Acids on ^{3}H ECD with 10% OV-101 and Mixed Column

GC column	compd	amt injected, μg	RRT/A	response, % (variable)
10% OV-101	palmitic acid	10	0.91	60
	oleic acid	10	1.69	72
	linoleic acid	10	1.64	52
10% OV-101 +	stearic acid	100	1.74	90
15% OV-210	lauric acid	20	0.23	47
	palmitic acid	100	0.98	80
	oleic acid	20	1.68	42
	linoleic acid	20	1.67	43
	linolenic acid	1	1.69	30
	myristic acid	20	0.45	41

Table II. Response of Fatty Acids and Their Methyl Esters on KCl-TD with 10% OV-101 Column

compd	amt injected, μg	RRT/A	response, %
palmitic acid	20	0.99	24
methyl palmitate	4	0.84	18
oleic acid	20	1.77	20
methyl oleate	4	1.53	8
linoleic acid	20	1.79	21
methyl linoleate	4	1.49	9

Table III. Response of Fatty Acids on ^{63}Ni ECD with 5% OV-101 Column

compd	amt injected, μg	RRT/A	response, % (variable)
palmitic acid	4	0.99	21
oleic acid	4	1.77	14
linoleic acid	4	1.77	7

Gaul (1978) reported that the unidentified responses were also observed during analysis of this rice bran using an HECD in the halogen mode. When a sufficient quantity of fatty acid was examined by HECD–GLC, a response was observed (Figure 6). The response on the HECD may be due to the formation of a large quantitiy of oxygen containing gases or the incomplete pyrolysis of the fatty acids, and this could result in the formation of species which cause a response.

Difficulties were observed in the chromatography of these acids. The GLC columns used in this work are not suitable for the chromatography of fatty acids. Contamination of the detectors occurred despite the cleanup because of the large quantity of material injected into the chromatograph. Considering the unsuitability of the columns and the detector contamination, it was not surprising to observe irreproducibility in peak sizes (heights or areas) with identical injections, especially on ECD. Changes in response to the subsequent injection of 1 ng of heptachlor epoxide into the same chromatograph were observed within 1 day as well as from day to day.

In a review of the capabilities of the ECD, Rowland and Burgett (1975) reported the response factors of various

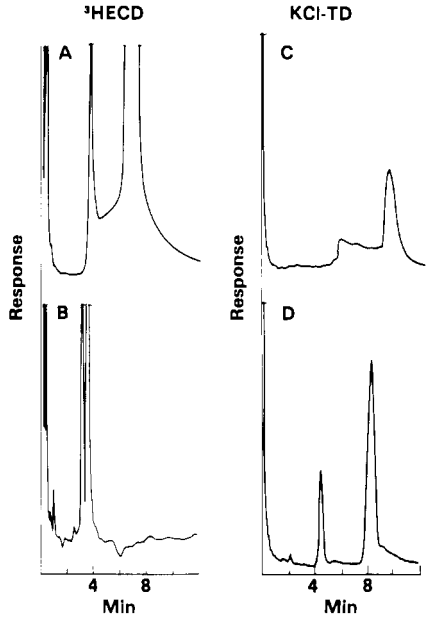

Figure 3. ^3H ECD and KCl-TD gas chromatograms with 10% OV-101 column. A and C are before methylation and B and D are after methylation, each equivalent to 0.6 mg of oil injected.

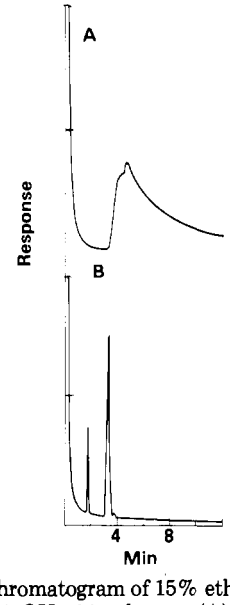

Figure 4. FID gas chromatogram of 15% ethyl ether in petroleum ether eluate with 2% OV-101 column: (A) before methylation, equivalent to 30 μg of oil injected and (B) after methylation, equivalent to 0.75 μg of oil injected.

organic functional groups. Alkyl carboxylic acids were not included in that report. However, in this work we determined that several grams of a free fatty acid produces a significant ECD response and, compared to the reference compound, benzene, has an approximate response factor of slightly less than 1.

The free fatty acid content of the sample was investigated to confirm their GLC interferences. The amount of free fatty acids in the oil extracted from most foods is usually small, but the oil extracted from rice bran contained a large quantity of these acids. The total amount of free fatty acids in the rice oil was determined by titration to be 54.4% as oleic acid. When a 3-g rice bran oil sample was analyzed, approximately 1.2 g of residual oil was placed on the Florisil cleanup column, following the acetonitrile partitioning steps outlined in the PAM method. The lauric acid value (Mills, 1968) of the Florisil used is 96.8. Consequently, 1 g of Florisil can adsorb 96.8 mg of lauric acid

Table IV. Palmitic Acid, Oleic Acid, and Linoleic Acid (mg)[a] Present in Florisil Eluates after Petroleum Ether–Acetonitrile Partitioning[b]

Florisil eluate, %	palmitic acid	oleic acid	linoleic acid	total
6	47	210	145	402
15	45	137	129	311
50	18	35	40	93
total, mg	110	382	314	806

[a] Calculated as free acids from methyl esters determined by FID-GLC with 3% OV-225 column. [b] Weight of cleaned-up rice oil was 3 g.

Table V. Response of Several Pesticides and Industrial Chemicals Eluting at or Near Free Fatty Acids

	RRT/A	
compd[a]	10% OV-101 column	10% OV-101 + 15% OV-210 column
pentachlorophenyl methyl sulfide	0.90	0.93
γ-chlordene	0.94	1.04
Aroclor 1248[b]	0.96	1.02
Aroclor 1242[b]	0.96	1.02
β-chlordene	0.96	1.05
Aroclor 1254[b]	0.98	1.02
aldrin	1.00	1.00

[a] Although some of these compounds may not be registered for use on rice, the possibility of misuse or of occurrence of these residues in other commodities having moderate amounts of fatty acids is not unreasonable. [b] Multiple responses.

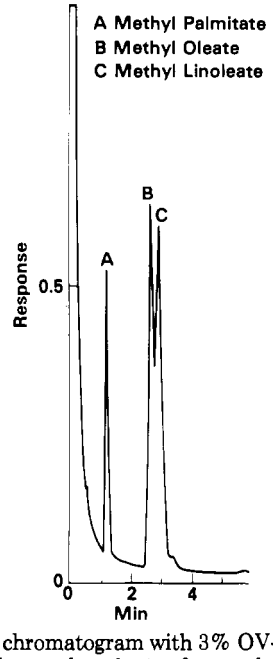

Figure 5. FID gas chromatogram with 3% OV-225 column; 15% ethyl ether in petroleum ether eluate after methylation, equivalent to 3 μg of oil injected.

from a hexane solution. The quantity of Florisil used (19 g) should have been sufficient to remove 1.84 g of fatty acids from hexane, more than the quantity of residual oil placed on the column. However, the Florisil column was not eluted with hexane but with 6, 15, and 50% ethyl ether in petroleum ether, solvent systems more polar than

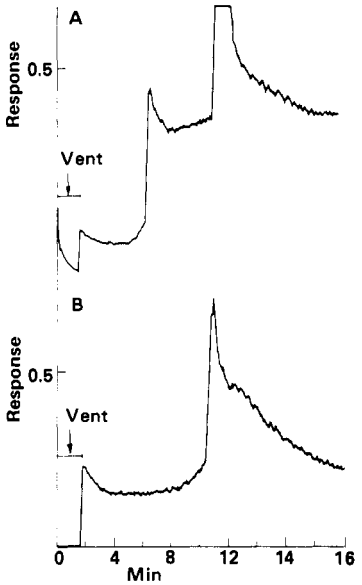

Figure 6. HECD gas chromatogram with 10% OV-101 column: (A) 15% ethyl ether in petroleum ether eluate, equivalent to 0.3 mg of oil injected and (B) 20 μg of linoleic acid.

hexane. Thus, the column would retain less than 1.84 g of fatty acids. The actual weights of fatty acids found in each Florisil eluate are listed in Table IV. Petroleum ether–acetonitrile partitioning and Florisil column chromatography should not be considered effective for cleanup of samples containing high quantities of free fatty acids. Under certain storage conditions, the oil in rice bran is subject to rapid hydrolysis, which increases the free fatty acid content of oil (Loeb et al., 1949). Since palmitic, oleic, and linoleic acid are the major free fatty acids present in rice bran (Iverson, 1965), it might be expected that these three fatty acids are the cause of interfering GLC responses.

Pesticide residue analysts should be aware of the possibility that free fatty acids may interfere with the GLC determination of residues of pesticides and industrial chemicals such as those in Table V and other organic chemicals which may be as yet uncharacterized under these conditions. Erroneous results might possibly be obtained if the presence of fatty acids is ignored. Furthermore, analysts using other chromatographic columns or conditions should be aware that free fatty acids may cause similar effects with those columns or conditions. Fatty acids determined by GLC cause a positive response by ECD and HECD, and overloading of the GLC column with fatty acids or methyl esters leads to a positive response by KCl-TD.

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