containing inorganic base may also yield pyruvic acid as a hydrolytic byproduct. Furthermore, the storage of concentrated aqueous solutions of sodium dalapon prior to application will necessitate more material than generally required to obtain a desired result. The preparation of concentrated stock solutions will result in at least an initial 3 to 5% loss of material by hydrolysis and more upon storage. Therefore, stock solutions at high concentration which are diluted prior to application should not be prepared and stored for long periods (Melnikov, 1971).

It appears that sodium dalapon solutions with concentrations greater than about 0.9 *m* form basic solutions when freshly prepared (Figure 1). Upon standing, however, these solutions become acidic from the hydrochloric acid released by the decomposition of the dalapon salt. Therefore, the pH value of 6.0 reported for a 50% solution must be the observed pH for sodium dalapon and its hydrolytic byproducts rather than sodium dalapon alone. Although the quantity of material lost through hydrolysis to reduce the hydroxide ion concentration to an acidic pH is only 3 to 4%, this loss may become significant for certain material balance studies.

The pH values have been reported here for sodium dalapon solutions with respect to different concentrations. These values should not be considered as the true pH of the solution due to hydrolysis prior to measurement. The results may be used, however, as a general guideline to indicate the approximate pH expected for a solution of given concentration.

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Received for review June 26, 1972. Accepted January 3, 1972. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

The *p*-Value Approach to Quantitative Liquid–Liquid Extraction of Pesticides and Herbicides from Water. 2. Selection of Water:Solvent Ratios and Number of Extractions

Irwin H. Suffet

The general goal of aqueous pesticide residue analysis is the recovery of 100% of a pesticide and its degradation products for qualitative and quantitative analysis. The *p*-value concept has previously been used to determine the pH and solvent to approach 100% pesticide recovery. In this paper, equations are developed from liquid–liquid extraction theory for the number of extractions and water:solvent ratios for maximum recovery for typical *p*-values. A computer program analysis of the equations developed indicates that a pesticide which has a *p*-value of ≥0.90 in an aqueous solvent system can be extracted from the aqueous phase with 95% recovery in ≤5 successive extractions of ≥50 ml with a total volume ≤500 ml of solvent. The equations were successfully tested with serial extraction of 2,4-D by separatory funnel and vortex stirring extraction procedures. The contribution to the total error of initial LLE step was estimated to be less than 20% for a four-step serial extraction.

Aqueous pesticide residue analysis is concerned with the reproducible minimum detectable concentration of a given analytical procedure (Brown and Nishioka, 1967; Faust and Suffet, 1969; Nicholson, 1967). The general goal of aqueous pesticide residue analysis is to recover 100% of a pesticide and its metabolites, if present, for quantitation and/or identification. Some quantity of a compound is lost during each analytical step (*i.e.*, extraction, concentration, clean-up, etc.) of a residue method. It is necessary to minimize these losses.

The general practice is to report recovery efficiencies by fortification techniques, that is, the addition of a known quantity of a pesticide to water in a laboratory test prior to processing the sample through each step of the analytical procedure. Fortification techniques provide data only on the theoretical recovery efficiency of the total analytical procedure and not on the liquid–liquid extraction (LLE) step alone.

The question remains: do the percent recoveries from the fortification procedure represent the actual recovery efficiencies from field samples? The pitfalls of the fortification procedure have been discussed for plant and soil pesticide residues (Gunther, 1962; Wheeler and Frear, 1966). Hermann and Post (1968) have demonstrated the extraction of model pollutants from distilled water to be different than their extraction from natural water. Gunther (1962) concluded that the fortification process is "illusory except in a few instances." A completely homogeneous system such as a true solution may approach actual field recovery. However, field samples are "weathered" (subject to physical, chemical, and metabolic transformations) and may be in aggregate or molecular form in or on

Department of Chemistry, Environmental Studies Institute, Drexel University, Philadelphia, Pennsylvania 19104.

plant tissue or soil. Therefore, the field residue may not be in the same form as the fortified sample. The actual recovery of the field sample can be determined by isotope tracers applied to the field (Gunther, 1962).

Fortification of laboratory water samples will approach actual recovery of field samples if a pesticide is completely dissolved and not associated with suspended matter and the water properties are similar to natural water (pH, temperature, and ionic strength). In another approach, natural water characteristics can be altered to laboratory fortification specifications in order that efficiencies can be compared and maximized. This second approach is considered here.

The optimization of the liquid–liquid extraction (LLE) step will maximize recovery. The criteria to achieve 100% recovery have been presented in previous work (Faust and Suffet, 1972). The choices of pH and solvent have been considered in Part 1 of this series (Suffet and Faust, 1972). A simple and direct experimental method quantitates an isolated liquid–liquid extraction step for dissolved compounds. This method develops a p-value, "the fraction of the total solute that distributes itself in the nonpolar phase of an equivolume solvent pair" (Beroza *et al.*, 1969). A p-value, therefore, presents the partition coefficient on a fractional basis. The parameters of temperature and ionic strength are kept constant while the best solvent to extract a pesticide from water was determined. The choice of a solvent for which the pesticide has a high p-value relative to water enables the use of small aqueous samples and/or less solvent.

The choice of water:solvent ratios and the number of successive extractions required for maximum recovery of a pesticide will be considered herein. Equations are developed from LLE theory that can be utilized to choose these parameters for quantitative extraction of a compound under selected conditions. These equations are based upon the p-value. Organophosphate systems utilizing the parent, oxon, and hydrolysis products of Diazinon, Baytex (Fenthion), Parathion, and Malathion (Table I, Part 1, Suffet and Faust, 1972) are discussed to show the capability of the p-value approach.

Direct analysis of 2,4-D from an isolated LLE step is utilized as a model aqueous residue to test the equations of successive extraction by separatory funnel and vortex stirring extraction procedures.

EXPERIMENTAL SECTION

All p-values are determined at a temperature of 25 ± 0.5°, with an aqueous ionic strength of 0.2 M orthophosphate buffer relative to an immiscible organic solvent. The pH is adjusted by the choice of phosphate salts to make up the 0.2 M buffer. The preparation of sample, reagents, and the p-value method utilized have previously been described (Suffet and Faust, 1972).

Pesticide and herbicide chemicals were obtained from their manufacturer. 2,4-D was recrystallized before use.

Volume correction factors were determined for pure solvent and buffered water under the condition of the p-value method in calibrated 1-l. graduated cylinders fitted with ground-glass stoppers. Solvents were well mixed and left to equilibrate in a water bath for 24 hr at 25 ± 0.5°. The volume correction factors for serial liquid–liquid extractions were determined with pure solvent and buffers saturated with that particular solvent.

Two methods of serial liquid–liquid extraction were used for fortification studies—classical separatory funnels (2-l. capacity separatory funnels with Teflon stopcocks) and the vortex stirring method (American Public Health Association, 1971). Widemouthed glass jars which contain 0.25, 1, or 3 l. when three-fourths full and a magnetic stirrer were used at laboratory temperatures (24–27°) in the vortex stirring method. A separatory funnel was then used to separate the water from the solvent layer before successive extractions. 2,4-D was serially extracted by both methods from pH 2, 0.2 M orthophosphate buffer made up in distilled water. Benzene was used to extract 50 mg/l. of 2,4-D at a water:solvent ratio of 10:1. The residual 2,4-D in the water layer was measured spectrophotometrically in matched 1-cm far uv silica cells at 284 nm, after purging benzene with a stream of nitrogen gas for 10 min.

A difference of transmittance of 2% T (0.0086 absorbance units) above the instrument noise level is considered an acceptable response in the uv. The molar absorptivity as determined in the present study was 1640 for 2,4-D. The theoretical lower limit of detectability of 2,4-D therefore is 1.2 mg/l. for a 1-cm path length with this molar absorptivity. When the %T of the residual 2,4-D was more than 80% T, 2,4-D was determined by a standard additions procedure to minimize spectroscopic error. Spectroscopic error is minimal in the 20–80% T region. In the standard additions procedure, the concentration of the 2,4-D present in a sample is equal to the concentration originally in the sample plus the concentration of a standard addition, each times a correction factor for its volume change. A statistical comparison of the standard addition method *vs.* direct uv analysis of 2,4-D indicated no significant differences (t-test, >0.99) (Suffet, 1973).

THEORY

The equation to calculate the total fraction of solute extracted into the solvent phase for one extraction of unequal phase volumes and/or originally unequilibrated aqueous and solvent phase (E) (Bowman and Beroza, 1965) is

$$E = \frac{\alpha p}{\alpha p - p + 1} \quad (1)$$

This is the same equation that is utilized to calculate p-values but is rearranged in terms of the E-value. α is a volume correction factor. α is equal to the volume of solvent (V_n) divided by volume of water (V_p) after LLE.

Table I. Volume Correction Factors for the First Step of LLE 40:1, 20:1, and 10:1 Water:Solvent Ratios

System	Final volume, ml	V_n/V_s	V_p/V_h	$\alpha_1 = V_n/V_p$
Water:solvent ratio 40:1[a]				
Benzene and	V_n = 19.5			
		0.98	1.00	0.024
pH 4.3 buffer	V_p = 800.5			
Hexane and	V_n = 20.1			
		1.00	1.00	0.025
pH 4.3 buffer	V_p = 800			
Water:solvent ratio 20:1[b]				
Benzene and	V_n = 39.3			
		0.98	1.00	0.049
pH 4.3 buffer	V_p = 800.5			
Hexane and	V_n = 40.1			
		1.00	1.00	0.050
pH 4.3 buffer	V_p = 800			
Water:solvent ratio 10:1[c]				
Ethyl ether and	V_n = 18.4			
		0.27	1.06	0.026
pH 4.3 buffer	V_p = 851.5			
Ethyl acetate and	V_n = 22.0			
		0.27	1.06	0.026
pH 4.3 buffer	V_p = 850.5			
Chloroform and	V_n = 67.0			
		0.96	1.00	0.095
pH 4.3 buffer	V_p = 703			

[a] V_s = 20 ml, V_h = 800. [b] V_s = 40 ml, V_h = 800 ml. Ethyl ether, V_p = 834 ml, ethyl acetate, V_p = 838 ml (both completely dissolved). [c] V_s = 80 ml, V_h = 800 ml for ethyl ether and ethyl acetate. V_s = 70 ml, V_h = 700 ml for chloroform.

Table II. Amount of Solvent Required to Saturate 1 l. of 0.2 *M* Orthophosphate Buffer at 25 ± 0.5°

Solvent	Saturation volume (ml/l.)[a] *ca.* 25°	Solvent solubility in H_2O (g/l.)[b]
Benzene	0.75	0.80 *ca.* 25°F
Hexane	0.13	0.138 *ca.* 15.5°
Ethyl acetate	74	69–75 *ca.* 20–30°
Ether	79	72–86 *ca.* 20–30°
Chloroform	6.80	8.2 *ca.* 20°

[a] From experimental α_1-value data extrapolated to 1 l. from 40, 20, and 10:1 ratios (Suffet and Faust, 1972). [b] After Marsden and Mann (1963).

The theoretical calculation of multiple extractions of unequal phase volumes can be considered from successive steps 1, 2, 3 .... utilizing eq 1. The first step extracts the fractional amount of solute E_1 as outlined above. Successive steps extract fractions of the remaining solute from the aqueous phase which is saturated with solvent E_2, E_3 ... Therefore

$$F_n = E_1 + E_2 + E_3 + \ldots \quad (2)$$

$$F_n = 1(E_1) + A(1 - E_1) + B(1 - E_1 - E_2) + \ldots \quad (3)$$

$$F_n = \frac{\alpha_1 p}{\alpha_1 p - p + 1} + \frac{\alpha_2 p}{\alpha_2 p - p + 1}(1 - E_1) + \frac{\alpha_3 p}{\alpha_3 p - p + 1}[1 - (E_1 - E_2)] + \cdots \quad (4)$$

where F_n is the total fraction after n extractions and A, B ... are the fractional parts of solute extracted in successive steps. Equations 3 and 4 express the theoretical quantitative extraction of any water residue sample. α_1, α_2 etc. are volume correction factors for each successive LLE step. Equation 4 has been computer programmed to enable selection of optimum parameters for quantitative serial LLE. The computer printout will appear following these pages in the microfilm edition of this volume of the journal.

An individual $\alpha p/(\alpha p - p + 1)$ term can be calculated by eq 1 or its graphical solution for a known α-value. After the initial extraction, the volume of water phase changes, depending upon the solubility of the solvent in the aqueous phase and *vice versa*.

The α-values for the solvents almost completely insoluble in water (hexane and benzene) are constant. Therefore, for a constant α-value, eq 2 can be written for n successive extractions

$$F_n = E_1 + E_1[(1 - E_1) + (1 - E_1)^2 + (1 - E_1)^3 \ldots . (1 - E_1)^{n-1}] \quad (5)$$

which is equivalent to

$$F_n = 1 - (1 - E_1)^n \quad (6)$$

Equation 6 is in the same form as Bowman and Beroza's equation for repeated extractions with equilibrated solvents of equal volume (Bowman and Beroza, 1965).

$$E_u = 1 - (1 - p)^n \quad (7)$$

where E_u equals the fraction extracted into the upper phase. E_1 is related to p by eq 1. When $\alpha = 1$ (equilibrated phases of equal volume extraction), $E_1 = p$, wherefrom eq 7 is developed.

After the second extraction step (water saturated with solvent and unsaturated solvent), the α_2-values for solvents that are somewhat more soluble in water (ethyl acetate and ether) vary within the experimental error of the theoretical α_2-value. Therefore, the theoretical α_2-value could be used for all successive α_2-values after the first extraction step.

Successive extractions where the water:solvent ratio remains constant after the first step of extraction can be calculated from the following equation.

$$F_n = E_1 + A(1 - E_1)[1 + (1 - A) + (1 - A)^2 + (1 - A)^3 \ldots (1 - A)^{n-1}] \quad (8)$$

which is equivalent to

$$F_n = E_1 + (1 - E_1)[1 - (1 - A)^{n-1}] \quad (9)$$

α_2 can be calculated by the following equation.

$$\alpha_2 = V_{s_2}/V_{p_1} \quad (10)$$

V_{s_2} is the volume of solvent utilized for the second extraction step and V_{p_1} is the final water volume of extraction step 1. For variable water:solvent ratios, for this case eq 4 must be used.

Equation 9 also can be used for the case of solvents almost completely insoluble in water which uses one α-value for the first extraction and a second α-value for successive steps. These equations are modifications of classical partition work (Craig and Craig, 1950) in terms of the p-value of Beroza *et al.* (1969) as applicable to aqueous residue analysis.

RESULTS

Experimental α_1-values for the first step of LLE for the solvents studied have been presented for 1:1, 5:1, and 10:1 water:solvent ratios (Suffet and Faust, 1972). Table I gives experimental α_1-values for other common first steps of 10, 20, and 40:1 water:solvent ratios. Volume correction factors of V_n/V_s and V_p/V_h, the changes of volume before and after extraction in the solvent and water phases, respectively, are also calculated for reference. V_n and V_p have been defined. V_s and V_h are the volumes of the solvent and water phases before LLE, respectively.

Table II shows the amount of solvent required to saturate 1 l. of 0.2 *M* orthophosphate buffer at 25 ± 0.5°. These volumes were extrapolated from experimental α_1-value data of Suffet and Faust (1972) and Table I. The experimental α_1-values compare favorably to the solvent's solubility in water (Marsden and Mann, 1963). Therefore, the solubility of a solvent in water can be utilized as a first approximation of the saturation volume at a particular temperature for 0.2 *M*, pH 4.3 orthophosphate buffer.

Experimental α_2-values for the second and/or successive steps of LLE have been determined for the water:solvent ratios of 10, 20, and 40:1 in Table III for water saturated with solvent and unsaturated solvent. After the initial extraction and associated solvent changes, the experimental α_2-values are almost the same as theoretical α_2-values (Table III).

Parameter Choice for Quantitative LLE of Organic Pesticides from Aqueous Systems. The general criteria for quantitative extraction of pesticides from aqueous systems have been shown (Faust and Suffet, 1972). The parameters of choice for aqueous extraction based upon

these general criteria are the choice of a LLE solvent for which the solute has a p-value near 1.00, the adjusting of aqueous properties to stabilize the solute, and the adjusting of aqueous properties for best recovery; *i.e.*, pH and ionic strength. The parameters chosen for pH and ionic strength were the same as used for determining p-values.

The major choices of parameters for a serial LLE procedure would be, in order of importance: the smallest aqueous volume which will give a sufficient amount of pesticide for quantitative analysis; the minimum number of extraction steps; the minimum total volume of solvent used; and the largest solvent extraction volumes to be utilized in the earlier extraction steps. The choices of a water: solvent ratio, minimum volumes, and/or minimum steps to give acceptable recoveries are presented, based upon a 1-l. aqueous sample volume. A computer evaluation to determine the best recovery methodology was developed for successive extractions, varying the solvent volumes used in multiples of 50 ml, setting maximum number of extractions at 5 and maximum total volume at 500 ml. Acceptable pesticide recovery levels of 0.95 or 0.99 were arbitrarily chosen.

Table IV presents a summation of computer readouts, giving the choices for serial LLE procedures on the basis of least steps and/or least solvent for different typical p-values (0.90, 0.95, and 0.99). Two groups of solvents are considered, solvents almost completely insoluble in water (hexane, benzene, and chloroform) and solvents somewhat more soluble in water (ethyl acetate and ether). In the latter case, the computer program adjusts for changes from α_1 to α_2 for the change of water:solvent volumes after the first extraction. The first choice for a serial LLE procedure in each group is primarily determined by the use of the largest solvent extraction volumes in the early extraction steps.

Table IV shows that hexane, benzene, and chloroform can serially extract from water 95% of a pesticide whose p-value in an aqueous solvent system is ≥ 0.90. Ether and ethyl acetate require solutes with a p-value of >0.90.

Method of Evaluation of the Equations for Successive Extraction. Evaluation of the theoretical equations and p-values determined can be done by literature comparison, laboratory fortification procedures at high concentrations for a specific testing method, and fortification at field concentrations. The p-value gives a theoretical guidepost for the setting up of an aqueous extraction procedure. The value must be checked by fortification procedures. Fortification of water under the conditions of the p-value is the recommended procedure; *i.e.*, a completely dissolved system not associated with suspended matter.

The error associated with a p-value was reported as ± 0.02 obtained for equal volumes of the two equilibrated solvents (Bowman and Beroza, 1965). The p-values error could be less in the present study, as p-values are determined from E-values with unequal phase volumes. This is presently under statistical investigation. Table IV indicates that in any serial LLE, 99% of a pesticide will be extracted from 1 l. of water adjusted to p-value conditions with two 100-ml portions of solvent. If the p-value is off by 0.01 (p = 0.98), after two extractions 97% will be extracted. If the p-value is off by 0.02 (p = 0.97), after two extractions 94% will be extracted. On the other hand, if the p-value is off by 0.01 or less in the positive direction (p = 0.99–1.00), two 10:1 water:solvent extractions will give greater than 99% recovery.

Comparison of F_n and Experimental Data on Fractions Recovered. Table V presents a comparison between the theoretical calculated F_n from p-value data (Suffet and Faust, 1972) and experimental recovery data from the literature for organophosphate pesticides. F_n values and recovery data are both presented on a fractional scale. The literature recovery values are based upon extraction from many different types of aqueous samples, whereas the F_n values are based upon calculation from one type of water and a fixed extraction condition. Therefore some variation is expected between F_n and recovery data. There are two conditions where F_n calculations appear inconsistent with actual recovery data: where the actual water character extracted is different, and where p-values are >0.99 and small water:solvent ratios (α-values) are used.

In the first category cases, Table V, ref *c*, were extracted after the addition of a strong acid. Apparently the lower pH value increases the efficiency of extraction. The p-value of the solute therefore, if determined under these conditions, should be higher than at the pH values studied herein. In the second category are cases where α = 0.05, Table V, ref *b*. A pH effect may also be present here.

Table III. α-Values for Multiple LLE Steps, Water (Saturated with Solvent) to Unsaturated Solvent

Ratio of water: solvent[a]	System	Experimental α_2-value	Theoretical α_2-value
40:1	Ethyl acetate and pH 4.3 buffer	0.029	0.025
20:1	Ethyl acetate and pH 4.3 buffer	0.048	0.050
10:1	Ethyl acetate and pH 4.3 buffer	0.097	0.100
40:1	Ethyl ether and pH 4.3 buffer	0.026	0.025
20:1	Ethyl ether and pH 4.3 buffer	0.049	0.050
10:1	Ethyl ether and pH 4.3 buffer	0.096	0.100
40:1	Benzene and pH 4.3 buffer	0.025	0.025
20:1	Benzene and pH 4.3 buffer	0.050	0.050
10:1	Benzene and pH 4.3 buffer	0.100	0.100
40:1	Hexane and pH 4.3 buffer	0.025	0.025
20:1	Hexane and pH 4.3 buffer	0.050	0.050
10:1	Hexane and pH 4.3 buffer	0.100	0.100
40:1	Chloroform and pH 4.3 buffer	0.025	0.025
20:1	Chloroform and pH 4.3 buffer	0.050	0.050
10:1	Chloroform and pH 4.3 buffer	0.100	0.100

[a] Original volumes: 40:1, V_h = 700 ml; V_n = 17.5 ml. 20:1, V_n = 700 ml; V_n = 35 ml. 10:1. V_h = 700 ml; V_n = 70 ml.

Recovery Study of an LLE Step at High Concentration. Two methods of serial liquid–liquid extraction are in use: the classical manual separatory funnels method and the vortex stirring method (American Public Health Association, 1971; Kawahara *et al.*, 1967; Schafer *et al.*, 1969). Vortex stirring occurs when volumes of solvent and water are stirred at a sufficient rate to produce bubbles of air escaping from the base of the vortex.

The time for partition equilibrium for both methods was followed. The amount of 2,4-D extracted from 0.25, 1, and 3 l. of 0.2 M orthophosphate buffers at pH 2 remained constant from 2 to 60 min. Benzene was the solvent utilized at a 10:1 water:solvent ratio. The p-value of 2,4-D in the binary solvent system of benzene and 0.2 M orthophosphate buffer at pH 2 is 0.915 (Suffet, 1973). This p-value was determined by the methodology described in Part 1 of the series (Suffet and Faust, 1972).

Figure 1 shows plots of F_n *vs.* n for the extraction of 50 mg/l. of 2,4-D from 0.2 M pH 2 orthophosphate buffer with benzene for theoretical, vortex mixing, and separatory funnel analysis. Each point is the average of three individual runs.

The F_1 value from serial extraction of 2,4-D [water:benzene (10:1)] is equivalent to the E-value determined for 2,4-D in this system. These F_1 values fall well within the 99% confidence interval for the E-value determined (Suffet, 1973). The vortex and the separatory funnel methods are shown to be equivalent procedures for extraction efficiency of a direct LLE analysis system.

Previous data on residue level samples of chlorinated hydrocarbon pesticides at 200 and 400 ng/850 ml in distilled water illustrated a recovery advantage afforded by a

Table IV. Guide to Selection of Optimum Parameters for Quantitative Serial LLE of Organic Pesticides from Water

Solvents	p-Value	Fraction recovery	Steps	Total volume, ml	Serial extraction procedure, ml	F_n				
						F_1	F_2	F_3	F_4	F_5
Solvents almost completely insoluble in water										
Hexane, benzene, and chloroform	0.90	0.95	4	500	150, 150, 100, 100	0.57	0.82	0.90	0.95	
			4	500	150, 150, 150, 50	0.57	0.82	0.92	0.95	
			4	500	200, 100, 100, 100[a]	0.64	0.81	0.90	0.95	
	0.90	0.99		None possible with 8 steps and 500 ml of solvent; therefore none are recommended.						
	0.95	0.95	2[b]	350	200, 150[a]	0.79	0.95			
			2	400	200, 200	0.79	0.95			
			2	400	250, 150	0.83	0.96			
			2	400	300, 100[c]	0.85	0.95			
	0.95	0.99	3	500	200, 150, 150[c]	0.79	0.95	0.99		
			3[b]	500	200, 200, 100[a]	0.79	0.95	0.99		
			4[b]	400	100, 100, 100, 100	0.66	0.88	0.96	0.99	
	0.99	0.95	1	200	200	0.95				
			2	100	50, 50[a]	0.83	0.97			
			2	150	100, 50	0.91	0.99			
	0.99	0.99	2	200	100, 100	0.91	0.99			
			2	200	150, 50[a]	0.94	0.99			
			3	150	50, 50, 50	0.83	0.97	0.995		
Solvents somewhat more soluble in water										
Ethyl acetate and ethyl ether	0.90	0.95		None possible with 8 steps and 500 ml of solvent; therefore none are recommended.						
	0.90	0.99		None possible with 8 steps and 500 ml of solvent; therefore none are recommended.						
	0.95	0.95	3	400	150, 150, 100	0.57	0.88	0.96		
			3	400	200, 100, 100	0.69	0.89	0.96		
			3	400	200, 150, 50	0.69	0.92	0.96		
			3	400	250, 100, 50[a]	0.76	0.91	0.95		
			4	350	100, 100, 100, 50	0.32	0.75	0.91	0.95	
			4	350	150, 100, 50, 50	0.57	0.85	0.92	0.96	
			4	350	200, 50, 50, 50	0.69	0.84	0.92	0.95	
	0.95	0.99	4	500	200, 100, 100, 100[a]	0.69	0.89	0.96	0.99	
			5	500	250, 100, 50, 50, 50	0.76	0.91	0.95	0.98	0.99
			5	500	200, 150, 50, 50, 50	0.69	0.92	0.96	0.98	0.99
			5	500	200, 100, 100, 50, 50	0.69	0.89	0.96	0.98	0.99
			5	500	150, 150, 100, 50, 50	0.57	0.88	0.96	0.98	0.99
			5	500	150, 100, 100, 100, 50	0.57	0.85	0.94	0.98	0.99
			5	500	100, 100, 100, 100, 100	0.32	0.75	0.91	0.97	0.99
	0.99	0.95	2[d]	150	100, 50[a]	0.71	0.95			
			2	200	100, 100	0.71	0.97			
			2	200	150, 50[e]	0.88	0.98			
	0.99	0.99	2	250	150, 100	0.88	0.99			
			2	250	200, 50[a]	0.92	0.99			
			3	200	100, 50, 50	0.71	0.95	0.99		

[a] First choice. [b] Not applicable to $CHCl_3$ at less than acceptable recovery. The solubility of $CHCl_3$ in 0.2 *M* orthophosphate buffer is 7.4 ml/l. at 25°. [c] First choice for chloroform. [d] Not applicable to ethyl ether at less than acceptable recovery. The solubilities of ethyl acetate and ethyl ether in 0.2 *M* orthophosphate buffer are 74 and 79 ml/l., respectively, at 25°. [e] First choice for ether.

vortex device over the classical separatory funnel method (Kawahara *et al.*, 1967). The recovery efficiency cited was for the whole procedure (extraction, concentration of sample, and gas chromatographic analysis). Extraction time was 30 min with an 8.5:1 water:solvent ratio. Assuming equilibrium, the partition law should be constant for vortex mixing and the manual method in a constant volume. Supersaturation of the solvent is unlikely.

Table VI shows a statistical description of the results of serial LLE by both the manual and vortex methods. The total error concept (McFarren *et al.*, 1970) is employed for the direct analysis of a set of LLE steps to assess the contribution of this step to the total error of a complete residue procedure. The LLE step in aqueous residue analysis appears to contribute substantially to the total error (McFarren *et al.*, 1970) as the common procedures of gas chromatographic analysis after recovery and cleanup of residues from crops and soils have acceptable total errors (Farrell, 1972). Aqueous residue analysis including the same steps does not (McFarren *et al.*, 1970). Therefore the evaluation of the contribution of the LLE step to the total error is desirable. When the causes of these error contributions have been determined, corrective action can be employed.

Table VI shows that a total error of up to 17.4% was found for four successive extraction steps. The error can be attributed to the LLE step. Table VI also shows that the total error associated with a multistep procedure decreases as the number of steps increases; *e.g.*, F_4, a four-step analysis has a lower total error than a F_3, a three-step analysis. This is because the predicted and actual fraction extracted (F_n) asymptotically converge to 1.00 (the maximum fraction extracted) as the number of steps (n) increases. This finding appears contrary to the general criteria for quantitative extractions of trace organics, that is, the minimum number of different steps, is best for analysis, but the importance of additive errors with each successive

Table V. Theoretical Calculation of F_n (Recovery) from p-Value Data of Suffet and Faust (1972) vs. Experimental Recovery Data from the Literature

Solvent	F_n calculated from p-value: p-Value	pH	F_n calculated	F_n recovery observed from literature: Observed F_n recovery	pH	Water:solvent ratio	Reference
Malathion							
Hexane	0.98	6.0	0.99	0.88–0.996[a]	6.6–6.9	0.05	Ragab (1968)
Benzene	0.99	6.0	0.91	0.983–1.006		0.10	Pionke *et al.* (1968)
Benzene[b]	0.99	6.0	0.83	0.97–1.05		0.05	Konrad *et al.* (1969)
Diazinon							
Hexane	0.95	7.4	0.95	0.99	7.0–7.4	1.00	Gomaa (1970); Gomaa *et al.* (1969)
Benzene	0.99	7.4	0.91	0.972–0.992		0.10	Pinoke *et al.* (1968)
Benzene[b]	0.99	7.4	0.83	0.92–1.04		0.05	Konrad *et al.* (1969)
Diazoxon							
Hexane	0.84	7.4	0.84	0.84	7.0–7.4	1.00	Gomaa (1970)
Hexane	0.84	7.4	0.57	0.57	7.0–7.4	0.25	Gomaa *et al.* (1969)
IMH							
Chloroform	0.44	7.4	0.44	0.44	7.0–7.4	1.00	Gomaa (1970)
Parathion							
Hexane[c]	0.89	3.1	0.45	>0.90	Strong acid	0.10	Warnick and Gaufin (1965)
Benzene[c]	0.88	3.1	0.78	0.99–1.00	Strong acid	0.05	Mulla *et al.* (1966)
Benzene[b]	0.88	3.1	0.78	0.93–1.00		0.05	Konrad *et al.* (1969)
Ethyl acetate[c]	0.84	3.1	0.84	0.99	1–1.5	1.00	Gomaa (1970)
Paraoxon							
Ethyl acetate	0.98	3.1	0.98	0.987	1–1.5	1.00	Gomaa (1970)
p-Nitrophenol							
Ethyl acetate	0.99	3.1	0.99	0.996	1–1.5	1.00	Gomaa (1970)
Baytex							
Hexane[c]	0.93	3.4	0.57	>0.90	Strong acid	0.10	Warnick *et al.* (1966)

[a] Five extractions of water phase, with saturated NaCl. [b] Case where $\alpha = 0.05$; F_n calculated is much less than observed F_n recovery. [c] Case, strong acid; F_n calculated is much less than observed F_n recovery.

extraction becomes insignificant at the high levels studied. The procedures employed a high residue concentration (50 mg/l.) and additive errors occurring at field concentration levels (ng/l.) are not observed. Trace analysis errors due to laboratory manipulations at low levels (*e.g.*, sorption losses on glassware, spills, and pipetting errors) are comparatively small. Since intermediate steps between LLE and quantitation such as evaporation and clean-up are not used, these errors are not present.

Therefore the total error concept (Table VI) describes the optimum value with minimum error for quantitative analysis. These are the goals toward which performance at trace concentration levels should strive. There is an extraction efficiency (signal) to error (noise) ratio which occurs during a trace analysis.

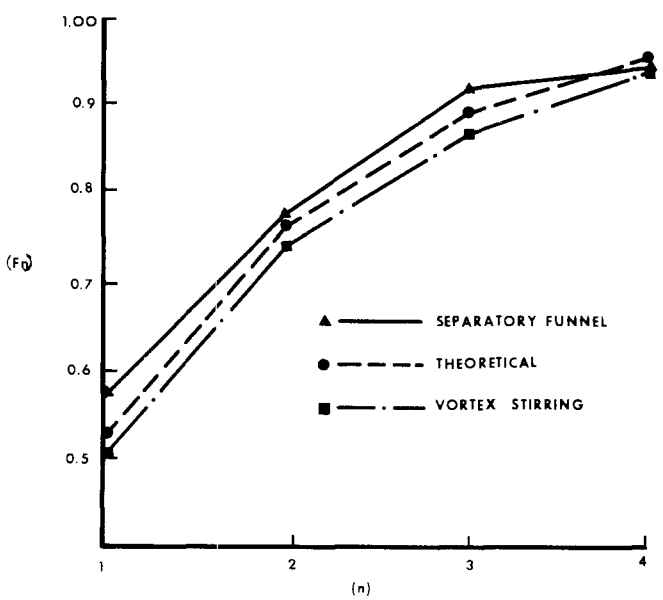

Figure 1. Number of extractions (n) *vs.* the total fraction extracted (F_n) for serial extraction of 50 mg/l. 2,4-D from 1 l. of pH 2.0, 0.2 *M* orthophosphate buffer at a water:benzene ratio of 10:1. The p-value of 2,4-D equals 0.915. Each point is the average of three runs.

DISCUSSION

Binary solvent systems in which the p-values of the solute approach 1.00 are sought. Table IV shows that a p-value of 0.90 can be considered a lower limit to ensure 95% of recovery of a pesticide from 1 l. of water with nonpolar solvent in ≤500 ml of solvent and ≤5 extraction steps. An accurate determination of the p-value is critical when the p-value is ≥0.90. For example, if a p-value of a pesticide in an aqueous solvent system is really 0.02 lower than the

Table VI. Assessment of Total Error[a] Associated with a Serial LLE Step; Extraction of 2,4-D from Water by Benzene[b]

Average of set of 3	Std dev	Theoretical F_n (p = 0.915)	Mean error	Total error, %
Method of vortex stirring				
$F_1 = 0.496$	0.034	0.518	−0.022	17.4
$F_2 = 0.736$	0.014	0.768	−0.032	7.81
$F_3 = 0.866$	0.003	0.888	−0.022	3.15
$F_4 = 0.932$	0.007	0.946	−0.014	2.96
Separatory funnel method				
$F_1 = 0.566$	0.016	0.518	+0.048	15.4
$F_2 = 0.777$	0.021	0.768	+0.009	6.64
$F_3 = 0.914$	0.013	0.888	+0.026	5.86
$F_4 = 0.934$	0.008	0.946	−0.012	2.96

[a] Total error = absolute value of mean error + 2 × standard deviation × 100 divided by the true value (McFarren *et al.*, 1970). [b] Water:benzene 10:1, 0.2 *M*, pH = 2 orthophosphate buffer, 25°.

p-value of 0.94 for a water:solvent ratio (*i.e.*, 10:1) F_1 would contain 0.51 and not 0.60. Therefore it is necessary to assure that the p-value is correct by the fortification technique.

Since a 100% extraction is the goal of analysis, liquid–liquid extraction procedures should utilize multiple extractions. Multiple extractions will decrease error associated with p-value determinations. The greater the number of extractions for the same total volume of solvent, the more solute will be extracted, and the use of large initial volume extracts a major portion of solute on the first extraction (eq 4). Equation 4 is a nonlinear function which approaches asymptotically an F_n value of 1.00 as the number of successive extractions increases. Many aqueous residue procedures have recommended a large volume of solvent for the first step, since the additional extractions are performed on partially depleted aqueous solutions and do not involve the portion of solute which has already been extracted. Computer calculations can optimize the best water:solvent ratio and number of steps of extraction, as exemplified by Table IV.

The equations developed for the fraction extracted, F_n (in a serial LLE recovery), for a specific pesticide are as accurate as the determined p-value. Future work should delineate the error in the p-value determination statistically to determine its capabilities and thereby see if the 0.98–1.00 p-value $-$ E-value region can be expanded to give an accurate approximation of a theoretical F_n-value. Recovery data and p-values should also be statistically compared using constant water properties.

ACKNOWLEDGMENT

The author wishes to express his appreciation to M. G. Harasewych for his efforts in the development of the computer program and to C. Marcinkiewicz for his useful suggestions while critically analyzing the manuscript. The author also wishes to acknowledge Samuel D. Faust, in whose laboratory the work was initiated.

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Received for review May 25, 1972. Accepted December 14, 1972. This research was supported by Research Grant No. 801179 (formerly 16020FYT), Office of Water Research, Environmental Protection Agency, Washington, D. C. Computer program of equation 4 will appear in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-73-288.