avoid losses in the vapor phase by flash evaporation.

In view of the wide range of ethanol concentration in the distillery products, it seems important to use a modular sampling and chromatographic setup.

CONCLUSION

This study has enabled us to test a quick method (5 min) for evaluating the amount of ethanol in complex products. Losses per analysis are low. The results with a beet wine are comparable to the pycnometric reference laboratory method.

This technique has been tested with many products of grape or beet distillery. It may be applied to other ethanol-containing products, in other fields. By changing sensitivity, other volatile constituents may be analyzed. Automation is possible, and the apparatus can be implemented on derivation of the process for continuous analysis and control.

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Supercritical Methanol: An Efficacious Technique for the Extraction of Bound Pesticide Residues from Soil and Plant Samples

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Soil and plant samples containing bound ^{14}C residues of a number of pesticides and/or their metabolites were extracted with supercritical methanol. In a parallel experiment they were subjected to the high-temperature distillation technique. The extracts or the distillates were purified and analyzed by gas chromatography and gas chromatography–mass spectrometry. A comparison between the results obtained with both the techniques revealed that better recoveries of ^{14}C and higher concentrations of residues identified were obtained by the extraction with supercritical methanol. The work demonstrates the feasibility of supercritical fluid technique for the extraction of bound pesticide residues from soil and plants often not detectable in routine residue analysis.

INTRODUCTION

Studies using radioisotopes as tracers within pesticide molecules have revealed that a considerable portion of pesticide residues may remain bound (nonextractable) in soil and plants (Khan, 1982b; Huber and Otto, 1983). Bound residues in soil and plants are not generally detected in routine residue analysis. Thus, for a long time the possible soil or plant burden of total pesticide residues has been underestimated.

During the past few years determination of the nature and quantities of bound pesticide residues in soil and plants has been a challenging problem for a number of research workers. In most of the studies reported in the literature, quantification of ^{14}C-bound residues in soil or plants has been achieved by combustion. This method is limited to the determination of total ^{14}C bound residues and cannot be used to identify the chemical form of the bound residues. Attempts have also been made to extract and/or release bound pesticide residues by the milder to harsher methods. Drastic extractive procedures destroy the structure of soil or plants by solubilizing the materials, and strong acid or base hydrolysis often results in the destruction of the identity of bound residues (Khan, 1982b; Huber and Otto, 1983).

A pyrolysis technique for estimation of bound residues of chloroaniline compounds in plants was reported by Balba et al. (1979). Similar technique was developed by Khan and Hamilton (1980) involving high-temperature distillation (HTD) of the solvent-extracted soil or plant material to release bound ^{14}C residues. The released bound ^{14}C residues were collected in different solvents and analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). In recent years this technique has been widely used to determine the chemical identity of bound ^{14}C residues of pesticides and/or me-

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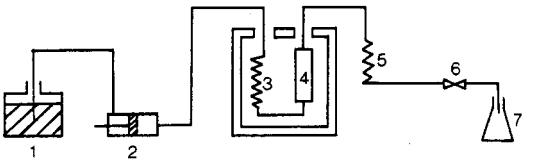


Figure 1. Schematic diagram for the apparatus used for extraction by supercritical methanol: 1, solvent; 2, HPLC pump; 3, capillary; 4, HPLC column; 5, cooler; 6, regulating value; 7, flask.

tabolites in soils (Khan and Hamilton, 1980; Zhang et al., 1984; Capriel et al., 1985), humic materials (Khan, 1982a; Capriel et al., 1985), and plant samples (Khan, 1980; Khan et al., 1984a,b). While the HTD technique provides a possible means for the chemical identification of bound ^{14}C residues of certain pesticides, the thermal decomposition of bound ^{14}C residues to $^{14}CO_2$ during distillation often results in considerably lower recoveries of the bound pesticide and/or metabolites.

A technique that involves extraction by supercritical fluids for the isolation of thermal labile natural products (Stahl et al., 1978; Hubert and Vitzthum, 1978) has been recently used for the extraction of soil organic matter (Spiteller, 1982). A fluid phase at the supercritical condition is considered neither exclusively liquidlike nor gaslike but more of an intermediate state. The advantages of using mobile phase at supercritical conditions are low viscosity, high diffusion coefficients, and variation of density and dielectric constant as a function of pressure. This paper describes the application of this technique to the extraction of bound (nonextractable) ^{14}C residues from soil and plant samples. The extracts obtained by supercritical fluids were subsequently analyzed by GC and GC–MS for determining the identity of the bound ^{14}C residues. For comparison, results obtained by the HTD technique are also reported.

MATERIALS AND METHODS

Soil and Plant Samples. The samples used in this study, the ^{14}C-labeled compounds, and their purity, methods of treatment, and procedures for exhaustive extraction with suitable solvents in order to remove any extractable ^{14}C residues have been described in earlier publications (Khan and Hamilton, 1980; Capriel and Haisch, 1983; Khan et al., 1984a; Khan et al., 1985). The soil and plant samples containing only bound (nonextractable) residues were finely ground (soil, 0.2 mm; plant, 0.5 mm) in a Retsch ultracentrifugal mill and prior to extraction by supercritical methanol were dried for 24 h over phosphorus pentoxide in a vacuum desiccator.

Chemicals. Anhydrous methanol (Merck) was used as supercritical fluid. All other solvents were of pesticide grade and were used as received.

Extraction of Bound ^{14}C Residues by Supercritical Methanol. The apparatus depicted in Figure 1 was used for extraction by supercritical methanol (Koll and Metzger, 1978). The solvent (anhydrous methanol) was compressed by the high-pressure liquid chromatography (HPLC) pump (Gynkotek, Model 300 B) up to 150 bar through the preheated capillary (stainless steel, 0.5 mm i.d., length 2 m) into the HPLC column (stainless steel, 5 mm i.d., length 12.5 cm; stainless-steel frits 2 μm) that contained the sample material to be extracted. The preheating of the capillary and the HPLC column was carried out in a gas chromatograph oven maintained at 250 °C and purged continuously with nitrogen. The latter was necessary in order to avoid the formation of explosive mixture of air–methanol. The extracts were passed through the cooler (water-cooled stainless-steel tube $^1/_8$ in.) and then through the regulating valve (Whitey SS-21RS2) and finally collected in an Erlenmeyer flask with a flow rate of 1 mL/min. The extraction was carried out for about 2 h, and the extracts were then concentrated to a small volume, subjected to liquid scintillation counting (LSC), column cleanup, and derivatization, and finally analyzed by GC and GC–MS.

Determination of Bound ^{14}C Residues by HTD. the bound ^{14}C residues from soil and plant samples were released by HTD technique described earlier (Khan and Hamilton, 1980). The solutions from each trap were subjected to LSC, column cleanup, and derivatization and then analyzed by GC and GC–MS.

Determination of Radioactivity. Combustion of the extracted soil or plant material was done in a sampel oxidizer, Oxymat Model JA 101, to produce $^{14}CO_2$. Aliquots of the various extracts and $^{14}CO_2$ released by combustion were analyzed in a Beckman Series 8000 liquid scintillation spectrometer, using an external standard and correcting the data for quenching.

Chromatography and Analysis. The supercritical methanol extracts and the HTD distillates were subjected to column chromatography for cleanup as descried earlier for prometryn (Khan and Hamilton, 1980), atrazine (Khan and Akhtar, 1983), deltamethrin (Zhang et al., 1984), dieldrin (Khan et al., 1984), carbofuran (Khan et al., 1984), and diuron, 2,4-D, and methylparathion (Sherma and Beroza, 1979). Extracts of samples, treated with prometryn, atrazine, deltamethrin, carbofuran, and 2,4-D were also derivatized prior to GC.

The gas chromatograph used was a Varian Model 3700 equipped with a ^{63}Ni and an AFID detector. The GC conditions were similar to those described earlier for various pesticide-treated soil and crop samples (Khan, 1975; Khan etal., 1976; Lichtenstein et al., ,1977; Zhang et al., 1984; Khan and Akhtar, 1983; Khan et al., 1984). The identity of the compounds was confirmed by comparing the GC retention times with those of authentic samples, by cochromatography, and finally by GC–MS. A high-resolution mass spectrometer, Model VG 2AB-2F, connected to a Varian GC Model 3700 was used. The mass spectra were recorded at 70 eV.

All samples were analyzed in triplicate, and average values are reported. The results are not corrected for recovery.

RESULTS AND DISCUSSION

With the exception of the soil treated with methylparathion, the recoveries of ^{14}C in extracts obtained from the soil samples by supercritical methanol were better than 60% (Table I). Less than 10% remained as residual ^{14}C in the extracted soil material treated with atrazine, prometryn, and diuron. The residual ^{14}C in the soil samples treated with deltamethrin, 2,4-D, and methylparathion amounted to 32, 27, and 34%, respectively, which represent about one-third of the total ^{14}C in the corresponding samples (Table I). It should be noted that the deltamethrin-treated soil is an organic (40.6% C) whereas the other two treated with 2,4-D and methylparathion are mineral soils. Thus, it appears that the amounts of residual ^{14}C remaining after supercritical extraction depend on the chemical structure of the compound rather than the organic matter content of the soil.

While the ^{14}C losses from the methylparathion-treated soil amounted to 28%, in general, about 10% of the total ^{14}C could not be accounted for by the residual and extractable bound residues. It is likely that the unaccounted ^{14}C was lost in the form of $^{14}CO_2$ and/or other volatile ^{14}C-containing products formed during the extraction with supercritical methanol. The average recovery of ^{14}C from

Table I. Recovery of ^{14}C from Soil and Crop Samples after the Extraction with Supercritical Methanol

sample no.	sample	treatment	^{14}C in sample after exhaustv solv extrctn, dpm/g	rec of ^{14}C after extrctn with supercrit methanol, % bound ^{14}C		
				methanol extr	resid in extr sample	un-accounted
1	soil (Munich, Germany)[b]	[^{14}C]atrazine (1.7 kg/ha)[d]	1.32×10^4	87	5	8
2	humic acid (Munich, Germany)[a]	[^{14}C]atrazine	2.76×10^5	82	8	10
3	humin (Munich, Germany)[a]	[^{14}C]atrazine	1.16×10^5	80	7	13
4	soil (Munich, Germany)[b]	[^{14}C]atrazine (1.6 kg/ha)[d]	1.76×10^4	83	9	8
5	soil (St. Jean, Canada)[c]	[^{14}C]prometryn (12.4 mg/kg)[e]	5.99×10^4	96	0.5	3.5
6	soil (St. Jean, Canada)[c]	[^{14}C]deltamethrin (10.0 mg/kg)[e]	7.00×10^3	63	32	5
7	soil (Florida, U.S.A.)[b]	[^{14}C]diuron (10.0 mg/kg)[e]	1.43×10^5	86	8	6
8	soil (Florida, U.S.A.)[b]	[^{14}C]-2,4-D (10.0 mg/kg)[e]	7.04×10^4	58	27	15
9	soil (Beltsville, U.S.A.)[b]	[^{14}C]methylparathion (10.0 mg/kg)[e]	3.0×10^3	38	34	28
10	corn (Ottawa, Canada)	[^{14}C]atrazine (5.0 mg/L)[f]	7.1×10^5	95	0.5	4.5
11	radishes (Florida, U.S.A.)	[^{14}C]dieldrin (11.1 kg/ha)[f]	2.1×10^4	94	1.0	5.0
12	radishes (Florida, U.S.A.)	[^{14}C]carbofuran (11.1 kg/ha)[f]	5.5×10^4	95	1.8	3.2
13	soil (London, Canada)[b]	unlabeled atrazine (100 mg/kg)[e]				

[a] Humic acid and humin were extracted from sample 1. [b] Mineral soil. [c] Organic soil. [d] Field application. [e] Soil incubated in the laboratory. [f] Plants grown in sand pots and irrigated with nutrient solution containing the pesticide.

Table II. Recovery of ^{14}C after Extraction with Supercritical Methanol and by the High-Temperature Distillation Technique

sample no.	sample	treatment	rec of ^{14}C, % bound ^{14}C	
			supercrit[a] methanol	high-temp[b] distilln
1	soil (Munich, Germany)	[^{14}C]atrazine	87	55
2	humic acid (Munich, Germany)	[^{14}C]atrazine	82	70
3	humin (Munich, Germany)	[^{14}C]atrazine	80	65
4	soil (Munich, Germany)	[^{14}C]atrazine	83	62
5	soil (St. Jean, Canada)	[^{14}C]prometryn	96	81
6	soil (St. Jean, Canada)	[^{14}C]deltamethrin	63	35
7	soil (Florida, U.S.A.)	[^{14}C]diuron	86	62
8	soil (Florida, U.S.A.)	[^{14}C]-2,4-D	58	
9	soil (Beltsville, U.S.A.)	[^{14}C]methylparathion	38	31
10	corn (Ottawa, Canada)	[^{14}C]atrazine	95	78
11	radishes (Florida, U.S.A.)	[^{14}C]dieldrin	94	95
12	radiehs (Florida, U.S.A.)	[^{14}C]carbofuran	95	66

[a] ^{14}C in the methanol extract. [b] The $^{14}CO_2$ is not included in these values.

Table III. Bound Residues in the Pesticide-Treated Samples Determined by the Supercritical Methanol Extraction and the High-Temperature Distillation Technique

sample no.	sample	bound residues,[a] ppm	
		supercrit methanol extrctn	high-temp distilln
1	soil (Munich, Germany)	I, 0.1; II, 0.5; V, 0.4; VI, T[d]	I, 0.1; II, 0.1; III, T; IV, T; V, 0.1; VI, 0.1
2	humic acid	I, 0.3; II, 0.2; V, 0.5	I, 0.3; II, 0.3; III, T; IV, 0.1; V, 0.2; VI, 0.2
3	humin	I, 0.1; II, 0.4	II, 0.1; V, T; VI, T
4	soil (Munich, Germany)	I, 0.3; II, 0.5	I, 0.3; II, 0.2
5	soil (St. Jean, Canada)	prometryn, 1.8; hydroxypropazine, 0.5; V, 0.6; deisopropylprometryn, 0.3	prometryn, 2.1; hydroxypropazine, 0.5
6	soil (St. Jean, Canada)	Br_2CA, 0.4[b]	Br_2CA, 0.1[b]
7	soil (Florida, U.S.A.)	diuron, 0.3; DCA, 1.5[c]	
8	soil (Florida, U.S.A.)	2,4-D, 0.9	
9	soil (Beltsville, U.S.A.)	methyl parathion, 0.58	
10	corn (Ottawa, Canada)	I, 0.1; II, 0.5; V, 2.3; VI, 2.7; VII, T	I, 0.1; II, 0.2; III, 0.4; IV, 0.6; V, 2.0; VI, 1.8; VII, 0.2; VIII, 0.1
11	radishes (Florida, Canada)	dieldrin, 11.3	dieldrin, 8.1
12	radishes (Florida, Canada)	carbofuran, 0.5; 3-hydroxycarbofuran, 0.3	3-ketocarbofuran, 0.2; 3-hydroxycarbofuran, 0.1
13	soil (London, Canada)	I, 0.6; II, 3.9; V, 0.7; VI, 1.5	I, 1.7; II, 3.0; V, 1.0; VI, 0.2; VIII, T

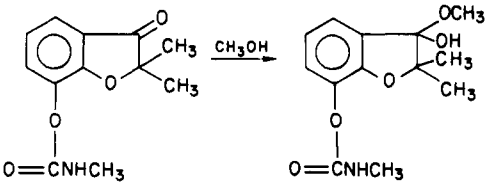
[a] Key: I, atrazine; II, hydroxyatrazine; III, deethylatrazine; IV, deisopropyloatrazine; V, deethylhydroxyatrazine; VI, deiospropylhydroxyatrazine; VII, 2-chloro-4,6-diamino-*s*-triazine; VIII, 2-hydroxy-4,6-diamino-*s*-triazine. Calculated on the basis of air-dried weight of the solvent-extracted plant material; oven-dry weight of soil and dry ash-free weight of humic materials. [b] 3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid. [c] 3,4-Dichloroaniline. [d] T = trace amounts, <0.05 ppm.

the plant material was 95% (Table I). The residual ^{14}C (1–2%) in the extracted plant samples was significantly less than in the soil samples. The relative standard deviations of the values presented in Table I were ≤5%, indicating a considerably good reproducibility by the supercritical methanol technique.

Table II shows comparative ^{14}C recoveries obtained by using the two techniques. It is obvious that better recoveries of ^{14}C from samples containing bound ^{14}C residues were obtained by the supercritcal methanol than the HTD technique. The loss of bound ^{14}C in the form of $^{14}CO_2$ due to thermal decomposition during HTD often resulted in low recoveries of the released materials in the solvent traps (Khan, 1982b). Such loss is not envisaged in the extraction of bound ^{14}C by supercritical fluids.

Under the conditions used to generate supercritical methanol (250 °C (150 bar)), reaction may take place between methanol and the pesticide molecules and/or their metabolities. Thus, atrazine and its monodealkylated products present as bound (nonextractable) ^{14}C residues

in the soil and plant materials reacted with methanol to yield their corresponding methoxy analogues. On the other hand the OH groups in hydroxyatrazine and in its monodealkylated products cannot be replaced by the OCH_3 group under these conditions. Thus, in the extracts of samples 1, 2, and 10 (Table III) obtained by supercritical methanol extraction we were unable to detect the presence of deethylatrazine (III) and deisopropylatrazine (IV). Most likely these compounds were converted to there OCH_3 analogues during supercritical extraction. It should be noted that the methylthio substituent in prometryn (sample 5, Table II) or in its monodealkylated metabolites is relatively very stable and cannot be displaced by the methoxy group. It was observed that, under the supercritical conditions used, 3-ketocarbofuran (sample 12, Table III) is conerted in the following manner to its methoxy derivative: This will also explain the absence



of 3-ketocarbofuran in the supercritical methanol extract of radishes (sample 12, Table III).

The concentrations of the compounds found in the extracts obtained by the HTD and supercritical methanol techniques are presented in Table III. In general the supercritical methanol showed higher concentrations than the HTD. In the soil samples treated with diuron, 2,4-D, and methylparathion, bound residues were not detected by the HTD technique presumably because of thier thermal decomposition to $^{14}CO_2$. Furthermore, the concentrations of hydroxyatrazine, hydroxypropazine, and their monodealkylated derivatives should be regarded only as qualitative because of the poor efficiency of the methylation of the hydroxy group with diazomethane (Khan et al., 1975). The amounts shown in Table III may not account entirely for the ^{14}C residues present in the supercritical extracts (Table I). In some cases a considerable proportion of the ^{14}C residues in the methanol extract might be present as unknown metabolites or may have been included in macromolecular type structures that could not be identified by the analytical methods used in this study.

The results of this study suggest that supercritical fluid extraction can provide an alternative technique for the determination of bound residues in soil and plants. Although some chemical alteration of pesticides and/or metabolites due to supercritical organic solvents is possible, this method does not undergo drastic conditions used in earlier studies for the determination of bound residues in soil and plants.

Registry No. 2,4-D, 94-75-7; MeOH, 67-56-1; atrazine, 1912-24-9; prometryn, 7287-19-6; deltamethrin, 52918-63-5; diuron, 330-54-1; methylparathion, 298-00-0; dieldrin, 60-57-1; carbofuran, 1563-66-2.

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