New Extraction System for Residue Analyses

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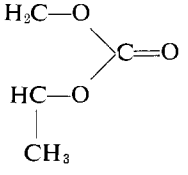
The need for a "universal" procedure for extraction of residual agricultural chemicals in the field of pesticide residue analysis has been magnified by the development of highly selective and sensitive pesticide detection systems. Propylene carbonate is an efficient extractant for residues of many classes of pesticides in a wide variety of materials. Data are presented on the extraction of chlorine-containing organics, organothiophosphates, urea derivatives, and carbamates from soils, fruits, vegetables, and animal products. Thin-layer chromatography, electron-capture, thermionic, and microcoulometric gas chromatography detection systems are applied to cleaned-up propylene carbonate extracts.

The literature contains several procedures for the extraction of various classes of pesticide compounds. Acetonitrile or mixed solvent systems, consisting of isopropyl alcohol in combination with hexane or benzene, are widely employed for many chlorine- and thiophosphorus-containing organic pesticides (*4*, *6*, *16*, *17*, *20*). Various other organic solvents have been used for the extraction of some organochlorine or phosphorus compounds, certain carbamates, and urea-type compounds (*3*, *8*, *19*). While these procedures have produced satisfactory results for their particular purposes, a "universally" applicable extracting system is needed in the field of residue analyses. This need has been magnified by the development of selective and highly sensitive screening systems for detecting certain classes of pesticide compounds.

The properties of propylene carbonate indicate it to be a potentially good broad spectrum extracting agent. Recently this material became commercially available, and an investigation of its possible application to pesticide residue analyses was undertaken.

These studies were conducted on internally fortified crop extracts because experimentally treated samples on such a wide variety of crops and compounds were not available. Although this is the usual practice in methods development, it is considered necessary to determine the application of propylene carbonate for extraction of field-weathered residues prior to its use (*8*, *9*).

Propylene carbonate is a stable, neutral compound, commercially available from the Jefferson Chemical Co., Houston, Tex. Its molecular structure (*17*) is:



4-Methyl 2-dioxolone

M.W. = 102.09

Table I depicts some of its physical properties. Propylene carbonate has a Tag Open Cup (T.O.C.) flash point of 270° F. and therefore would not be classified as a flammable solvent. At room temperature it does not ignite when a lighted match is immersed in it. Propylene carbonate is essentially nontoxic (*7*), either in large oral doses or by skin absorption, and prepurification is not required.

The high boiling point of propylene carbonate precludes the use of evaporation to concentrate or separate residues contained in sample extracts. Column chromatography and solvent partitioning, techniques normally employed for sample cleanup, are used to concentrate and separate residues from propylene carbonate.

Some additional solvent properties of this material are contained in Tables II and III. Most organic solvents are soluble in propylene carbonate, except the saturated aliphatic hydrocarbons. Thus liquid-liquid partitioning between propylene carbonate and hexane or isooctane is possible.

Propylene carbonate is not hygroscopic; however, water is 8.3% soluble in this solvent. This is probably the main reason why both polar and nonpolar compounds can be extracted with propylene carbonate. However, the dissolved water in carbonate extracts must be removed prior to chromatographic cleanup

Table I. Physical Constants of Propylene Carbonate (*7*)

Freezing point, ° C.	−49.2
Boiling point, ° C.	241.7
Specific gravity (20/4° C.)	1.2057
Flash point (T.O.C.), ° F.	270
Refractive index, n_D^{20}	1.4209
Specific heat, 20° C.	0.615
Heat of vaporization, 150° C.	13.200
Dielectric constant, 23° C.	69.0

Table II. Propylene Carbonate Solubility

Liquids Completely Miscible with Propylene Carbonate	Liquids Not Miscible with Propylene Carbonate
Acetone	Petroleum ether
Benzene	*n*-Hexane
Chloroform	Isooctane
Ethanol	Castor oil
Toluene	Corn oil
Xylene	Mineral oil

Residue Laboratory, Campbell Soup Co., Camden, N.J.

Table III. Solubility of Various Materials in Propylene Carbonate

Material	G. Solute in 100 G. of Propylene Carbonate
Carbon tetrachloride	100
Water	8.3
DDT	17
Lindane	18
Polyoxyethylene glycols	>100
Urea	<1
Acetylene	0.59
Methyl chloride	4.1
Sulfur dioxide	23.42

Table IV. Elution of Insecticides through Florisil[a]

Fraction I, 7% Et_2O in Petroleum Ether	Fraction II, 25% Et_2O in Petroleum Ether
Aldrin	Thimet
DDT, TDE, DDE	Diazinon
Lindane	Methyl parathion
Heptachlor	Parathion
Heptachlor epoxide	Methyl trithion
Dieldrin	Trithion
Endrin	Ethion
Methoxychlor	

[a] Deactivated with 5% added water.

and in order to measure volume *vs.* sample weight. This is accomplished by storing extracts over sodium sulfate.

Procedure

Three procedures have been found applicable for the extraction of chlorine-containing organics and organothiophosphorus compounds from fruits, leafy vegetables, cole crops, root crops, soils, grains, dehydrated products, meats, dairy products, and fats and oils.

Fruits, Vegetables, Grains, Meats, and Dairy Products. EXTRACTION. Representative samples are finely chopped and mixed prior to subsampling. A subsample is then macerated for 1 minute with 2 ml. of propylene carbonate per gram of sample; usually a 50- or 100-gram sample is used. The macerate is vacuum-filtered through a fritted-glass Buchner funnel containing glass wool and a $^1/_2$-inch layer of granular sodium sulfate. The filtered extract is dried over approximately 20 grams of sodium sulfate for a minimum of 10 minutes. This procedure is satisfactory for all the products examined except fresh milk.

CLEANUP. Florisil chromatography (*14*, *15*, *18*) is generally applicable for separation of organic chlorine and organothiophosphorus compounds from propylene carbonate extracts.

The deactivated Florisil is prepared according to the procedure described by Langlois, Stemp, and Liska (*13*). Thirty grams are added to a chromatographic column (25-mm. o.d. × 400-mm. length) containing 5 grams of sodium sulfate. An additional 5 grams of sodium sulfate is added on top of the Florisil. The column is prewashed with 50 ml. of petroleum ether, and the washings are discarded. Five milliliters of extract, representing 2.5 grams of sample, are transferred to the column and allowed to penetrate the upper portion of the Florisil. The sample is eluted with successive and separate 200-ml. portions of 7% diethyl ether in petroleum ether followed by 25% diethyl ether in petroleum ether. Table IV lists pesticides eluted by two ether solutions through Florisil deactivated with 5% water. One fraction contains organic chlorine compounds and the other contains organothiophosphorus compounds. This separation is preferred, because it simplifies applications of the multiple detection systems used.

DETECTION. Propylene carbonate extracts following cleanup were analyzed by electron-capture, thermionic, microcoulometric gas chromatography, and thin-layer chromatographic procedures.

The electron-capture detection system consisted of a Barber-Colman Model 10 gas chromatograph, equipped with high temperature ^{63}Ni detectors (*1*). A $^1/_4$-inch glass column containing a mixed liquid phase of 10% QF-1 and 5% SE-30 on Gas Chrom Q 100/120 was used. The temperatures of the column, detector, and inlet were 205°, 300°, and 250° C., respectively. Nitrogen was used as the carrier gas, at flow rate of 50 to 60 ml. per minute.

A thermionic detection system consisting of a MicroTek 220 gas chromatograph equipped with a dual stacked thermionic flame detector was employed for determination of organophosphorus compounds extracted with propylene carbonate. A bead was formed on the upper coil with a 1 to 1 mixture of potassium chloride and boric acid for thermionic detection. A column containing 10% SE-30 on 80/100 Chromosorb AW was used. The temperatures of the column, detector, and inlet were 190°, 285°, and 260° C., respectively. Nitrogen was used as the carrier gas at a flow rate of 100 ml. per minute.

Propylene carbonate extracts following cleanup were also analyzed by the TLC procedures described by Kovacs for organochlorine and organothiophosphorus compounds (*11*, *12*). A Dohrmann Model C-200 microcoulometric sulfur cell was used in conjunction with a MicroTek 220 gas chromatograph for analyzing sulfur-containing organics.

Soils and Dehydrated Products. EXTRACTION. A 100-gram sample is weighed in a 500-ml. Erlenmeyer flask and mixed with 200 ml. of propylene carbonate on a mechanical shaker for 2 hours. The mixture is filtered with the aid of suction and dried over sodium sulfate.

CLEANUP. Cleanup for dehydrated products is achieved using the previously described Florisil column. However, soils are first eluted with petroleum ether until a 200-ml. fraction is collected in a graduated cylinder. This fraction contains aldrin, chlordan, and the aggregate DDT compounds. Two hundred milliliters of 7% ethyl ether is then collected to remove the remaining chlorine-containing organic compounds, such as endrin and dieldrin. In the detection systems used,

it is advantageous to separate DDT compounds from naphthalenic members of the chlorine class and most organophosphorus compounds.

DETECTION. The detection techniques described above were also applicable to propylene carbonate extracts of soil and dehydrated products.

Fats and Oils. EXTRACTION. The following procedure is applicable for the extraction of chlorine-containing organic compounds from oils and from fats or red meat, poultry, and butter: Twenty grams of melted fat or oil and 20 ml. of propylene carbonate are combined and then shaken gently for 30 seconds in a 125-ml. separatory funnel, and the layers are allowed to separate. In some instances, centrifugation may be required to separate emulsions. The bottom layer, propylene carbonate, is filtered through a powder funnel containing a small plug of glass wool into a 100-ml. bottle. The fat layer is re-extracted with another 20-ml. portion of propylene carbonate. The extracts are then combined and stored over 5 grams of anhydrous sodium sulfate.

CLEANUP. Aliquots equivalent to 2 to 5 grams of sample are taken through the same cleanup as described for vegetables.

DETECTION. The detection systems previously described were applicable for this group of foods extracted with propylene carbonate.

Results

Table V lists results obtained when some representative commodities were extracted with propylene carbonate before and after fortification. Recoveries ranging from 80 to 95% were obtained for commodities containing both high and low fat content. Unlike current procedures, each commodity was extracted in a similar manner without adjustment for fat or water content.

To evaluate the effectiveness of propylene carbonate as a broad extractant, a composite celery sample was fortified with a wide variety of chlorine and thiophosphorus-containing organic compounds at the 1-p.p.m. level, and then extracted with propylene car-

Table V. Recovery of Pesticides Added to Foods Prior to Extraction with Propylene Carbonate

Commodity	Fortification		Residue Found		Recovery, %
	Compound	P.p.m.	Apparent	Corrected	
Animal feed	None	...	0.00	...	...
	Dieldrin	1.0	0.85	0.85	85
Beef	None	...	0.13	0.00	...
	DDE	1.0	0.97	0.84	84
Butter	None	...	0.00	0.00	...
	DDT	0.1	0.09	0.09	90
Cabbage	None	...	0.02	0.00	...
	Dieldrin	0.5	0.48	0.46	92
Cheese	None	...	0.00	0.00	...
	DDT	1.0	0.85	0.85	85
Chicken meat	None	...	0.00	0.00	...
	Dieldrin	0.1	0.08	0.08	80
Green beans	None	...	0.00	0.00	...
	Malathion	1.0	0.80	0.80	80
Parsley	None	...	0.06	0.00	...
	Diazinon	1.0	0.92	0.86	86
Peaches	None	...	0.00	...	...
	Lindane	1.0	0.85	0.85	85
Dried milk	None	...	...	...	...
	TDE	0.1	0.08	0.08	80
Egg yolk	None	0.0	0.00	...	...
	Heptachlor	1.0	0.82	0.82	82
	Heptachlor epoxide	1.0	0.95	0.95	95
Corn oil	None	...	0.00	0.00	...
	DDT	0.5	0.46	0.46	92
Poultry fat	None	...	0.00	0.00	...
	Dieldrin	0.05	0.45	0.45	90
Soil	None	...	0.03	0.00	...
	Endrin	0.10	0.12	0.09	90

bonate. The recovery studies contained in Table VI indicate this solvent to be applicable for extraction of these classes of compounds.

A statistical comparison of the propylene carbonate *vs.* mixed solvent extraction system was made (Table VII). A composite sample of celery was fortified at levels of 1 and 2 p.p.m. with DDT and parathion, respectively. Electron-capture gas chromatographic analyses were then performed on five separate subsamples. Previous analysis established the celery to contain less than 0.01 p.p.m., if any, DDT or parathion. The data contained in Table VII indicate that propylene carbonate is as effective an extractant as the mixed solvent system. There is no statistical difference between the results for the two systems even at one standard deviation.

Examples of comparison with other extraction procedures are shown in Table VIII. Fortified green beans, soil, and animal fat were extracted using the carbonate solvent and another solvent system suitable for the commodity and compound being analyzed. These results indicate propylene carbonate to be an effective extractant.

Partitioning. Since the solubility properties of propylene carbonate appeared well suited for solvent partitioning of insecticide residues, the distribution of several pesticides in certain solvent pairs was measured. The procedure employed was an adaptation of the one described by Beroza and Bowman (*2*). In order to apply solvent partitioning to the cleanup of propylene carbonate extracts, it is necessary, in most instances, to add water to the extract.

Table IX lists the fractional amount of pesticide partitioned into the hydrocarbon phase from the indicated system. Maximum partitioning values and effective cleanup are obtained when the following procedure is used: Ten milliliters of propylene carbonate extract and 50 ml. of distilled water are combined in a 250-ml. separatory funnel. Fifty milliliters of either petroleum ether or isooctane are added, and the funnel is shaken for 30 seconds. After the layers separate, the aqueous phase is drawn off and discarded. The hydrocarbon phase is filtered through sodium sulfate into a 250-ml. beaker and concentrated to a volume suitable for the detection system.

Experimental work was performed on the extraction of some nonthiophosphorus, certain urea compounds, and carbamates with propylene carbonate.

Propylene carbonate was previously found to be an effective extractant for residues of the organothiophosphorus compounds. To determine its possible use as an extractant for nonthio organophosphorus com-

Table VI. Propylene Carbonate Extraction of a Variety of Pesticides Added to Celery

Compound	Amount Added, P.P.M.	Residue Found, P.P.M.		Recovery, %
		Apparent	Corrected	
Lindane	1.0	0.87	0.87	87
Aldrin	1.0	0.78	0.78	78
Heptachlor epoxide	1.0	0.84	0.84	84
Dieldrin	1.0	0.85	0.85	85
DDT	1.0	(0.05)[a] 0.96	0.91	91
Diazinon	1.0	0.90	0.90	90
Parathion	1.0	(0.24)[a] 1.18	0.94	94
Malathion	1.0	0.74	0.74	74
Ethion	1.0	0.89	0.89	89
Trithion	1.0	0.83	0.83	83

[a] Amount found prior to fortification, p.p.m.

Table VII. Comparison of Extraction of Fortified Celery with Propylene Carbonate *vs.* Mixed Solvent

Fortification, P.P.M.		Hexane–IPA (2 to 1), P.P.M.		Propylene Carbonate, P.P.M.	
DDT	Parathion	DDT	Parathion	DDT	Parathion
1	2	0.98	1.89	0.88	1.79
1	2	0.86	1.82	0.93	1.89
1	2	1.02	1.76	0.97	1.82
1	2	0.98	1.94	0.96	1.96
1	2	0.92	1.79	0.86	1.76
Maximum		1.02	1.94	0.97	1.96
Minimum		0.86	1.76	0.86	1.79
Mean		0.95	1.85	0.92	1.85
Recovery		95%	92.5%	92%	92.5%
Mean error		0.05	0.15	0.08	0.15
Std. deviation		±0.06	±0.07	±0.05	±0.08
Rel. std. deviation		6.3%	3.8%	5.4%	4.3%

Table VIII. Comparison of Propylene Carbonate with Other Extraction Procedures

Commodity	Fortification Compound	Fortification P.p.m.		Extraction System, P.P.M. Prop. Carb.	(2 to 1) Hexane–IPA	Procedure (*11*)	Shell–10% acetone in pet. e. (*19*)
Green beans	Malathion	1.0		0.80	0.77	...	...
Soil	Endrin	0.1		0.09	...	...	0.09
			DDE	0.27	...	...	0.15
			DDT	0.58	...	...	0.60
			TDE	0.20	...	...	0.15
Animal fat	Dieldrin	0.1		0.08	...	0.03	...
			DDE	0.26	...	0.25	...
			DDT	0.43	...	0.35	...
			TDE	0.09	...	0.10	...

Table IX. Fractional Amount of Pesticide Partitioned into Hydrocarbon Phase

Pesticide	Petroleum Ether–Propylene Carbonate 1 to 1	5 to 1	Petroleum Ether–Propylene Carbonate–Water 5:1:1	5:1:2	5:1:5	5:1:5
Lindane	0.16	0.20	0.29	0.50	0.95	...
Aldrin	0.68	0.70	0.95	0.92	0.95	...
DDT	0.14	0.25	0.63	0.68	0.95	...
Dieldrin	N.d.[a]	0.20	0.45	0.50	0.95	...
Diazinon	...	...	...	...	0.65	0.70
Methyl parathion	...	...	...	...	0.18	0.48
Parathion	...	...	...	...	0.37	0.64
Malathion	...	...	...	...	0.25	0.64
Trithion	...	...	...	...	0.64	0.73
Ethion	...	...	...	...	0.77	0.75

[a] Not determined.

pounds from an aqueous medium the following experiment was performed:

Twenty milliliters of a Phosdrin standard (10 mg. per ml.), prepared in H_2O, were measured into a 250-ml. separatory funnel and then extracted with 20 ml. of propylene carbonate. The carbonate phase was diluted to 200 ml. and then dried over sodium sulfate.

Results of analysis of the carbonate layer by direct injection into the stacked flame thermionic detection system indicated a partition coefficient value of 0.52 for Phosdrin. This is not considered to be a satisfactory approach for residue determination. However, it demonstrates that Phosdrin can be extracted into propylene carbonate from an aqueous solution.

Several unsuccessful attempts were made to separate small quantities of Phosdrin from propylene carbonate. These included Florisil chromatography, a few solvent-partitioning procedures, sweep codistillation (*22*), and column chromatography with other adsorbents.

Urea-Type Compounds (Linuron). To determine whether propylene carbonate could be used as an extractant for free linuron, representative potato and carrot samples were fortified with linuron at a level of 1 p.p.m., then extracted with propylene carbonate as described for vegetables.

Linuron was separated from propylene carbonate when 2 ml. of extract (1 gram) were chromatographed through Florisil deactivated with 10% water and eluted with 300 ml. of 25% diethyl ether in petroleum ether.

The thin-layer procedure described by Kovacs (*11*) for organic chlorine compounds was used for detecting linuron. Using a mobile solvent of 10% acetone in *n*-heptane, an R_f value of 0.40 for linuron was observed. Recovery of free linuron from potato and carrot extracts was approximately 70%.

Carbamates (Carbaryl). To determine whether propylene carbonate could be used as an extractant for carbaryl, a composite sample of peaches was fortified at a level of 5 p.p.m. and extracted with propylene carbonate using the procedure described for vegetables.

The cleanup described for linuron above and the TLC procedure described by Finocchiaro (*5*) were employed for measuring carbaryl. The recovery for carbaryl from peaches was approximately 50%.

Discussion

In the electron-capture and thermionic detection systems employed, propylene carbonate emerges as a single peak approximately 1 minute after injection.

Although propylene carbonate and pesticide compounds can be separated by electron-capture gas chromatography, electron-capture peak suppression occurred when a concentration of 1% propylene carbonate

was added to cleaned up extracts. One per cent propylene carbonate in a cleaned up celery extract containing 1 p.p.m. DDT and 2 p.p.m. parathion caused a suppression of 5 and 15%, respectively, for these compounds. However, no suppression occurred at a 0.1% carbonate concentration. These data indicate that the application of high temperature electron-capture gas chromatography requires that injected solutions contain not more than 0.1% propylene carbonate.

The use of Florisil as described will separate the pesticides tested from a maximum of 10 ml. of propylene carbonate. If larger aliquots are desired, it is necessary to adjust the chromatographic conditions by a proportionate increase of Florisil and eluants.

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References

(1) Ahren, A. W., Phillips, W. F., Abstracts, A 043, 152nd Meeting, ACS, New York, N.Y., September 1966.
(2) Beroza, M., Bowman, M. C., *J. Assoc. Offic. Agr. Chemists* **48,** 358 (1965).
(3) Bleidner, W. E., Baker, W. E., Levitsky, M., J. AGR. FOOD CHEM. **2,** 476 (1954).
(4) FDA Pesticide Manual, Vol. **1.**
(5) Finocchiaro, J. M., Benson, W. R., *J. Assoc. Offic. Agr. Chemists* **48,** 736 (1965).
(6) Oetz, M. E., *Ibid.*, **45,** 393 (1962).
(7) Jefferson Chemical Co., "Propylene Carbonate Technical Bulletin," Houston, Tex., 1965.
(8) Johnson, D. P., *J. Assoc. Offic. Agr. Chemists*, **47,** 283 (1964).
(9) Klein, A. K., *Ibid.*, **41,** 551–55 (1958).
(10) Klein, A. K., Long, E. P., Sheehan, J. O., Jr., *Ibid.*, **42,** 539–44 (1959).
(11) Kovacs, M. F., *Ibid.*, **46,** 884 (1963).
(12) *Ibid.*, **47,** 1008 (1964).
(13) Langlois, B. E., Stemp, A. R., Liska, B. J., J. AGR. FOOD CHEM. **12,** 243 (1964).
(14) McKinley, W. P., Malion, J. H., *Ibid.*, **6,** 915 (1959).
(15) Mills, P. A., *Ibid.*, **42,** 734 (1959).
(16) Mills, P. A., Only, J., Gaither, R., *J. Assoc. Offic. Agr. Chemists* **46,** 186 (1963).
(17) Moddes, R. E., Cook, J. W., *Ibid.*, **42,** 208 (1959).
(18) Ordas, E. P., Smith, V. C., Meyer, C. F., J. AGR. FOOD CHEM. **4,** 444 (1956).
(19) Shell Chemical Co., "Manual of Methods for Determination of Residues of Shell Pesticides," Analytical Method **MMS-1/63.**
(20) *Ibid.*, Analytical Method **MMS-6/64.**
(21) *Ibid.*, Analytical Method **MMS-43/64.**
(22) Storherr, R. W., Watts, R. R., *J. Assoc. Offic. Agr. Chemists* **48,** 1154 (1965).

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