alteration of L or Z. The E_s values for the chloro and ethyl moieties are -1.16 and -1.62, respectively. Changing the ring substituent from chloro to ethyl, therefore, can be compensated for by substituting dichloromethyl ($E_s = -2.78$) for trichloromethyl ($E_s = -3.30$) in the α position.

From the preceding discussion, it is obvious that a large number of new DDT analogs still remain to be synthesized and examined for insecticidal activity. The model that we have developed in predicting the insecticidal activity of DDT analogs is by no means infallible but it provides a more systematic approach to the design of new compounds. The synthesis of new DDT analogs with predictable activity from our analysis is currently in progress.

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The p-Value Approach to Quantitative Liquid-Liquid Extraction of Pesticides and Herbicides from Water. 3. Liquid-Liquid Extraction of Phenoxy Acid Herbicides from Water

Irwin H. Suffet

The goal of aqueous herbicide analysis is the recovery of 100% of an herbicide for qualitative and quantitative analysis. Liquid-liquid extraction is the method of choice for quantitative recovery from water. The p-value concept is useful in developing an understanding of the liquid-liquid extraction process in order to select optimum experimental conditions to approach 100% herbicide recovery. The best solvents for extraction of phenoxy acid herbicides are ethyl acetate and ether. The best solvents for simultaneous extraction of 2,4-D or 2,4,5-T and their n-butyl and isopropyl esters are ether and ethyl acetate (2,4-D and esters) and benzene (2,4,5-T and esters). Possible variation of the p-value caused by alteration of natural water characteristics was tested. The apparent p-values for 2,4-D obtained with waters from different sources and the p-value found in distilled water were found to be consistent. Adsorption characteristics of herbicide esters were changed upon adjustment of turbid water to the aqueous characteristics of the p-value method. Therefore the aqueous sample should be filtered before adjustment of aqueous conditions for liquid-liquid extraction. The p-value gives a theoretical guide (an F_n value) for development of an aqueous residue procedure. Recovery data from the literature and a recovery study at high concentration confirmed the calculated F_n value. A two-step serial extraction with 200 and 50 ml of ethyl acetate under p-value conditions is the choice for extracting 99% of 2,4-D from 1 l. of aqueous solution.

The first step of aqueous residue analysis consists of liquid-liquid extraction (LLE). It is critical to know the efficiency of the LLE step if the data are to be valid as "actual" aqueous concentration data for interpreting ecological problems such as persistence and transport of herbicides in aquatic environments (Faust and Suffet, 1966, 1969, 1972; Suffet and Faust, 1972a). In fact, at low herbicide levels (ng/l.), the efficiency of the LLE step can set the lower limit of detectability of the analytical method. Similar emphasis on the initial extraction step of residues from plant and soil material has been stated by Wheeler and Frear (1966) and Chiba and Morley (1968), respectively.

At the pH of natural waters (pH 5 to 9), chlorinated phenoxy acid herbicides can exist as ionized salts, amine salts, or esters. Since phenoxy acid herbicides are primarily applied as ester derivatives, it appears important to know the concentration of both ester and anion of the free acid to assess herbicide fate. Therefore analytical procedures should individually determine both the free acid herbicide and its ester forms.

A "standard method" of aqueous herbicidal analysis has not been developed which meets the criterion of analytical acceptability of less than 50% total error (McFarren *et al.,*

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Table I. Conditions Used for Several Liquid–Liquid Extractions of Herbicides and Herbicide Esters from Water

Herbicides	Solvents	Sample volume, ml	Solvent volume, ml	pH	Range of recovery, %	Reference
2,4-D, MCPA, 2,4,5-T	CHCl₃	25	3 × 10	<2	84–102	Erne (1963)
2,4-D	1:3 ether–CHCl₃	250	3 × 50	5-ml HCl concn	96.7–104	Aly and Faust (1963)
2,4-D, 2,4,5-T, MCPA, MCPB, 2,4-DB, Dalapon	Ether	100	100	2-ml H₂SO₄ concn	85–100	Abbott et al. (1964)
Silvex, Silvex PGBE	CHCl₃	2000	1 × 100 3 × 50	3.0	65–95	Pope et al. (1966)
2,4-D	CHCl₃	50	3 × 20	<2	85	Erne (1966)
2,4-D, 2,4,5-T, Silvex	Ether	800	150, 2 × 50	2.0	81–110	Goerlitz and Lamar (1967)
2,4-D, 2,4,5-T, Silvex, MCPA, isopropyl and butyl esters of 2,4-D	Benzene	1000	1 × 100 2 × 50	≈2	49–96	Devine and Zweig (1969)
2,4-D, 2,4,5-T, Silvex, 2,4-DB, MCPA, MCPB and esters	1:1 ether– petroleum ether	500	2 × 100 1 × 50	5-ml HCl concn	74–100	Mestres et al. (1969)

Table II. Experimental Conditions for the *p*-Value Determination of Herbicides and Herbicide Esters[a]

Name	Chemical name	pKₐ[b]	Experimental concentration,[c] mg/l.	Method of quantitation[d]
2,4-D	(2,4-Dichlorophenoxy)acetic acid	2.64–3.31	50.0	uv
2,4,5-T	(2,4,5-Trichlorophenoxy)acetic acid	2.88–3.46	38.6	uv
MCPA	(2-Methyl-4-chlorophenoxy)acetic acid	2.90–3.40	74.5	uv
2-CPA	2-Chlorophenoxyacetic acid	2.92–3.05	53.4	uv
4-CPA	4-Chlorophenoxyacetic acid	2.36–3.56	61.8	uv
MCPB	(2-Methyl-4-chlorophenoxy)butyric acid	4.80–6.21	23.8	uv, std additions
2,4-DB	4-(2,4-Dichlorophenoxy)butyric acid	4.58–5.90	16.6	uv, std additions
Isopropyl ester of 2,4-D	Isopropyl ester of (2,4-dichlorophenoxy)acetic acid		1.01	glc, internal std
n-Butyl ester of 2,4-D	n-Butyl ester of (2,4-dichlorophenoxy)acetic acid		0.94	glc, internal std
Isopropyl ester of 2,4,5-T	Isopropyl ester of (2,4,5-trichlorophenoxy)acetic acid		0.80	glc, internal std
n-Butyl ester of 2,4,5-T	n-Butyl ester of (2,4,5-trichlorophenoxy)acetic acid		0.97	glc, internal std

[a] Amchem Products Inc., anal grade. All solids were recrystallized before use. [b] After Nelson and Faust (1969). [c] All compounds are soluble at these concentrations. [d] All uv analyses on the water phase after solvent evaporation step (Suffet and Faust, 1972b).

1970). The total error is 100 times the absolute value of mean error plus two times the standard deviation divided by the true value. Table I shows a summation of some of the phenoxy acid herbicide methods utilized. These procedures use different parameters; e.g., solvents, number of extractions, and aqueous sample–solvent volumes. Some hydrolyze the ester to the acid before analysis. Usually an herbicide acid and the ester of the herbicide are not quantitated individually.

The general criteria for quantitative recovery of organic pesticides from aqueous systems have been described by the author in part 2 of the series (Suffet, 1973). A simple partition function, the *p*-value, sets the partition coefficient on a fractional basis. The *p*-value has been found useful for studying the parameters of the LLE process for aqueous samples (Suffet and Faust, 1972b). The *p*-value enables the direct study of the LLE step. The *p*-value approach to partition was first introduced by Bowman and Beroza (1965, 1966) for two different residue procedures, namely, cleanup by partition and identification by partition at the ng/l. level. Part 1 of the series (Suffet and Faust, 1972b) described the experimental design, method of partition, calculation, and analytical approaches utilized to obtain data for the selection of pH and solvents for LLE of selected organophosphates, their oxons and hydrolysis products. Part 2 of the series (Suffet, 1973) described a computer method of choosing the number of ex-

traction steps and water to solvent ratio for a serial LLE method if the *p*-value is known. It was concluded that the *p*-value of a solute in a water and solvent system must be above 0.90 to recover 95% or more of the solute from 1 l. of water phase in five or less steps using a maximum 500 ml of solvent. The solvents hexane, benzene, chloroform, ether, and ethyl acetate were considered. Only the first three of these solvents could recover 95% at a *p*-value of exactly 0.90. Therefore, a *p*-value is sought that is above 0.90 and is as close to 1.00 as possible. The computer program can delineate the serial extraction process if the *p*-value is known.

This paper will isolate the LLE step from the aqueous residue analysis of herbicides and study it in detail. It will also show the use of the *p*-value for selection of the best solvent for herbicide extraction from water when aqueous characteristics are known. The paper will then show the applicability of the *p*-value for determining other parameters of LLE after testing the effect of natural water properties on the *p*-value obtained. These parameters will be aqueous volume, water:solvent ratio, and the number of steps of serial extraction for maximum recovery. The effect of natural water properties, including the effect upon LLE of turbid water (water of high turbidity) which contains suspended matter (particles) such as clay, finely divided organic matter, plankton, and other microscopic organisms, will also be discussed.

Table III. Natural Water Characteristics of the Schuylkill River Water and Its Tributary, Wissahickon Creek

Natural water characteristics	Schuylkill River and Wissahickon Creek	Schuylkill River[a] high turbidity water
Alkalinity (as $CaCO_3$)	52–99 mg/l.	70 mg/l.
Hardness (as $CaCO_3$)	160–212 mg/l.	181 mg/l.
pH (units)	7.6–8	7.8
Specific conductance, μmho	407–519	565
Cl^-	23–51 mg/l.	46 mg/l.
COD	8.3–9.2 mg/l.	6.8 mg/l.
BOD	2.4–12.0 mg/l.	2.1 mg/l.
SO_4^{-2}	18–112 mg/l.	75 mg/l.
Dissolved solid	244–405 mg/l.	520 mg/l.
Total solids	254–452 mg/l.	1040 mg/l.
Turbidity	3–4 JTU	395 JTU

[a] Collected after a storm event.

Table IV. Experimental Conditions for the Study of the Effect of Suspended Particles on the LLE Step of Aqueous Residue Analysis

Run	Experimental mixture	Initial aqueous concentration, mg/l.	Temp, °C	Turbidity,[a] JTU	pH
1.	Isopropyl ester of 2,4-D	2.41	24.5–25.5	358	7.8
	n-Butyl ester of 2,4-D	2.24			
	n-Butyl ester of 2,4,5-T	1.93			
2.	Isopropyl ester of 2,4-D	2.41	23–24.5	46	2.0[b]
	n-Butyl ester of 2,4-D	2.24			
	n-Butyl ester of 2,4,5-T	1.93			
3.	Isopropyl ester of 2,4-D	2.41	21–24.5	46	2.0[b]
	n-Butyl ester of 2,4-D	2.24			
	n-Butyl ester of 2,4,5-T	1.93			
4.	Isopropyl ester of 2,4-D	4.02	22–26	46	2.0[b]
5.	Isopropyl ester of 2,4-D	4.02	25.5–26	58	7.8
6.	Isopropyl ester of 2,4-D	4.02	25.5–26	65	7.8

[a] Schuylkill River high turbidity water. Water characteristics listed on Table III. [b] pH = 2, 0.2 M orthophosphate buffer.

EXPERIMENTAL SECTION

p-Value Determination. Table II shows the experimental conditions for the p-value determination of each phenoxy acid herbicide and herbicide esters studied. The pH must be adjusted to below the pK_a to have the acid in the molecular form (the extractable form). The pH was set at 0–2 with 0.02 or 0.2 M orthophosphate buffer (Christian and Purdy, 1962). Table I shows that pH 2 is a primary pH utilized by other workers. The pK_a's of phenoxyalkyl acid herbicides range from 2.73 to 4.80 (Nelson and Faust, 1969). In the present study, no statistical difference was found between the amount extracted between pH 0 and 2.

The p-values were determined in the highest practical water:solvent ratio. The highest water:solvent ratio will give the most accurate p-value, and will also simulate the usual extraction conditions for residue samples (Suffet and Faust, 1972a).

The p-value determination consists of shaking 20 ml of aqueous buffer containing an herbicide with a 2-4-ml volume of solvent in a glass-stoppered graduated cylinder. After the phases are separated, the solvent phase or aqueous phase is analyzed. The amount extracted into the solvent phase is corrected for volume changes. This is the E-value. The E-value is the fractional amount of pesticide extracted into the solvent phase from the water phase under any specific condition. From the E-value, the p-value is calculated (Suffet and Faust, 1972b).

The effect of natural water characteristics upon the p-value method was investigated. Table III shows the natural water characteristics of the river water, creek water, and river water containing high turbidity used in the study.

Recovery Studies. Two methods of serial liquid–liquid extraction were used for recovery studies—the classical manual separatory funnel method and the vortex stirring method (American Public Health Association, 1971; Kawahara et al., 1967; Schafer et al., 1969). 2,4-D (100 mg/l.) was serially extracted by both methods under p-value conditions from distilled water. Ethyl acetate was used to extract the 2,4-D in a serial extraction procedure. Samples extracted with ethyl acetate showed emulsification and 10-min stabilization was used before separation. The residual 2,4-D in the water layer was measured by uv spectrophotometry after a 10-min purge with nitrogen to eliminate residual ethyl acetate. The theoretical lower limit of uv detectability of 2,4-D in aqueous pH 2 buffer is 1.2 mg/l. for the 1-cm pathlength uv cell used. Standard additions were used to minimize spectroscopic error. The time to develop an equilibrium for both methods with 1 l.

of 0.2 M orthophosphate buffer at pH 2 was set at 10 min. The amount extracted was constant from 2 to 60 min.

Studies of the Effect of Suspended Particles on the LLE Step. Table IV summarizes a series of experimental conditions for the study of the effect on the LLE step for suspended particles in natural water. Experiments were performed under two conditions, natural pH and ionic strength, and under the conditions of a p-value determination (0.2 M orthophosphate buffer at a pH of 2).

The amount of suspended particles present in the natural water was lowered as desired by filtering a portion of the turbid natural water, Table III, and using the filtrate to dilute the sample to the desired experimental turbidity. The suspended particles present were measured by their turbidity with a Hellige Turbidimeter, standardized in Jackson Turbidity Units (JTU) (American Public Health Association, 1971).

Herbicidal esters were shaken with suspended particles in a 1-l. flask at room temperature. The shaker was stopped only when a sample was withdrawn. The herbicide left in the aqueous phase was determined after a 25-ml aliquot was collected and immediately filtered through a 0.45-μ millipore filter. A 20-ml aliquot of the filtrate was analyzed at 25 ± 0.5° by the p-value method after adjustment of the filtrate (if necessary) to a pH of 2 and 0.2 M ionic strength with orthophosphate buffer. Benzene and ethyl acetate were utilized as the extraction solvents at 10:1 and 5:1 water:solvent ratios, respectively. Runs 1–3 used both solvents; the remaining runs used only benzene. Quantitation was completed by glc as indicated in Table II.

Analytical. The method of quantitation of esterified herbicides was gas chromatography. Ultraviolet spectrophotometry was employed to quantitate all free acid herbicides.

The gas–liquid chromatograph used was a Tracor Model MT-220 equipped with 10 mCi ^{63}Ni electron-capture detector. The detector was operated in a DC voltage mode at 32 V which was found best for reproducible response to phenoxy acid esters. An applied voltage of 25 V was maximum response. The linearity of response was from 0.7 to 2.7 ng for each ester. The glc column used was a 6-ft length of 4 mm i.d. borosilicate glass. The column was packed with 3% OV-1 on 80/100 mesh acid-washed Chromosorb W. Chromatograms were recorded on a 1-mV Beckman recorder with chart speed maintained at 0.5 in./min. Nitrogen, used as a carrier gas, was predried by passage through a filter dryer. The carrier gas flow rate was

Table V. Liquid–Liquid Extraction of Phenoxyalkyl Herbicides from Orthophosphate Buffer at 25 ± 0.5°

					p-Value			
Compound	pH	Ionic strength, M	Ethyl acetate	Ether	Benzene	Chloroform	Carbon tetrachloride	Hexane
2,4-D	0–2	0.02–0.2	0.996	0.99+	0.915	0.95[a]	0.51[b]	<0.04[b]
2,4,5-T	0–2	0.02–0.2	0.99	0.99+	0.98	0.98[a]	0.81[b]	0.24[b]
MCPA	0–2	0.02–0.2	0.99+	0.99+	0.95	0.97[a]	0.69[b]	(0.16)[b]
MCPB	2	0.02	0.99	0.99	0.98	0.99	0.96[a]	0.81[a]
2,4-DB	2	0.02	0.99	0.99	0.98	0.98	0.97[a]	0.81[a]
2-CPA	2	0.2	0.99	0.99	(<0.50)	0.81[a]	(0.14)[b]	(<0.04)[b]
4-CPA	2	0.2	0.99	0.99+	0.63	0.86[a]	(0.23)[b]	(<0.04)[b]

All values are the average of at least three determinations except those in parentheses, with a water:solvent ratio of 10:1.
[a] Water:solvent ratio changed to 5:1. [b] Water:solvent ratio changed to 1:1.

Table VI. Liquid–Liquid Extraction of 2,4-D and Some of Its Esters from Orthophosphate Buffer at 25 ± 0.5°

			p-Value (average of at least three determinations)			
Compound	pH	Ionic strength, M	Ethyl acetate	Ether	Benzene	Hexane
		Water:solvent ratio of 10:1				
2,4-D	0–2	0.02–0.2	0.996	0.99+	0.915	<0.04
Isopropyl ester of 2,4-D	2	0.2	0.97[a]	0.97[a]	0.99	0.99
n-Butyl ester of 2,4-D	2	0.2	0.96[a]	0.96[a]	0.96	0.96

[a] Water:solvent ratio changed to 5:1.

Table VII. Liquid–Liquid Extraction of 2,4,5-T and Some of Its Esters from Orthophosphate Buffer at 25 ± 0.5°

			p-Value (average of at least three determinations)			
Compound	pH	Ionic strength, M	Ethyl acetate	Ether	Benzene	Hexane
		Water:solvent ratio of 10:1				
2,4,5-T	0–2	0.02–0.2	0.99+	0.99+	0.98	0.24[b]
Isopropyl ester of 2,4,5-T	2	0.2	0.96[a]	0.95	0.97	0.97
n-Butyl ester of 2,4,5-T	2	0.2	0.96[a]	0.96[a]	0.98	0.99

[a] Water:solvent ratio changed to 5:1. [b] Water:solvent ratio changed to 1:1.

maintained at an optimum value of 120 ml/min, as determined from a Van Deemter plot. The column temperature was maintained at 180°. The inlet temperature was 240° and detector temperature was 255°. The relative retention times compared to the isopropyl ester of 2,4-D were 1.72, 1.60, and 2.81 for the n-butyl ester of 2,4-D, isopropyl ester of 2,4,5-T, and n-butyl ester of 2,4,5-T, respectively. The attenuation ratio was set at 64 × 10², where 0.3 ng produces a 12-mm peak height of the isopropyl ester of 2,4-D. The n-butyl ester of 2,4-D and isopropyl ester of 2,4,5-T appeared as one peak.

An internal standard of one herbicide was used to correct for any variations in the absolute standard curve of the herbicide studied. A plot of the ratio of response of the herbicide studied to the internal standard vs. the concentration of the herbicide studied was used for all standard curves.

A Beckman DK-2A spectrophotometer with matched 1-cm far uv silica cells was utilized for all ultraviolet measurements. The most accurate quantitative procedure was utilized for all uv determinations.

When the %T of the residual herbicide was more than 80% T, the herbicide was determined by a standard additions procedure to minimize spectroscopic error. Spectroscopic error is minimum in the 20–80% T region. In the standard additions procedure, the concentration of the

herbicide 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 their volume change. A statistical comparison of the standard additions method and the direct uv measurement of 2,4-D indicated no significant differences (t-test > 0.99).

RESULTS

Solvent Choice. Table V shows the p-values determined for some phenoxy acid herbicides. Tables VI and VII show the p-values determined for 2,4-D and 2,4,5-T and their n-butyl and isopropyl esters, respectively. Erne (1963) has determined the distribution coefficient of some of these herbicides in 0.1 M buffers with 1:1 water:solvent ratios which were presaturated with solvent. Erne's distribution coefficients were used to calculate corresponding p-values. The p-values calculated from Erne and the p-values determined here were the same for 2,4-D, MCPA, 2,4,5-T, and MCPB in $CHCl_3$; 2,4-D in benzene showed a p-value of 0.89, compared to 0.915 determined in this study.

The best solvents for extraction of phenoxy acid herbicides are ethyl acetate and ether. Chloroform and benzene, although frequently used, are far less efficient. Hexane is a poor solvent choice. The best solvents for simultaneous extraction of 2,4-D or 2,4,5-T and their respective

Table VIII. The Effect of Different Natural Waters on the p-Value Determination of 2,4-D from 0.2 M, pH 2 Orthophosphate Buffer at 25 ± 0.5°

Type of water	Benzene			Ethyl acetate		
	Average p-value	Standard deviation	Number of deter-minations	Average p-value	Standard deviation	Number of deter-minations
Water:solvent ratio of 10:1						
Distilled water	0.915	0.008	11	0.996	0.002	13
Schuylkill River water	0.925	0.007	13	0.996	0.0005	11
River water (filtered)	0.911	0.007	2	0.997	0.0010	3
Wissahickon Creek water (high organic load)	0.920		1	0.998		1

n-butyl and isopropyl esters are ether and ethyl acetate (2,4-D and esters), and benzene (2,4,5-T and esters).

A trend in polarity is indicated; the more polar an herbicide molecule is, the lower is its p-value for a particular solvent. This is shown by the 2-methylphenoxyalkyl acetic and butyric acids MCPA and MCPB, whose p-values are higher than their respective 2-chloro derivatives 2,4-D and 2,4-DB and the p-values of the corresponding phenoxyacetic acids 2,4-D and MCPA, which are lower than the phenoxy-butyric acids 2,4-DB and MCPB. The p-value data for carbon tetrachloride appear useful as a relative polarity scale of these compounds.

The Relationship Between p-Values Determined in Distilled Water and Natural Waters. Table VIII shows the relationship between the p-values as determined in distilled water and apparent p-values determined in actual natural waters of different types; e.g., unfiltered river water, filtered river water, and a creek water of high organic load. Natural water characteristics are adjusted to those utilized for the p-value technique in distilled water. In this way, quantitative comparison between p-values in distilled water and natural water can be observed directly. 2,4-D was used as a model compound partitioned between water and benzene (p-value 0.915) or ethyl acetate (p-value 0.996). These two solvents represent a spread of p-values over the range of desirable partitioning solvents (Suffet, 1973).

The student t-test was used to determine whether the differences of apparent p-values using river water and p-values using distilled water are due to random error. The t-test shows that the p-values obtained for river water and distilled water are consistent within random errors at the 3S confidence level for 2,4-D extracted with benzene or ethyl acetate. Similar results have been obtained with the isopropyl ester of 2,4-D and 2,4,5-T and the n-butyl ester of 2,4-D for these waters with four different solvents.

The critical finding is the consistency of the apparent p-value for 2,4-D found with water from different sources and the p-value found in distilled water having similar water characteristics of temperature, ionic strength, and pH. Hermann and Post (1968) described the extraction of model water pollutants from distilled water to be different than it is when one extracts them from natural waters. Therefore, adjustment of water characteristics to p-value pH and ionic strength are important parameters for control of LLE efficiency alone with the choice of solvent.

Precision of p-Value Determination of Phenoxy Acid Herbicides. The reproducibility of the p-value was tested with a minimum of ten determinations. p-Values near 0.90 and 0.99 were chosen for statistical analysis. Table IX gives the statistical analysis of E-values and corresponding p-values for the extraction of 2,4-D from distilled water and Schuylkill River water under p-value conditions with benzene and ethyl acetate at water:solvent ratios of 10:1 (α = 0.10 and 0.026, respectively).

Table IX shows that a p-value in the range of 0.90–0.92 would be ±0.02 at the 3S confidence interval (99.7%), and

Table IX. Precision of E- and p-Values for the Liquid–Liquid Extraction of 2,4-D from 0.2 M pH 2 Orthophosphate Buffer at 25 ± 0.5°

Benzene	Avg E	Standard deviation	3S confidence interval (99.7%)	Number of deter-mina-tions
Water:solvent ratio of 10:1				
Distilled water	0.520	0.027	0.081	11
River water[a]	0.555	0.026	0.078	13
	Avg p			
Distilled water	0.915	0.008	0.024	11
River water[a]	0.925	0.007	0.021	13
Ethyl acetate	Avg E			
Distilled water	0.856	0.047	0.141	13
River water[a]	0.910	0.018	0.054	11
	Avg p			
Distilled water	0.996	0.002	0.006	13
River water[a]	0.997	0.0005	0.0015	11

[a] Schuylkill River water (filtered). Water characteristics listed on Table III.

that a p-value of greater than 0.99 would be ±0.01 at the 3S confidence interval. The corresponding 3S confidence intervals for E-values are ±0.08 and ±0.05–0.14, respectively.

Effects of Suspended Particles on the LLE Step of Aqueous Residue Analysis. The object of this study was to equilibrate an herbicide in a naturally turbid water caused by suspended particles and then to observe its effect upon the LLE step of aqueous residue analysis. These observations could aid in understanding the influence of suspended matter in natural water of high turbidity on herbicide recovery. Herbicide monitoring procedures attempt to extract the whole water sample as collected, whether or not it contains suspended particles. This seems strange when only the aqueous concentration is reported.

The initial run at natural turbidity showed that the concentrations of the herbicidal esters left in the aqueous phase were too low to be analyzed by the p-value method after filtration. Subsequent runs at adjusted lower turbidity also showed that the concentrations of the n-butyl esters of 2,4-D and 2,4,5-T left in the aqueous phase remained too low to be analyzed by the p-value method. Therefore, only the isopropyl ester of 2,4-D was utilized in runs 4–6.

Figure 1 illustrates the adsorption characteristics of mixtures of the isopropyl ester of 2,4-D (initial concentration of 4.02 mg/l.) in river water (58 JTU at a natural pH) and adjusted river water (46 JTU, pH 2, adjusted to 0.2 M with orthophosphate buffer). Analysis of the postfiltration aqueous phase demonstrated an immediate adsorption in
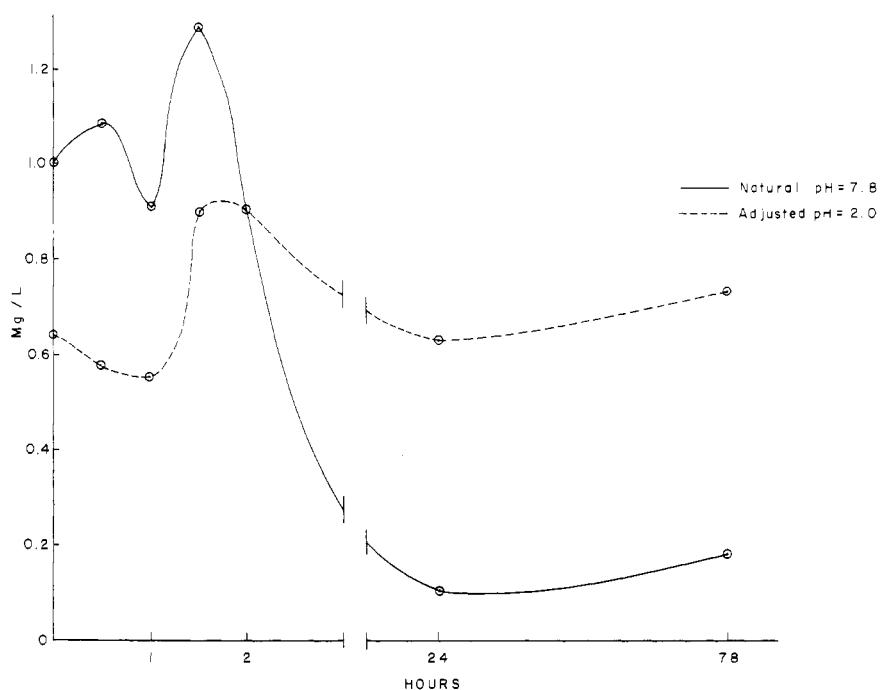
Figure 1. The adsorption characteristics of the isopropyl ester of 2,4-D in a turbid river water. Mg/l. left in solution *vs.* contact time with natural turbidity. Isopropyl ester of 2,4-D initial concentration = 4.02 mg/l. —, turbid river water, pH 7.8, natural ionic strength, turbidity = 58 JTU. ---, turbid river water adjusted to pH 2.0, and 0.2 *M* with orthophosphate buffer (*p*-value conditions), turbidity = 46 JTU.

Table X. Theoretical Calculation of F_n (Recovery) from *p*-Value Data *vs.* Experimental Recovery Data from the Literature

| | F_n calculated from *p*-value | | F_n recovery observed from literature | | | |
Solvent	*p*-Value[a]	F_n calculated	Observed F_n recovery	pH	Water:solvent ratio	Reference
2,4-D						
Chloroform	0.95	0.99+	0.92–0.94	<2	25:3 × 10	Erne (1963)
Ethyl ether	0.99+	0.99	1.00 ± 0.042–0.057		100:100	Abbott *et al.* (1964)
Ethyl ether	0.99+	0.99	0.82–0.99	2.0	800:150, 2 × 50	Goerlitz and Lamar (1967)
Benzene	0.915	0.80	0.799 ± 0.0386	≈2	1000:100, 2 × 50	Devine and Zweig (1969)
MCPA						
Chloroform	0.97	0.99+	0.92 ± 0.05	<2	25:3 × 10	Erne (1963)
Ethyl ether	0.99	0.99	1.00	*b*	100:100	Abbott *et al.* (1964)
Benzene	0.95	0.91	0.489	≈2	1000:100, 2 × 50	Devine and Zweig (1969)
2,4,5-T						
Chloroform	0.98	0.99+	0.93 ± 0.045	<2	25:3 × 10	Erne (1963)
Ethyl ether	0.99+	0.99	0.90		100:100	Abbott *et al.* (1964)
Ethyl ether	0.99+	0.99+	0.86–1.10	2.0	800:150, 2 × 50	Goerlitz and Lamar (1967)
Benzene	0.98	0.99	0.957 ± 0.0603	≈2.0	1000:100, 2 × 50	Devine and Zweig (1969)
MCPB						
Ethyl ether	0.99	0.99	1.00	*b*	100:100	Abbott *et al.* (1964)
2,4-DB						
Ethyl ether	0.99	0.99	0.95	*b*	100:100	Abbott *et al.* (1964)
n-Butyl ester of 2,4-D						
Benzene	0.96	0.94	0.92	≈2	1000:100, 2 × 50	Devine and Zweig (1969)
Isopropyl ester of 2,4-D						
Benzene	0.99	0.90+	0.93	≈2	1000:100, 2 × 50	Devine and Zweig (1969)

[a] 2 ml of H_2SO_4; *p*-values from Table V. [b] Concn.

excess of 50% of the added herbicide. Less adsorption was initially observed from the natural water sample than the sample adjusted to *p*-value conditions.

Blank samples which were dosed with herbicide but which did not contain suspended particles were analyzed in the same manner as the turbid samples. Analyses of

the blank showed that the concentration during the experimental run did not change. This indicated that adsorption did not occur on the millipore filter or glassware used.

The approach to equilibrium was not straightforward. The initial adsorption was followed by at least one cycle

of partial desorption and subsequent readsorption. The
time required for these systems to attain full equilibrium
was greater than the 2 days studied. These experiments
indicated that changing the pH and ionic strength of a
naturally turbid water before removal of suspended mat-
ter could effect herbicide distribution between liquid and
solid phases.

Herbicide distribution between particles and the aque-
ous phase would be upset if the aqueous characteristics
are changed and then filtered (Figure 1). The sample
should be filtered before adjusting aqueous characteris-
tics. The time of filtration of natural waters should be at
the time of collection. Then the actual distribution would
be noted. The effect of direct extraction of the natural
water sample with its suspended particles and a mass bal-
ance between phases remains to be studied.

Comparison of F_n and Experimental Recovery Data.
The p-value gives a theoretical guide for the evaluation of
the aqueous extraction procedure of phenoxy acid herbi-
cides. The p-value must be checked by fortification proce-
dures. 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.

Table X shows the theoretical calculated F_n values (the
total fraction extracted with a serial extraction) (Suffet,
1973) calculated for phenoxy acid herbicides from p-value
data and a comparison with experimental recovery data
from the literature for phenoxy acid herbicides. The litera-
ture recovery values are based upon extraction under many
different water characteristics, whereas the F_n values are
based upon calculation of one type of water and LLE condi-
tion, namely p-value conditions (Table II). Therefore, some
variability is expected between F_n and recovery data.

The comparison of F_n and recovery data shows that F_n
values are within the range of all the recovery data from
the literature. The comparison confirms the experimental
observation that benzene is a poor solvent for extraction of
2,4-D and MCPA from water, although the recovery of
MCPA was reported as 40% less than expected (Devine
and Zweig, 1969). The comparison of F_n and recovery also
confirms the p-value results; ether is the best of the ex-
traction solvents studied for the phenoxy acid herbicides
under p-value conditions (Table V).

**Recovery Study of an LLE Step at High Concentra-
tions.** The p-value can be used to check or develop a
quantitative serial LLE procedure based upon general cri-
teria for quantitative extraction of pesticides from aque-
ous solution (Suffet, 1973). First, adjust the natural water
quality to the water quality used for the p-value method and
then define an acceptable recovery level (e.g., 99%) and
aqueous sample volume (e.g., 800 ml) (Table X, Goerlitz
and Lamar, 1967). Then, by a computer program readout
(Suffet, 1973), select the serial LLE procedure by choosing
the procedure that uses the least number of steps and/or
the least solvent volume. If two or more serial LLE pro-
cedures are possible then make a final choice of procedure
by picking the serial extraction which used the largest sol-
vent volumes in the earlier LLE steps.

Table XI presents a summation of a computer readout
giving the choices for serial extraction steps and total sol-
vent volumes needed to collect 99% of 2,4-D with ether
(p-value 99+) from 800 ml of water in a total solvent vol-
ume of 250 ml or less using solvent volumes in multiples
of 50 ml. Line 3 of the computer readout suggests a serial
extraction of 150 ml, and 50 ml theoretically would ex-
tract 99% of the herbicide. This procedure is comparable
to that of Goerlitz and Lamar (1967) (Table X), who used
800 ml of water in a serial extraction of 150, 50, and 50
ml. Therefore, according to the above, the third extraction
is unnecessary and a two-step procedure is suggested.

A recovery study of successive LLE steps at high con-
centrations has been performed previously (Suffet, 1973). A

Table XI. Computer Readout of p-Value Program to Determine the Best Serial LLE Parameters[a]

[a] Suffet (1973). [b] Best choice.

comparison between the theoretical F_n and actual vortex
mixing and separatory funnel analysis for a four-step serial
LLE of 100 mg/l. of 2,4-D from 0.2 M pH 2 orthophosphate
buffer with benzene (10:1 benzene:water) was statistically
compared (Suffet, 1973). The total error concept was em-
ployed (McFarren et al., 1970). A total error of up to 17.4%
was found attributable to the LLE step. The vortex stirring
and the separatory funnel procedures were shown to be
equivalent.

In the present work, a two-step serial extraction with
200 and 50 ml of ethyl acetate or ether under p-value con-
ditions is theoretically needed to extract 99% of 2,4-D
from 1 l. of aqueous solution. This was determined by a
computer readout in Table XI. The p-values of ethyl ace-
tate and ether are 0.99+. A series of three classical
separatory funnel and three vortex extractions under the
p-value procedure was run with ethyl acetate at a 2,4-D
level of 100 mg/l. The residual aqueous 2,4-D was mea-
sured by uv spectrophotometry. A recovery of 99% ± 0.02
was determined confirming the recovery.

DISCUSSION

It is hoped that valuable insights have been obtained by
isolating the LLE step in residue analysis. The determina-
tions of the p-value, E-value, and theoretical F_n value are
aids for the selection of optimum conditions for a particu-
lar analysis. These are the attainable efficiencies of the
LLE step to which the analyst should strive. Actual forti-
fication procedures are necessary for confirmation of theo-
retical F_n values. In actual residue analysis, successive
LLE steps extract successively a smaller amount of the
original total weight of pesticide present. Therefore, a
slight error due to separation of the solvent and water
phases would diminish with successive extractions.

This paper has shown the use of the p-value in devel-
oping the ability to select the best solvent for the extrac-
tion of a particular herbicide or a combination of herbi-
cides. An aqueous volume:solvent volume ratio and a se-
rial extraction procedure for maximum recovery with the
use of minimum aqueous and solvent phases has been de-
scribed using the example of 2,4-D. The effects of natural
water characteristics on serial extraction procedure have
been indicated. A judicious choice of solvent according to
the p-value approach and the use of a computer program
can give an initial approximation for recovery of these
herbicides. Future work should delineate statistically the
relationship between an F_n value and actual recovery
values at aqueous residue levels (ng/l.).

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Photolysis of the Herbicide Dinitramine (N^3,N^3-Diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine)

Herbert C. Newsom* and William G. Woods

The photolysis of the herbicide dinitramine (N^3,N^3-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) in methanol and in water was investigated. The compound was found to degrade rapidly through reductive cyclization of a nitro group and an adjacent N-ethyl group to give the following products: 6-amino-1-ethyl-2-methyl-7-nitro-5-trifluoromethylbenzimidazole; 5-amino-1,2-dihydroxy-3-ethyl-2-methyl-4-nitro-6-trifluoromethylbenzimidazoline; 1-ethyl-6-hydroxylamino-2-methyl-7-nitro-5-trifluoromethylbenzimidazole; and 6-amino-2-methyl-7-nitro-5-trifluoromethylbenzimidazole.

N^3,N^3-Diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine, dinitramine (1), is a new preplant incorporated selective herbicide which is effective on broadleaf weeds and grasses in cotton, soybeans, and other crops. The products of photolysis of this compound and an idea of the rate of this type of degradation are of interest from environmental and agronomic aspects.

The effect of ultraviolet irradiation on phytotoxic amines with ortho–nitro substituents has been previously studied (Crosby and Li, 1969; Probst and Tepe, 1969). Trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) underwent loss in herbicidal activity and a change in its ultraviolet absorption on exposure to sunlight, both on soil and on glass plates (Wright and Warren, 1965). Unpublished data (Day, 1969) indicate that photolysis of trifluralin in methanol gave at least ten products. Two of these apparently were the monodealkylated and didealkylated compounds α,α,α-trifluoro-2,6-dinitro-N-propyl-p-toluidine and α,α,α-trifluoro-2,6-dinitro-p-toluidine. A preliminary report of a more extensive reexamination of trifluralin photolysis was presented while this manuscript was in preparation (Leitis and Crosby, 1972).

The present paper describes the degradation rate and products obtained on photolysis of dinitramine (1).

EXPERIMENTAL SECTION

The dinitramine used was recrystallized several times from cyclohexane and ethanol to give >99.6% purity. Solvents were AR grade and melting points were uncorrected.

Rate of Sunlight Degradation of Aqueous Dinitramine. An acetone solution of dinitramine (0.00142 g/cm³) was stirred into 12 l. of water and three 5-ml samples were withdrawn and analyzed (Newsom and Mitchell, 1972) for