The *p*-Value Approach to Quantitative Liquid–Liquid Extraction of Pesticides from Water. 1. Organophosphates: Choice of pH and Solvent

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Beroza and Bowman have developed the idea of *p*-value for confirmation of insecticide identity and for cleanup of insecticides at the nanogram level. In this paper the concept of using the *p*-value for determination of the parameters of solvent choice for quantitative liquid–liquid extraction of organophosphate pesticides from water are reported. The *p*-value method is modified to suit the present application. The *p*-value can be used to determine the quantitative extraction of the parent, oxon, and hydrolysis products of Diazinon, Parathion, Malathion, and Baytex.

The first step of aqueous residue analysis consists of pesticidal extraction. It is recommended that any pesticide analysis of water should consider the quantitative aspects of the extraction as well as the subsequent determinative steps. Thus, a more complete recovery picture of the pesticide and its environmental fate can be followed.

A *p*-value approach for the determination of the parameters for quantitative liquid–liquid extraction (LLE) of some organophosphate pesticides will be outlined. *p*-Values have previously been utilized by Bowman and Beroza (1965, 1966) for confirmation of insecticide identity and for cleanup at the nanogram level. Suffet and Faust (1971) have outlined the theoretical *p*-value approach for application to LLE of pesticides from aqueous solution. The *p*-value is defined as "the fraction of the total solute that distributes itself in the nonpolar phase of an equivolume solvent pair" (Beroza *et al.*, 1969).

This paper deals with the selection of pH value and solvent from *p*-values for organophosphate systems, consisting of the parent molecule, oxon, and hydrolysis products of Diazinon, Baytex (Fenthion), Parathion, and Malathion (Table I).

The authors have reviewed the subject of liquid–liquid extraction of organophosphate pesticides from water (Faust and Suffet, 1966, 1969, 1972). Serial and continuous extractions of organophosphate pesticides were employed in several papers that were concerned with laboratory and/or field studies. Tables in these reviews showed the available percent recovery data by fortification procedures for several pesticides.

The methods of extraction were concerned with one pesticide, a related group of pesticides, or a pesticide and its degradation products. Each procedure arbitrarily utilized a particular solvent and specified the number of extractions and various solvent to water ratios. In some cases, percent recovery, as determined by a technique of fortification, was the only experimental justification for selection of the solvent.

Many questions can be asked of these procedures: What dictated the selection of the solvent? What are the effects of such variables as pH, ionic strength, temperature, and turbidity on efficiency of extraction? What is the proper pH for sample storage between the time of sampling and extraction? What is the optimum pH for extraction? What is the most efficient solvent to water ratio to use? How many times must the sample be reextracted for maximum recovery? For the most part, answers to these questions were not offered. It is entirely possible that the most efficient extractive parameters were not used.

EXPERIMENTAL

Reagents and Solvents. Water doubly distilled from alkaline permanganate was utilized for all aqueous solutions. All solvents were of "pesticidal quality" grade. Reagent grade H_3PO_4, KH_2PO_4, Na_2HPO_4, $Na_3PO_4 \cdot H_2O$ were used for all orthophosphate buffers (Christian and Purdy, 1962). Reagent grade Na_2SO_4 (anhydrous crystals) was utilized as a drying agent. Glassware was soaked in an acid bath (1:10 HNO_3:H_2SO_4) for at least 24 hr after cleaning with a detergent and before use. Glass wool was preextracted with solvent.

Analytical. Two gas chromatographs were used, a Research Specialities 600 Series with a flame ionization detector and a Microtek MT-220 with a 10 mCi of ^{63}Ni electron capture detector. Reoplex-400 gas–liquid chromatographic (glc) columns were utilized (Suffet and Faust, 1967). Diazinon, Diazoxon, IMHP, and MMTP were resolved and were quantitated under these conditions with a 4-ft column. Baytex, Bayoxon, Malathion, Parathion, and Paraoxon were quantitated individually with a 2-ft Reoplex-400 column under a flow rate of 100 ml/min under the chromatographic conditions previously described for the Ni^{63} detector. The absolute retention time of these compounds was thereby halved. Diethyl fumarate and diethyl maleate were quantitated on the 2-ft column and Ni^{63} detector at 90° C and 75 ml/min nitrogen. *p*-Nitrophenol was quantitated with the flame ionization detector and a 1-ft Reoplex-400 column under the conditions previously reported.

A Beckman DK-2A spectrophotometer with matched 1-cm far uv silica cells was utilized for all ultraviolet measurements.

Aqueous Sample Conditions. Experimental conditions for determination of the *p*-values were: temperature 25° C; ionic strength 0.2 *M*; and the pH value was set with the 0.2 *M* orthophosphate buffer. All aqueous solutions were spiked by transferring an aliquot of a stock solution of the

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pesticide in an appropriate solvent into a volumetric flask. If the transfer solvent was not water and the compound's vapor pressure was low, then solvent was evaporated slowly under an atmosphere of nitrogen. In the case of compounds with high vapor pressure (diethyl fumarate and diethyl maleate), an aliquot of a stock solution was transferred in ethanol so that the water solution contained less than 0.5% ethanol. Solubility of the compound of interest was tested by a modification of Bohen and Claussen's method (1951) at the concentration utilized for the experimental procedure. The specific experimental conditions for each compound are shown in Table II.

p-**Value Test Procedure.** The *p*-value determination consists of shaking known volumes of water containing a pesticide and a solvent in a glass-stoppered graduated cylinder. Then the phases separate and equilibrate in a water bath for at least 10 min.

A portion of the solvent phase could be analyzed directly after equilibration and compared to a standard curve with a flame ionization detector. Direct analysis of the equilibrated solvent is not possible with an electron capture detector as the Ni^{63} detector responded anomalously to repeated injections of water-saturated solvent. Therefore, the solvent is dried (on a 2-in. column made of a Pasteur pipette containing Na_2SO_4 and adjusted to volume) before injection onto the glc column.

For ultraviolet analysis, if the solvent does not absorb at or above the wavelength of maximum absorption of the compound of interest, a portion of the water phase is analyzed directly. If the solvent absorbs at the wavelength of maximum absorption, it must be stripped from the water phase with nitrogen (approximately 10 min). A portion of the solvent phase can be analyzed directly or after dilution if the solvent does not interfere with the compound's wavelength of maximum absorption.

Calculation of the *p*-**Value.** The *p*-value (for an equal, equilibrated, two-phase solvent system) is equal to

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

where the *E*-value is the fractional amount of pesticide extracted into the nonpolar phase of an unequal, unequilibrated, two-phase solvent system and α is a volume correction factor to place the *E*-value on a consistent *p*-value basis.

Calculation of the *E*-**Value.** If ultraviolet spectroscopy is employed, the *E*-value can be calculated from

$$E = 1 - \frac{A_s - A_n}{A_s} \times \frac{V_p}{V_h} \tag{2}$$

where $A_s - A_n$ is the amount (mg) in the water phase after extraction and equilibration, and A_s is the amount (mg) in the water phase before extraction and equilibration or the maximum amount possibly extracted into the nonpolar phase. V_h is the water volume phase to be extracted. V_p is the volume of water after extraction and equilibration. If the solvent absorbs at the wavelength maxima, it must be stripped from the water phase, whereupon

$$E = 1 - \frac{A_s - A_n}{A_s} \tag{3}$$

The number of micrograms that is left in the water phase ($A_s - A_n$) is the only experimental variable. A_s is known and $A_s - A_n$ is determined from a standard uv curve. The method of Beroza and Bowman (1965a,b, 1966; Bowman and Beroza, 1965, 1966; Beroza *et al.*, 1969) was utilized for gas chromatography analysis and uv analysis of the solvent phase.

$$E = \frac{A_n}{A_s} \times \frac{V_n}{V_s} \tag{4}$$

Table I. Organophosphate Parent Compounds, Their Oxons, and Hydrolysis Products

Name	% Purity	Generic name
Diazinon	99.0	*O,O*-Diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate
Diazoxon	96.97	*O,O*-Diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphate
IMHP	Recrystallized, mp 174.5–175.5° C	2-Isopropyl-4-methyl-6-hydroxypyrimidine
Baytex[a]	99.6	*O,O*-Dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate
Bayoxon		*O,O*-Dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphate
MMTP	Recrystallized, mp 54–56° C	3-Methyl-4-methylthiophenol
Parathion	99.5	*O,O*-Diethyl *O,p*-nitrophenyl phosphorothioate
Paraoxon		*O,O*-Diethyl *O,p*-nitrophenyl phosphate
p-Nitrophenol	Recrystallized, mp 114° C	*p*-Nitrophenol
Malathion	99.5	*O,O*-Dimethyl *S*-(1,2-dicarbethoxyethyl)phosphorodithioate
Malaoxon		*O,O*-Dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorothioate
Basic hydrolysis products of malathion	bp 217.9° C[b]	Diethyl fumarate (*trans*)
	bp 225° C[b]	Diethyl maleate (*cis*)

[a] Alternate name = Fenthion. [b] Eastman White Label Grade, Eastman Organic Chemicals.

Table II. Summary of Experimental Conditions for the LLE *p*-**Value Determination of Organophosphate Systems**

System	Concentration (mg/l.)[a] 1:1 Ratio	Concentration (mg/l.)[a] 5,10:1 Ratio	Method of quantitation	Sensitivity of quantitative method,[b] ng
Diazinon				
Diazinon	20	5	glc-ecd	40
Diazinon		20	uv[c]	
Diazoxon	40	10	glc-ecd	160
IMHP	200		glc-ecd	500
Baytex				
Baytex		5	glc-ecd	250
Baytex		5	uv[d]	
Bayoxon		28	glc-ecd	800
Bayoxon		40	uv[c]	
MMTP		62.5	glc-fid	1000
Parathion				
Parathion	14.4	2.9	glc-ecd	35
Paraoxon	30	6	glc-ecd	100
p-Nitrophenol	200		glc-fid	2000
p-Nitrophenol	200	20–200	uv[c]	
Malathion				
Malathion		12.5	glc-ecd	125
Malaoxon		80	glc-ecd	1350
Diethyl fumarate		9	glc-ecd	18
Diethyl maleate		24	glc-ecd	50

[a] All compounds are soluble in water at these concentrations. [b] Sensitivity of method = the maximum amount injected into a glc column assuming complete extraction. This is set at greater than 50% full scale deflection. [c] uv: 1 cm cells, water phase analyzed. [d] uv: 1 cm cells, hexane phase analyzed.

Table III. Volume Correction Factors for the One-Step LLE Method

1:1 water:solvent ratio[a]

System	Final volume, ml	V_n/V_s	V_p/V_h	$\alpha = V_n/V_p$
Ethyl acetate	V_n = 480			
+		0.96	1.04	0.93
pH 4.3 buffer	V_p = 518			
Ethyl ether	V_n = 464			
+		0.93	1.05	0.89
pH 4.3 buffer	V_p = 523			
Benzene	V_n = 495			
+		0.99	1.00	0.99
pH 4.3 buffer	V_p = 500			
Hexane	V_n = 497			
+		1.00	1.00	1.00
pH 4.3 buffer	V_p = 500			
Butanol	V_n = 440			
+		0.88	1.10	0.80
pH 4.3 buffer	V_p = 500			
Chloroform	V_n = 490			
+		0.98	1.01	0.97
pH 4.3 buffer	V_p = 505			
Carbon tetrachloride	V_n = 495	0.99	1.00	0.99
+				
pH 4.3 buffer	V_p = 500			

[a] $V_s = V_h$ = 500 ml.

Table IV. Volume Correction Factors for the One-Step LLE Method

A. 5:1 water:solvent ratio

System	Final volume, ml	V_n/V_s	V_p/V_h	$V_n/V_p = \alpha$
Ethyl acetate[a]	V_n = 99			
+		0.62	1.06	0.12
pH 7.4 buffer	V_p = 852			
Ethyl ether[a]	V_n = 96			
+		0.60	1.06	0.11
pH 7.4 buffer	V_p = 853			
Hexane[a]	V_n = 159			
+		0.99	1.00	0.20
pH 7.4 buffer	V_p = 800			
Benzene[a]	V_n = 157			
+		0.98	1.00	0.20
pH 4.3 buffer	V_p = 800			
Chloroform[b]	V_n = 134			
+		0.96	1.01	0.19
pH 4.3 buffer	V_p = 706			
B. 10:1 water:solvent ratio[c]				
Benzene	V_n = 87			
+		0.97	1.00	0.10
pH 7.4 buffer	V_p = 900			
Hexane	V_n = 89			
+		0.99	1.00	0.10
pH 7.4 buffer	V_p = 900			

[a] V_s = 160 ml; V_h = 800 ml. [b] V_s = 140 ml; V_h = 700 ml. [c] V_s = 90 ml; V_h = 900 ml.

V_n/V_s is a correction factor due to unequilibrated phase volumes and/or unequal original phase volumes. V_n is the volume of the nonpolar phase after extraction and equilibration. V_s is the original volume of the nonpolar phase before equilibration and extraction.

The admixture of two unequilibrated solvents at constant temperature will show that each solvent's volume will change after equilibration. This is caused by mutual solubility (Marsden and Mann, 1963). The mutual solubility phenomenon requires that appropriate correction factors must be determined and applied.

The volume correction factors (V_n/V_s, V_p/V_h, and $\alpha = V_n/V_p$) were determined by Bowman and Beroza (1966) simultaneously with each p-value. However, in the present p-value applications, it was deemed more precise and convenient to determine the volume changes independently. Correction factors were determined under the conditions of the p-value method in a 1-l. graduated cylinder fitted with a ground glass stopper. The solvents were mixed well and left to equilibrate for 24 hr. 0.2 M orthophosphate buffers were used.

Tables III and IV show the volume correction factors V_n/V_s, V_p/V_h, and α. These corrective factors for any unequilibrated binary system can be determined by this method. The original water to solvent ratios of 1:1, 5:1, and 10:1 were evaluated as these were utilized throughout the LLE p-value determinations. The correction factors are reported to two significant figures.

EXPERIMENTAL RESULTS

Tables V, VI, VII, and VIII show the p-values determined for the Diazinon, Baytex, Parathion, and Malathion systems, respectively. Beroza and Bowman (1965, 1966) stated that for single extractions the precision of the one-step p-value determination is ±2%. The triplicate p-values obtained in this study were within this range.

Effect of Solute Concentration, Ionic Strength, and Temperature on p-Values. Theoretically the distribution coefficient and the p-value are independent of concentration up to 10,000 mg/l. (Bowman and Beroza, 1966). The p-value of p-nitrophenol (0.2 M orthophosphate buffer:ethyl acetate) was studied from 20–200 mg/l. to determine if its partition isotherm was linear in this experimental setup. The p-value

Table V. Liquid–Liquid Extraction of Diazinon System from 0.2 M Orthophosphate Buffers at 25° C ± 0.5° C

Compound	pH	p-Value: Hexane	Benzene	Ethyl acetate	Ether	$CHCl_3$	CCl_4	n-Butanol
Diazinon	7.40	0.95	0.99	0.95	0.99+			
Diazoxon	7.40	0.84	0.99	0.95	0.95			
IMHP	2.05		(0.15)	(0.13)		(0.17)		
	3.00		(0.15)	0.41		0.36	(0.15)	0.65
	4.30		(0.15)	0.38	(0.14)	0.48	(0.15)	0.64
	6.40		(0.15)	0.41		0.49	(0.15)	0.66
	7.40	0.02[a]	(0.12)	0.38	(0.14)	0.44		0.66

[a] The difference of a chloroform extraction and a series of (A) hexane and (B) chloroform extractions was used to confirm the 0.02 p-value obtained by a hexane extraction. All values are the average of three determinations except those in parentheses.

Table VI. Liquid–Liquid Extraction of Baytex System from 0.2 *M* Orthophosphate Buffers at 25° C ± 0.5° C

Compound	pH	*p*-Value (average of three determinations)			
		Hexane	Benzene	Ethyl acetate	Ether
Baytex	3.40	0.93	0.97	0.93	0.95
Bayoxon	3.40	0.91	0.99	0.98	0.97
MMTP	3.40	0.58	0.98	0.98	0.98

Table VII. Liquid–Liquid Extraction of Parathion System from 0.2 *M* Orthophosphate Buffers at 25° C ± 0.5° C

Compound	pH	*p*-Value (average of three determinations)					
		Hexane	Benzene	Ethyl acetate	Ether	$CHCl_3$	CCl_4
Parathion	3.10	0.89	0.88	0.84	0.93		
Paraoxon	3.10	0.77	0.99	0.98	0.99		
p-Nitrophenol	3.10	<0.20	0.60	0.99	0.98	0.66	<0.30

Table VIII. Liquid–Liquid Extraction of Malathion System from 0.2 *M* Orthophosphate Buffers at 25° C ± 0.5° C

Compound	pH	*p*-Value (average of three determinations)			
		Hexane	Benzene	Ethyl acetate	Ether
Malathion	6.00	0.98	0.99	0.99	0.99
Malaoxon	6.00	<0.20	0.99	0.99	0.97
Diethyl fumarate	6.00	0.96	0.99	0.99	0.99
Diethyl maleate	6.00	0.75	0.97	0.98	0.97

Table IX. The Effect of Ionic Strength on the *p*-Value Determination of Organophosphate Pesticides

Compound	Concentration	Solvent	pH	Ionic strength	*p*-Value
p-Nitrophenol	50 mg/l.	Ethyl acetate[a]	3.00	0.05 *M*	0.99
				0.10 *M*	0.99
				0.20 *M*	0.99
Baytex	5 mg/l.	Hexane[b]	3.40	0.02 *M*	0.93
				0.2 *M*	0.93
Diazinon	20 mg/l.	Hexane[b]	7.40	0.02 *M*	0.95
				0.20 *M*	0.95

[a] Determined by uv examination of water phase. [b] Determined by uv examination of solvent phase.

Table X. The Effect of Temperature on the *p*-Value Determination of Diazoxon (Hexane:0.2 *M* Orthophosphate Buffer) (*p*-Value = 0.84, Table V)

Diazoxon pH 10.40 Adjusted to pH 7.40 for Extraction[a]			
Temperature, °C	E[b]	*p*-Value[c]	Half-life, hr
20	0.46	0.77	10.1
40	0.45	0.76	3.1
60	0.53	0.82	0.7
Diazoxon pH 3.14 Adjusted to pH 7.40 for Extraction[a]			
Temperature, °C	E[b]	*p*-Value[c]	Half-life, min
10	0.38	0.71	47.8
20	0.44	0.77	22.8
40	0.45	0.76	6.1
60	0.54	0.82	1.7

[a] Hydrolysis experiments were run in 0.02 *M* orthophosphate buffer. The pH was adjusted to 7.40 with NaOH or H_3PO_4 before extraction. [b] Time of extraction, 5 min. [c] Water:hexane ratio 4:1.

remained constant at 0.99 throughout this range of concentrations.

The effect of the ionic strength on the *p*-value of *p*-nitrophenol, Baytex, and Diazinon was studied under the conditions of Table IX. Table IX shows the results. *p*-Values did not change throughout the ionic strength range 0.02 *M* to 0.2 *M* for soluble species. Therefore, *p*-values can be determined with orthophosphate buffers in this range. Larger amounts of salting agents appear to be needed to produce a "salting-out" effect. The *p*-value method alters water conditions to an ionic strength of 0.2 *M*. This is from 4–20 times that of natural waters (Durfor and Becker, 1964; Rainwater, 1962).

The temperature was varied (10° C to 60° C) and solute concentration was kept constant to determine its effect on *p*-values during the study of the kinetics of hydrolysis of Diazoxon (Gomaa *et al.*, 1969). Table X shows the results. Although the half-life time was decreased significantly with temperature (thereby lowering the amount of Diazoxon in solution), the *p*-value was increased. Therefore, temperature must remain constant. Low temperature extraction is generally less efficient (Morrison and Freiser, 1963). This is illustrated as related to *p*-values by Diazoxon (Table X) (Gomaa *et al.*, 1969).

DISCUSSION

The Parathion and Baytex systems appear to be most stable in acid solution (Muhlmann and Schrader, 1957; Ruzicka *et al*, 1967; Weiss and Gakstatter, 1965). The phenolic

hydrolysis products of Parathion and Baytex are undissociated in acid solution (Lange, 1956). The Malathion system appears to be most stable at pH 6.0 (Bender, 1968; Spiller, 1961). Diazinon and Diazoxon are most stable at pH 7.4 (Gomaa *et al.*, 1969). The hydrolysis product IMHP has the ability to form tautomers, *i.e.*, form salts at high and low pH values.

The LLE of Diazinon, Baytex, Parathion, and Malathion, together with the oxons and hydrolysis products of these pesticides, was not considered in any of the studies reported by other investigators (Faust and Suffet, 1966, 1969, 1972).

At pH 7.4, benzene, ethyl acetate, and ether are excellent solvents for the extraction of Diazinon and Diazoxon from water (Table V). If it is desirable to leave IMHP in the water phase, hexane should be used. Butanol is the best solvent for IMHP but it is difficult to use as it has a high viscosity, a great water solubility, and a high boiling point. Therefore, chloroform is the solvent of choice for IMHP.

At pH 3.1, ether is the best solvent for the LLE of the Parathion system (Table VII). At pH 6.0, the Malathion system is best extracted by ethyl acetate followed by benzene and ether (Table VIII). Hexane is also a good solvent for Malathion and has been utilized by Ragab (1968) with 88–98% recoveries.

At pH 3.4, benzene, ether, and ethyl acetate are excellent solvents for the Baytex system (Table VI). Beroza and Bowman (1968) have reported *p*-values of Baytex and Bayoxon for confirmatory evidence in the hexane–water system. The *p*-values were reported as 1.00 for Baytex and 0.92 for Bayoxon. The *p*-values determined in this study were 0.93 for Baytex and 0.91 for Bayoxon in 0.2 *M* orthophosphate buffers of pH = 3.40. The discrepancy between these results and those of Beroza and Bowman (1968) may be due to differences of water quality, *i.e.*, the buffer used in this study.

The final choice of solvent depends upon the desired analysis. An example of the use of *p*-value data will be discussed now for a specific experimental situation. The gas chromatographic procedure of Suffet and Faust (1967) was utilized for the hydrolytic studies of Diazinon and Diazoxon. Since IMHP and Diazoxon are incompletely separated by the glc procedure, Diazinon and Diazoxon were simultaneously extracted by hexane and quantitated by the electron capture detector to which both have a greater sensitivity. This leaves IMHP in the aqueous phase, which was then extracted with chloroform and quantitated by the flame ionization detector, where it has the greater sensitivity (Gomaa *et al.*, 1969).

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