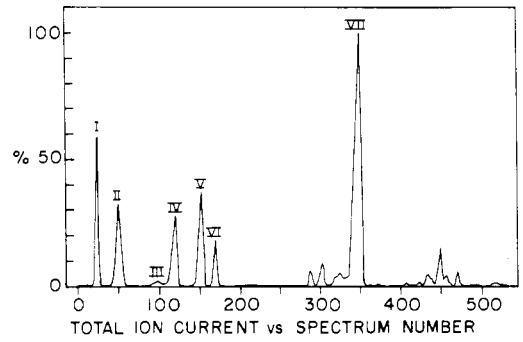

Figure 1. Reconstructed gas chromatogram. The abscissa is normalized to 100% for the ion current of the largest peak. The ordinate gives the spectrum number.

Table I. Composition of Oil

terpenes	time of elution, min	% of oil
α-pinene	21.97	8.0
β-pinene	34.50	7.2
α-terpinene	40.80	0.6
limonene	42.77	14.3
γ-terpinene	47.07	19.7
β-phellandrene + *p*-cymene	50.63	7.6
major sesquiterpene	93.93	34.6

983, 929, 887, 880, 868, 850, 832, 790, 578, 554, 510, 498, 489. In order of elution, the following monoterpenes have been identified by comparing elution times and quadrupole mass spectra with the reference compounds: α-pinene, I; β-pinene, II; α-terpinene, III; limonene, IV; γ-terpinene, V; and β-phellandrene cochromatographing with approximately 11% *p*-cymene, VI. Ikeda et al. (1962) have reported that *p*-cymene cannot be separated from terpinolene with a column using diethyleneglycol succinate as the stationary phase and we find that our LAC-446 column does not resolve *p*-cymene from β-phellandrene. All of the monoterpenes found in our leaf oil have already been reported by Minyard et al. (1965) to be present in the flower buds of Deltapine smoothleaf cotton, *Gossypium hirsutum*. α-Pinene and γ-terpinene were found in the air-dried powdered cotton leaf of *Gossypium barbadense* L. cv. Giza 69 by Hedin et al. (1972).

The composition of the oil, determined with the Varian Aerograph Series 1520, is given in Table I. Studies on the identification of our main sesquiterpene are still in progress.

ACKNOWLEDGMENT

We gratefully acknowledge receiving a file of high-resolution infrared spectra from J. A. Wenninger, Division of Colors and Cosmetics, Food and Drug Administration.

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Junji Kumamoto*[1]
J. Giles Waines[1]
J. Leland Hollenberg[2]
Rainer W. Scora[1]

[1]Department of Botany and Plant Sciences
University of California
Riverside, California 92521
[2]Department of Chemistry
University of Redlands
Redlands, California 92373

Received for review June 19, 1978. Accepted October 23, 1978.

Extraction of Chlorpyrifos-methyl Residues from Aqueous Solution and Analysis by Flame Photometric Gas Chromatography

Methods for extractions and analyses of samples of natural water and basal salt medium for bacterial growth containing traces of chlorpyrifos-methyl have been developed. Aqueous solutions were extracted by high-speed stirring after addition of a small volume of hexane in the sample. Extracts of lower concentration were concentrated by evaporation prior to analyses by flame photometric gas chromatography to yield a detection limit of 1 ppb. The extracts from all aqueous solutions were found to be relatively stable for 45 days. The efficiency of the extraction methods was near 100% for the water samples and near 95% for the basal salt medium samples under the experimental conditions described.

Chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) is similar in chemical structure to Dursban, the former being the *O,O*-dimethyl and the latter the *O,O*-diethyl form of *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate. Its broad spectrum of insecticidal activity and low mammalian toxicity make it a promising insecticide for many uses (Dow Chemical Co., 1973). The relatively few applications described thus far have been a field evaluation for the control of the spruce budworm (Hopewell, 1977), a study as a protector for hard winter wheat and seed corn by stored grain insects (LaHue, 1976, 1977), a study of its persistence in corn silage (Johnson et al., 1974), a comparison against malathion-resistant insects in wheat (Bengston et al., 1975), an evaluation for the control of *Dermestes maculatus deb.* (Coleoptera, Dermestidae) on sheep skins (MacQuillan and Shipp, 1976). Finally, an evaluation of its biological activity in soil (Harris, 1977) and metabolism studies (Bakke and Price, 1976; Whitten and Bull, 1974) were reported. Few chemical methods of analysis have been reported so far, except for a brief

0021-8561/79/1427-0204$01.00/0

description of two gas chromatographic methods (Leuck et al., 1975; Johnson et al., 1974).

As it seems relatively certain that the uses of chlorpyrifos-methyl as an insecticide will increase in the near future (Agriculture Canada is now conducting tests to use chlorpyrifos-methyl as a possible replacement for fenitrothion in the spruce budworm spraying program in the province of Quebec), it becomes necessary to evaluate a method for its extraction from aqueous solution and detection. This paper describes a method for extraction and detection using flame photometric gas chromatography. An evaluation of the stability of the extracts as well as the possibility of using the method in the field is discussed.

METHODS AND MATERIALS

Extraction of Aqueous Solution. Distilled water, natural water, and basal salt medium (Aaronson, 1970) were all extracted by a modification of a method reported by Miles et al. (1976). To a 500-mL glass-stoppered volumetric flask, containing solutions of 100 to 300 mL in which 1 mL of concentrated HCl had been added, 10 mL of hexane was added. The mixture was stirred at a high rate of speed for at least 15 min on an electric magnetic stirrer. After separation of the two phases 5 mL of hexane was recovered for analysis. As the studies of the degradation of chlorpyrifos-methyl by microorganisms and its degradation in the presence of metal ions in solution are of interest, smaller samples of 1 to 2 mL were also extracted by a similar method in which 0.1 mL of concentrated HCl and 1 mL of hexane were used. Using an electric shaker, the solutions were stirred for at least 20 min, then decanted. Half the original volume of hexane was recovered. The samples were dried with $CaCl_2$ and analyzed by the gas chromatographic procedure given below. The basal salt medium for bacterial growth was prepared as described in the literature (Aaronson, 1970). The following salts dissolved in 500 mL of twice-distilled water were added: 0.8 g of K_2HPO_4, 0.2 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 50 mg of $CaSO_4 \cdot 7H_2O$, 10 mg of $FeSO_4 \cdot 7H_2O$, and 1 g of $(NH_4)_2SO_4$. The solution was thoroughly mixed and the volume completed to 1 L.

Gas Chromatographic Analysis. A Varian Model 3700 gas chromatograph equipped with a flame photometric detector was operated with an interference filter for spectral isolation of phosphorus emission at 526 nm. The 60-cm glass column (2 mm i.d.) contained 4% SE30/6% OV-210 on 60–80 mesh Gas-Chrom Q and was operated isothermally at 180 °C after conditioning for 24 h at 225 °C. Flow rates of the gases in milliliter/minute were: nitrogen (carrier) 30 ± 1 at 60 psi, hydrogen 140 ± 3 at 40 psi, air (1) 180 ± 5 at 60 psi and air (2) 85 ± 2 at 60 psi. The temperature of the injector and the detector were set at 220 °C throughout the experiment. The attenuation was set at (256–1024) × 10^{-10}.

A solution of 1000 ng/μL in acetone of chlorpyrifos-methyl (Dowco 214, analytical grade 99.2%) was prepared and used as a primary standard. By proper dilution with hexane, secondary standards ranging in concentration from 1.0 to 120 ng/μL were injected into the gas chromatograph. Plot of peak heights vs. nanograms injected were done to obtain a standard curve which was linear over the range 1 to 120 ng. Injections of the extracts into the chromatograph gave peak heights that were compared to the standard curve in order to determine the corresponding quantities.

RESULTS AND DISCUSSION

Extraction from Aqueous Solutions. Water samples (distilled and natural) and basal salt medium samples fortified with chlorpyrifos-methyl at concentrations ranging from 1.0 to 8.0 ppm were extracted as described above. Recovery ranged from 98–101% for water samples and from 89–94% for basal salt medium as shown in Table I. The basal salt medium solution is not a clear solution and some of the salts remain in suspension. Under these conditions, adsorption of chlorpyrifos-methyl is highly suspected, since recovery percentage increases with stirring time. But a plateau is reached after 20 min. A possible explanation for the differences observed in recovery percentage from the two medium is that the interaction of the chlorpyrifos-methyl with the metallic ions in the solution, especially Mg^{2+} and Fe^{2+}, is very likely. Interactions of organophosphorus pesticides in similar conditions have been investigated and increases in the rate of hydrolysis have been reported (Mortland and Raman, 1967). Without the addition of concentrated HCl, recovery percentage decreases by as much as 50% for solutions containing high concentrations of chlorpyrifos-methyl and by as much as 100% for solutions containing low concentrations of the pesticide. In all the aqueous solutions extracted with concentrations of 1 ppm or lower, no recoveries were possible without the addition of HCl. Extraction of natural waters was performed on the samples as taken from the field. Recovery percentage was the same on samples extracted with and without filtration which shows that adsorption on suspended particles in reasonably clear samples is not a factor. Concentrations down to 1 ppb were also extracted by this method with the same percentage of recovery.

Table I. Recovery of Chlorpyrifos-methyl from Aqueous Solutions with Hexane

sample	volume extracted	ppm added	volume[a] of hexane	recovery,[b] %
water[c]	1	1, 2	1	96-98.5
	2	1, 2	1	97-99.5
	100	1, 2, 5	10	98-100
	300	1, 2, 5, 8	10	97-101
basal salt medium	1	1, 2	1	89.5-92
	2	1, 2	1	90.5-92.5
	100	1, 2, 5	10	89-94
	300	1, 2, 5, 8	10	89.5-93.5

[a] The proper amount of concentrated HCl was added. [b] The recovery percentages reported here are the lowest and highest values of ten extractions. [c] The results for natural and distilled waters were combined as they were so close.

Table II. Stability of the Extracts

extracts from[b]	% recovery[a] 0 h	24 h	1 week	4 weeks	6 weeks
water (distilled)	98.5	98	97	97.5	95
water (natural)	98	98	96.5	96	93
basal salt medium	93	93.5	92	91.5	88

[a] The results shown are an average of five extracts. [b] Stability of the extracts is independant of the volume it has been extracted from and also independant of the volume of hexane used for the extractions.

Studies were undertaken to determine the stability of the extracts. As it can be seen from Table II, extracts from aqueous solutions seem to be stable at room temperature for 45 days. Furthermore, tests for evidence of the suspected products of degradation, the oxygen analogue and the 3,5,6-trichloro-2-pyridinol, were negative, suggesting that the degradation of chloropyrifos-methyl in dry hexane is negligible. Traces of moisture in the extracts reduce considerably the stability.

Gas Chromatographic Analysis. Under the conditions described above, chlorpyrifos-methyl has a retention time of 1.98–2.05 min with a deflection of 34% for 2-ng samples and 72% for 120-ng samples at the proper attenuation as described above. In the cases of samples with low levels of chlorpyrifos-methyl, the extracts were evaporated to smaller volume prior to injection into the gas chromatograph. The conditions for the chromatographic analysis were such that no improvement in precision was noted when using the area of the peak instead of the height of the peak. Retention time and deflection percentage obtained seem to be convenient for a fast and precise analysis. Although the method described herein for extractions of samples containing low levels of chlorpyrifos-methyl was designed for aqueous solutions, it seems that with appropriate uses of solvents and clean-up procedure the method could be adapted for extraction and analysis of samples of biological nature. The simplicity of the manipulation for extraction of natural waters show that the method is adaptable for field uses. Well-protected extracts could be analyzed with accuracy in the laboratory up to 4 weeks after the extraction.

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Pierre-F. Blanchet
Département de Chimie-Biologie
Université du Québec à Trois-Rivières
Trois-Rivières, Québec, Canada G9A 5H7

Received for review on June 26, 1978. Accepted September 29, 1978.

A Convenient Microfiltration Procedure for Purification of Bovine and Ovine Subcutaneous Tissue Lipid Extracts

A convenient and rapid microfiltration procedure for the extraction and purification of lipids in bovine and ovine subcutaneous tissues is described. The procedure is as efficient in the extraction of total lipid, free fatty acids, total sterols, and phosphorus-containing lipid as an extraction procedure employing an aqueous wash for purification. Using lipid calcium as a measure of nonlipid contamination, the microfiltration procedure is as effective as aqueous washing. Due to the avoidance of separatory funnel washing and lengthy phase separations, the microfiltration procedure is preferred if numerous samples are extracted.

Lipid extraction by chloroform–methanol mixtures followed by aqueous washing to remove nonlipid material is effective for many tissues (Johnston, 1971). Unfortunately, troublesome and persistent emulsions are frequently encountered during the aqueous wash. For example, during the extraction of butter and margarine it was suggested that after washing the systems be allowed to stand overnight for phase separation (Smith et al., 1978). This approach is suitable for the extraction of a few samples but is tedious when numerous samples are extracted. During the extraction of a considerable number of subcutaneous tissues from lamb and steer carcasses using the chloroform–methanol and aqueous washing procedure, stable emulsions were encountered. This prompted a search for a purification procedure other than aqueous washing and this paper is a description of an effective one using microfiltration.

The subcutaneous tissue samples studied were those taken over the longissimus muscle at the 12th rib. One sample of steer tissue and one of lamb tissue containing relatively high total lipid and one from each of the species containing relatively low total lipid were used for the comparison studies. Extractions and lipid determination for each of the four samples were triplicated. Analysis of variance was used to test differences of means between the two extraction procedures (Steel and Torrie, 1960).

The microfiltration procedure was performed in the following manner. About 1 g of tissue was weighed to nearest 0.02 g and transferred to glass homogenization flasks. Twenty-five milliliters of 2:1 (v/v) chloroform–methanol (CM) was added, and the contents were homogenized at full speed for 2 min in a Virtis "45" homogenizer (Virtis Co., Inc., Gardiner, N.Y.). The homogenate was filtered through a CM-washed folded filter paper (Schleicher and Schuell, Keene, New Hampshire, No. 588) into a 200-mL round-bottom flask. Another 25 mL of CM was used to rinse the homogenizer blades and flask, the filter paper and residue, and the funnel with the use of a dropping pipet. Visual examination of the filter and residue for oiliness after drying indicated little, if any, lipid present. The extract was evaporated in a rotary evaporator, using a 55 °C water bath to facilitate solvent removal. When it was apparent that water was being evaporated (solution becomes opaque), more chloroform was added to aid in water removal and prevent bumping. The lipid was extracted from the flask

0021-8561/79/1427-0206$01.00/0