Study of the Effects of Environmental Variables and Supercritical Fluid Extraction Parameters on the Extractability of Pesticide Residues from Soils Using a Multivariate Optimization Scheme

MIN ZHOU,*,† RICHARD K. TRUBEY,† ZENAIDA O. KEIL,‡ AND DONALD L. SPARKS§

DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19880-0402, School of Engineering, Rowan College of New Jersey, Glassboro, New Jersey 08028, and Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware 19717-1303

A multivariate optimization scheme (MOS) was used to investigate the effects of environmental variables and supercritical fluid extraction (SFE) parameters on the extractability of pesticide residues from soil samples. MOS is a highly efficient technique for studying a large number of variables and identifying optimal extraction conditions. MOS offered the opportunity to systematically and simultaneously examine the interaction and effect profiles among important soil variables and extraction parameters. Pesticide residence time in soil had a major influence on binding extent for all tested pesticides. Extractability as a function of soil composition was greatly dependent on the particular pesticide examined. Bensulfuron-methyl was the most difficult to extract from soils containing high levels of both organic matter and clay. Atrazine was extracted more easily than diuron. High organic matter and high clay content led to strong binding for diuron and atrazine, respectively. Effects of SFE parameters on extractability appeared to depend on the nature of residues (e.g., freshly fortified versus aged residues). For freshly fortified samples, analyte solubility in supercritical fluid and/or modified supercritical fluid was the critical factor as indicated by the strong influence of pressure on extraction efficiency. For aged samples, temperature was an important determinant of extraction efficiency, indicating that mass transfer or diffusion processes were rate-limiting. The presence of modifier and extraction duration also significantly impacted extractability of aged pesticides.

Introduction

Modern pesticides are generally recognized as significantly benefiting our ability to satisfy the world's need for abundant, safe, affordable food and fiber. Knowledge of environmental fate in soil is important to ensure the environmental safety of pesticide. Pesticides reach the soil environment by direct or indirect application from aerial and ground sprays. The main processes affecting potentially the ultimate fate of pesticides in soil are retention by soil materials (involving adsorption/desorption processes), transformation processes (biological and chemical degradation), and transport (throughout soil, to the atmosphere, and to surface or groundwater) (*1*).

The occurrence and significance of bound pesticide residues in soil have become important issues in dealing with persistence, degradation, and biological availability of pesticide residues (*2*). The determination of pesticide residues in soil is often quite challenging. The effect of soil composition on the extractability of pesticide residues usually is complex, due to the heterogeneous nature of most soils. Furthermore, the selection of optimum extraction conditions is also quite complicated. The large number and complicated nature of the variables make pesticide extraction from soils an ideal application for MOS.

The analysis of bound pesticide residues in soil has long posed many challenges (*3*). The use of supercritical fluid extraction (SFE) of soil is becoming increasingly popular, particularly as a means of maximizing the amount of analyte extracted (*4*).

The traditional approach to research in pesticide−soil interactions has involved holding all variables constant except one, which is typically examined across a range (*1*). This approach can be very costly, time-consuming, and inadequate to uncover information about interactions among variables (i.e., how variables interact in synergistic or antagonistic ways).

In contrast, a multivariate optimization scheme (MOS) is inherently more efficient and with proper design is very effective (*5*). In a MOS, a comparatively limited set of experiments is performed, with several independent variables modified in each experiment. Modern desktop computing tools enable the use of MOS to efficiently design experiments and evaluate statistically significant results, including the determination of possible inter-variable interactions and effects.

SFE experiments involving a MOS have rarely been pursued. A two-level, two-factor (2^2) factorial design approach was used to optimize the temperature and pressure for the SFE of amine hydrochloride (*6*). With this approach, however, only two variables were optimized simultaneously. Reindl and Hofler (*7*) used a single-variable approach for optimization of SFE parameters. Hitchen et al. (*8*) used an experimental design with multivariate linear regression to examine the relative contribution of the main experimental variables during SFE. More recently, Barnabas et al. (*9*) reported an experimental design approach to the investigation of the extraction of polycyclic aromatic hydrocarbons (PAH) from soil using supercritical fluid carbon dioxide.

In this study, a MOS based on quadratic and central composite design was used to systematically elucidate the influence of soil environmental variables and SFE parameters on the extractability of freshly fortified and aged pesticide residues from soil samples. The combination of statistically designed experiments and the versatility of SFE efficiently elucidated certain soil matrix effects, increasing our ability to probe important aspects of pesticide binding.

Experimental Section

There were two experimental designs in the multivariate optimization scheme: a screening design and a response surface design. The screening experimental design was used to define the significance of independent variables for both soil environmental variables and SFE parameters. The

* Corresponding author telephone: (302)695-2853; fax: (302)695-4296; e-mail: zhoum@esvax.email.dupont.com.

† E. I. du Pont de Nemours and Company.

‡ Rowan College of New Jersey.

§ University of Delaware.

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software was developed by ECHIP Inc. (Hockessin, DE) and was primarily used to narrow down the number of variables to several important ones for further response experimental design. The response surface design was then employed to elucidate the interactions and relationships among the independent variables and to lead to an optimization scheme. Two groups of independent variables (i.e., soil environmental variables and SFE parameter) were studied to determine the effects of environmental variables and SFE parameters on the extractability of pesticides in soils.

Experimental Design for Multivariate Optimization Scheme. Statistical experimental design and analysis provide an optimal method to discover the relationship between a response and one or more control variables. If y is a response (i.e., recovery or extraction efficiency) and X_1, X_2, X_3, ... are a set of control variables (i.e., temperature, pressure, time, etc.), then one can propose a functional relationship between the two:

$$y = f(X_1, X_2, X_3, ...) \quad (1)$$

Polynomial models may be used as an empirical model to approximate the true physical model over the range of the experimentation. The most common models are linear, interaction, quadratic, and recently a fourth type, partial cubic, which contains interaction terms of the third degree but does not contain cubic terms in a single variable. A quadratic model was selected in the studies, which is sufficient to provide both interaction and effect information among independent variables, while a cubic model may involve a lengthy experiments for optimization. Equations 2 and 3 were three- and four-variable quadratic models, respectively:

$$y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{23}X_2X_3 + a_{13}X_1X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{123}X_1X_2X_3 \quad (2)$$

$$y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{12}X_1X_2 + a_{14}X_1X_4 + a_{23}X_2X_3 + a_{13}X_1X_3 + a_{24}X_2X_4 + a_{34}X_3X_4 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{44}X_4^2 + a_{1234}X_1X_2X_3X_4 \quad (3)$$

The a values are coefficients and the X values represent experimental conditions (i.e., temperature, pressure, modifier, and length of extraction) expressed as percentages of the experimental ranges on the respective axes. The coefficients were obtained using a modified version of the ECHIP Basic program. The sequential trials are listed in Tables 1 (environmental variables) and 2 (SFE parameters for aged atrazine).

Sample Preparation for Independent Variable Studies. For the soil environmental variable experiments, mixed soils were air-dried; weighed (2 g) in a scintillation vial; fortified with either atrazine, diuron, or bensulfuron-methyl at 20 000 dpm/g (^{14}C radiolabeled compound), respectively; and dried under a stream of nitrogen to minimize microbial activity. Samples were stored at approximately 4−7 °C in a refrigerator to ensure minimum degradation. Fortifying solutions were prepared by reconstituting in water after evaporating off organic solvent in the standards. For example, 1.0 mL of standard solution (10 μg/mL or 100 000 dpm/mL in acetonitrile) was transferred into a 15-mL graduated tube and evaporated down to approximately < 0.2 mL and brought up to 10 mL with deionized water to yield 1.0 μg/mL or 10 000 dpm/mL standard.

Threshold values of extraction were determined from preliminary experiments. SFE parameters [300 atm, 60 °C, with a modifier of 20% (acetonitrile/acidic water with 0.1 N HCl/0.5% Triton X-100 surfactant, 80/10/10 v/v/v)] were selected for the study to minimize difference in extractability due to poor solubility of analyte in modified supercritical fluid carbon dioxide (SC-CO_2).

TABLE 1. Effect of Environmental Variables on the Extractability of Three Pesticides from Soils using SFE[a]

trail no.	pesticide	length (month)	clay (%)	OM (%)	recovery (%)
18	2	0	15	2	94
12	0	0	28	15	94
11	0	12	2	15	68
1	0	0	2	2	99
17	1	12	2	2	80
15	1	12	15	28	50
20	0	6	2	28	72
9	2	12	28	15	34
6	2	12	2	28	36
5	0	0	2	28	96
2	2	12	2	2	79
16	1	0	2	28	93
4	0	12	28	2	47
19	0	6	28	2	65
3	2	0	28	2	90
3	2	0	28	2	90
1	0	0	2	2	100
13	1	6	28	28	60
4	0	12	28	2	47
7	2	0	28	28	83
8	0	12	28	28	38
5	0	0	2	28	96
2	2	12	2	2	78
14	2	6	15	28	58
10	2	0	2	15	92

[a] Four environmental variables were pesticides (atrazine = 0, diuron = 1, and bensulfuron-methyl = 2), residence time (length, month), clay content (%), and organic matter (OM %).

TABLE 2. Effect of SFE Parameters on the Extractability of Aged Atrazine from Alliston Soil Using MOS[a]

trail no.	T (°C)	P (atm)	length (min)	modifier (%)	recovery (%)
10	150	140	10	17.5	75
12	40	140	30	17.5	42
3	150	140	30	5.0	82
4	40	352	30	5.0	54
16	95	140	10	30.0	75
14	150	246	20	30.0	83
17	95	352	10	5.0	74
13	95	246	30	30.0	86
1	40	140	10	5.0	35
8	40	352	30	30.0	53
5	40	140	10	30.0	43
2	150	352	10	5.0	80
2	150	352	10	5.0	80
20	40	246	10	30.0	39
4	40	352	30	5.0	54
11	40	352	10	17.5	46
1	40	140	10	5.0	35
19	40	246	30	5.0	41
15	95	352	20	30.0	80
6	150	352	10	30.0	79
3	150	140	30	5.0	82
18	150	140	20	5.0	79
9	150	352	30	17.5	90
5	40	140	10	30.0	43
7	150	140	30	30.0	88

[a] Four SFE parameter variables were temperature (T, °C), pressure (P, atm), length of extraction (length, min), and modifier (%).

For studies of effects of SFE parameters, freshly fortified samples were prepared by spiking with either atrazine, diuron, or bensulfuron-methyl at 20 000 dpm/g (^{14}C radiolabeled compound) onto soils and were extracted using static SFE. Aged field samples consisted of Danish soil [one plot had been treated with [^{14}C]diuron and one was left untreated (control)] and Canadian Alliston soil [one plot had been treated with [^{14}C]atrazine or one plot was left untreated (control)]. Samples were air-dried, sieved through 250 μm, and stored in a freezer (ca. −10 °C) until analysis.

Soil Environmental Variables Studies. Three soils [high OM (57.5%) from North Carolina, high clay (56.7%) from Spain, and high sand (91.6%) from Florida] were chosen. Nine composite soils were generated by mixing different ratios of the three soils yielding desirable pooled samples for the studies. The four variables studied were the contents of organic matter (%), clay minerals (%), the residence time of pesticide in the soil matrix (month), and the chemical species [(0), (1), and (2) were designated for atrazine, diuron, and bensulfuron-methyl, respectively] (Table 1). A comparison of the relative importance of test variables (i.e., influence of variable on analyte extractability) was conducted using a multivariate optimization scheme based on a quadratic model (ECHIP Inc., Hockessin, DE).

To investigate the potential difference in soil matrix−pesticide interaction (unextractability), SFE parameters were optimized, but extended extractions were not performed in this phase of the study. SFE conditions were identical for all three pesticide extraction. The SFE parameters were pressure (300 atm), temperature (60 °C), and modifier [20% (acetonitrile/acidic water with 0.1 N HCl/0.5% Triton X-100 surfactant, 80/10/10 v/v/v)] in CO_2. The extraction was initiated by 3 min static mode and followed by 7 min dynamic mode (flow rate of 0.5 mL/min).

Supercritical Fluid Extraction Parameter Studies. In a preliminary test, seven variables were screened for further response surface testing. SFE temperature, SFE pressure, extraction time (length), extraction modifier, sample size, analyte concentration level, and soaking time (if applicable involves pre-addition of modifier to extraction vessel). The multivariate optimization scheme for the screening experiments identified four major variables for further studies. The variables were pressure, temperature, modifier (%), and length of extraction.

Response surface design techniques were used to optimize the extraction procedure. In initial experiments it was found that the importance of variables was dependent on whether freshly fortified samples or aged samples were investigated. To accurately reveal the significance of variables, both freshly fortified and aged samples were extracted, and the data were evaluated separately. The test soil samples were weighed directly in an extraction vessel (0.25−2.0 g). Modifier was added to the sample vessel prior to SFE extraction and continuously delivered by pump during dynamic mode extraction. Experimental design and trials are listed in Table 2 (for atrazine from aged Alliston soil).

For the SFE parameter optimization experiments, preliminary tests suggested that the static mode with a relatively short extraction period (5−10 min) was sufficient for the freshly fortified samples. The dynamic mode with an extended extraction period (30 min) was necessary to obtain high extraction efficiency for field aged samples. The optimum experimental conditions depended on the nature of the aged residues. For individual analytes, the SFE parameters were 140−352 atm, 40−70 °C, 5−30% modifier for bensulfuron-methyl, they were 140−352 atm, 40−100 °C, 5-30% modifier for diuron, and they were 140−352 atm, 40−150 °C, 5−30% modifier for atrazine. Note that bensulfuron-methyl is thermally labile; therefore, the upper limit of the temperature was set at 70 °C. Diuron is relatively stable at temperatures up to 100 °C. Atrazine is very stable at an elevated temperature, but the maximum temperature for the SFE system was 150 °C.

Because of inherent limitation of two- or three-dimensional (2-D or 3-D) contour plots, only two variables vs response (extractability) could be plotted/viewed at a time. However, different variables could be shown in either 2-D or 3-D to demonstrate the influence of each variable on the extractability.

Samples were extracted by an ISCO SFE system 2300 (ISCO, Inc., Lincoln, NE), consisting of two Model 260D pumps; one system controller (electronic); one Model SFX 2-10 dual-chamber extractor module and associated valves, fittings, mixing "tee", and connecting tubing; two 10-mL stainless steel sample cartridges; and two extractant collection vials (20 × 150 mm) contained in plastic Erlenmeyer flasks. Extracts were measured by Liquid Scintillation Counter (LSC) Tracor Analytic Mark III Liquid Scintillation System (Model 6881, TM Analytic Inc., Elk Grove Village, IL) or analyzed by HP 1090M liquid chromatograph with UV DAD detector (Hewlett-Packard, Wilmington, DE). Liquid chromatographic conditions were as follows: column: Zorbax Rx-C_8 4.6 mm i.d., 250 mm, 5 μm (Mac-Mod Analytical); mobile phase: 35% acetonitrile/water buffered at pH 5.4 with NaH_2PO_4; flow rate: 1.0 mL/min; injection volume: 25−50 μL; UV detection: 254 nm.

In some cases, unextractable residues were combusted by Combustion Equipment−Packard Tri-Carb combustor (Model 306, Packard Instrument Co., Downers Grove, IL) using reagents (for CO_2 trapping and radioactivity analysis) and standard procedure supplied by the manufacturer. Radioactive quantities were measured by LSC. Material balances were used to calculate extraction efficiencies.

Chemicals and Reagents. Deionized water obtained from a Milli-Q water purification system (Millipore Corp., Milford, MA). Acetonitrile, methanol, hydrochloric acid (HCl), NaH_2-PO_4, and Triton X-100 (surfactant) were 'Baker analyzed' reagents grade (J. T. Baker, Philipsburg, NJ). Carbon dioxide was SFC grade CO_2 (Scott Specialty Gases, Plumsteadville, PA).

Reference Standards. The radiolabeled and nonradiolabeled reference standards applied in the study were [*phenyl*-^{14}C(U)]diuron with a radiochemical purity of 98.3%, [*phenyl*-^{14}C(U)]bensulfuron-methyl and [*pyrimidine*-2-^{14}C]bensulfuron-methyl with a radiochemical purity of 99%, and [^{14}C]atrazine with a radiochemical purity of 99% and were synthesized by DuPont New England Nuclear (NEN) Products (Billerica, MA).

Analytical standards of diuron (DuPont No. DPX-14740-149, 99.7% purity), bensulfuron-methyl (DPX-F5384, 99.3% purity), and atrazine (INY 0150, 99.7% purity) were synthesized by DuPont Agricultural Products, E. I. du Pont de Nemours and Company (Wilmington, DE).

Results and Discussion

A MOS makes possible the efficient evaluation of the impact of environmental variables and SFE parameters on the extractability of freshly fortified and aged pesticide residues from soils. The response surfaces for the quadratic design experiment, together with the full design, are documented in Table 1 for the evaluation of soil environmental variables on the extractability and in Table 2 for the evaluation of SFE parameters on the extractability of aged atrazine extraction (aged soil).

The order of significance among the independent variables was evaluated using a *t*-test to show confidence intervals (95, 99, and 99.99% CI). The lack of fit was tested and evaluated using analysis of variance techniques (i.e., ANOVA), and *F*-test, which includes the comparison of residual standard deviation (model errors) and replicate standard deviation (pure errors).

Effects of Environmental Variables on Extractability. In preliminary studies, the following independent variables were determined to be significant: organic matter content, clay content, analyte residence time (aging period) in soil, and type of pesticides. Systematic evaluation of the independent variables was conducted subsequently using a multivariate approach.

Analyte residence time was the most significant factor for extractability for all pesticides studied (Figure 1) with a 99.99% CI, according to the *t*-test used in the Echip software. The extractability decreased from approximately 93 to 54% for

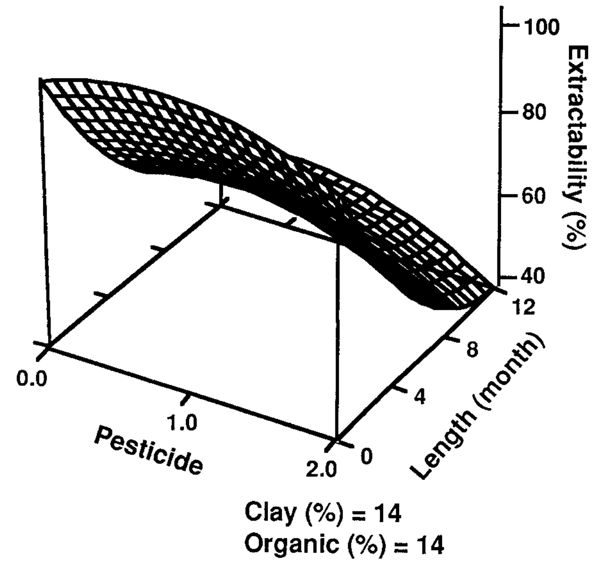


FIGURE 1. Effect of residence time (aging) on the extractability of three pesticides (atrazine = 0, diuron = 1, and bensulfuron-methyl = 2) from soils using SFE.

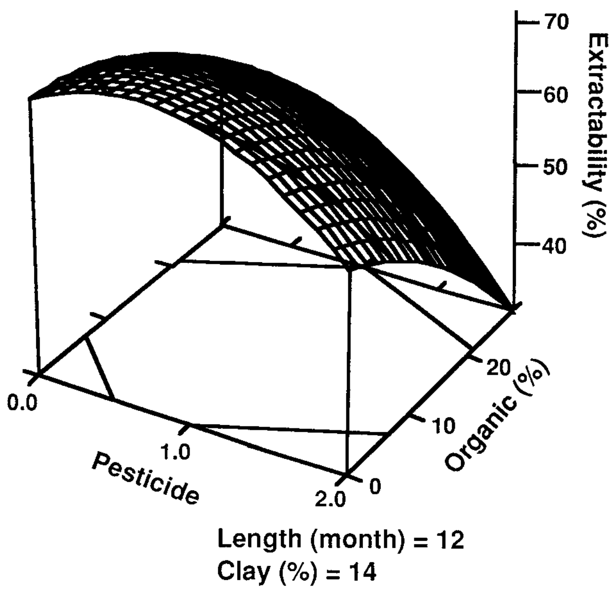


FIGURE 2. Effect of organic matter content (%) on the extractability of three pesticides from soils using SFE.

bensulfuron-methyl, from 98 to 68% for diuron, and from 100 to 73% for atrazine, respectively, with increase in residence time from 0 to 12 months. Other work in our laboratory indicated that the sorption mechanism for bensulfuron-methyl might be different from that of diuron and atrazine, probably associated with pH and cation exchange capacity (CEC) of the soil (work in process).

Both soil organic matter and clay minerals content (%) showed a significant impact on the extraction of bensulfuron-methyl in soil aged for 12 months. It appeared that bensulfuron-methyl binding is more dependent on the organic matter content than clay content in soil (Figures 2 and 3). With 12-month residence time, extractability of bensulfuron-methyl decreased to 32% when organic matter content increased to 28%. The strong effect of organic matter content on binding of bensulfuron-methyl is clearly shown in Figure 2. For the 6-month aging period, the effect of clay content as high as 15% was not significant. A large decrease in bensulfuron-methyl extractability was observed when clay content increased to 28% (Figure 3). Atrazine was the easiest analyte extracted; clay content had greater impact on extractability than did organic matter content (Figures 1−3). In the case of diuron, both organic and clay contents affected the extractability, but not as significantly as for bensulfuron-methyl.

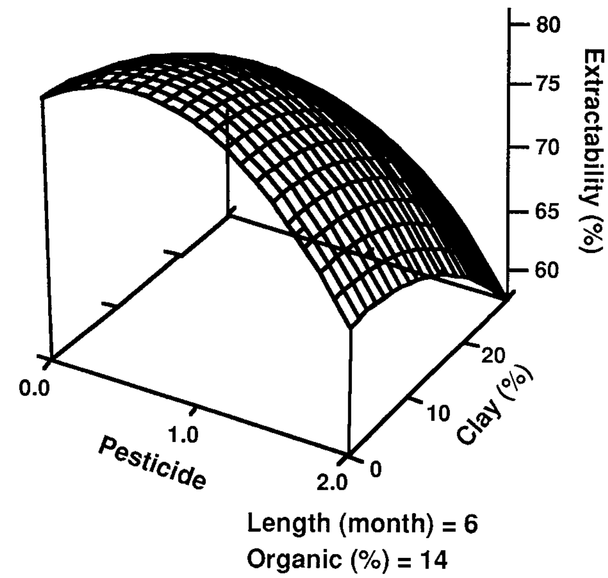


FIGURE 3. Effect of clay minerals content (%) on the extractability of three pesticides from soils using SFE.

To examine intervariable effect, two variables may be selected at a time while fixing a specific value for the other two resulting in a 3-D contour plot. For example, one may choose a fixed value for residence time (month) and organic matter (%) (i.e., 6 months and OM 14%; Figure 3) and then study the effect of other variables. Some of the extractability and variable values were not experimented but extrapolated using curve-fitting regression within the model. The effect of the variables studied would only suggest a ranking of the impact magnitude on extractability under a specific condition.

Mixtures of soil samples would be expected to differ from the original soils such characteristics as their cation exchange capacity (CEC), pH, and specific surface area, which are important to pesticide sorption. It is likely that such soil samples, prepared under laboratory conditions, would not truly reflect the natural soil environment. Therefore, the degree of significance among the soil environmental variables potentially indicate interactions and relationships among the test variables, but should not be taken as a quantitative evaluation of binding strength and mechanisms. However, these results should be useful in qualitatively understanding the nature of interaction of field aged residues with soil constituents and interpreting bound residues in the real soil environment.

Effects of SFE Parameters on Extractability. Studies of SFE optimization conducted in laboratories have traditionally dealt primarily with freshly fortified samples, which frequently behave very differently from environmental soil samples. Many pesticides bind to soils very tightly after aging for a period of time (*3*). Fortified samples are convenient, but often show higher recoveries than field aged samples. This is probably due to stronger matrix−analyte interactions (e.g., chemisorption and physisorption) in field aged samples.

Threshold values of temperature and pressure for extracting freshly fortified diuron, bensulfuron-methyl, and atrazine from inert (Celite 545) were obtained by adjusting the pressure and temperature within a reasonable range (140−352 atm for pressure, 40−70 °C for temperature). The values for either temperature or pressure that yielded recovery values above 90% were considered to be threshold values for each compound.

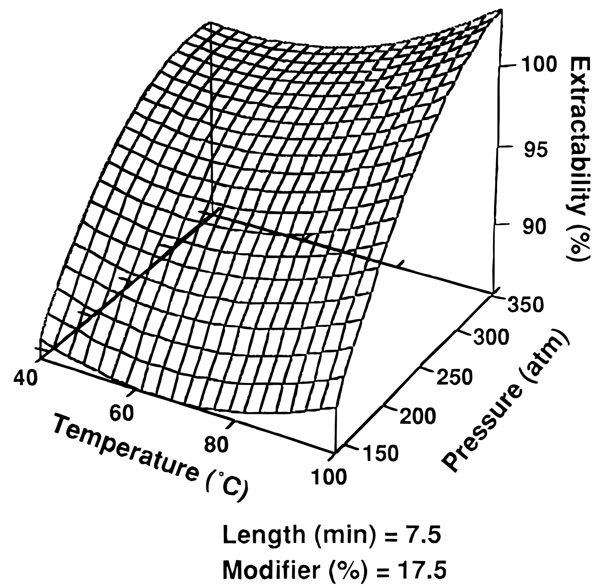
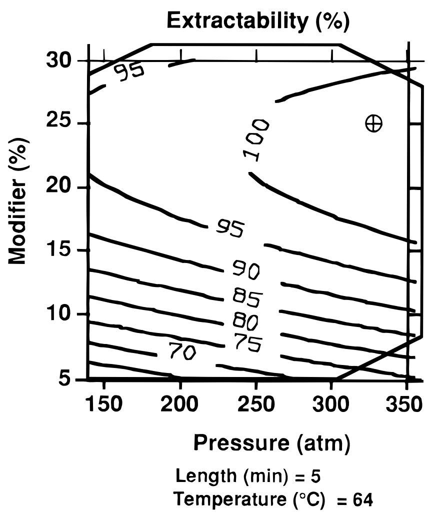


FIGURE 4. SFE extractability of fortified diuron from Danish soil with multivariate optimization scheme.



Pressure = 328		Modifier = 25
Value	Low Limit	High Limit
103	96	110

FIGURE 5. 2-D optimization for fortified bensulfuron-methyl (effect of pressure and modifier).

For a freshly fortified sample, recovery increased as both pressure and temperature increased, but it reached a plateau while both parameters exceeded threshold values for recovering a particular analyte. Beyond this threshold, an increase in either parameter was no longer important for the extraction process (Figures 4 and 5). Density of supercritical carbon dioxide is directly proportional to the increase of pressure, but inversely proportional to the increase of temperature. In fact, heterogeneous soil samples would behave differently as compared to the homogeneous matrix Celite 545. However, these threshold values would be expected to yield qualitative measurements in terms of desorption threshold for freshly fortified samples.

Recovery was enhanced with the increase in modifier percentage (5−30%) for all analytes. In particular, more than 15% modifier was required to yield an acceptable extractability of freshly fortified bensulfuron-methyl (Figure 5), as indicated also in the threshold evaluation. Recently, more studies have been reported using a relatively higher percentage of modifier to improve extraction efficiencies, particularly for recovering

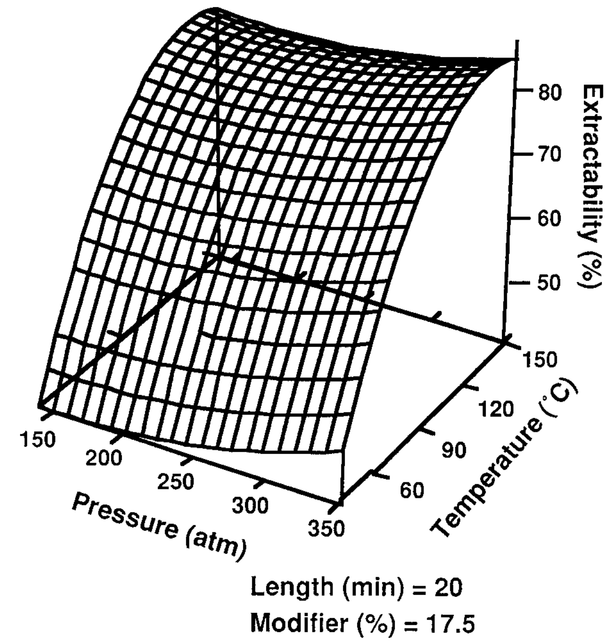


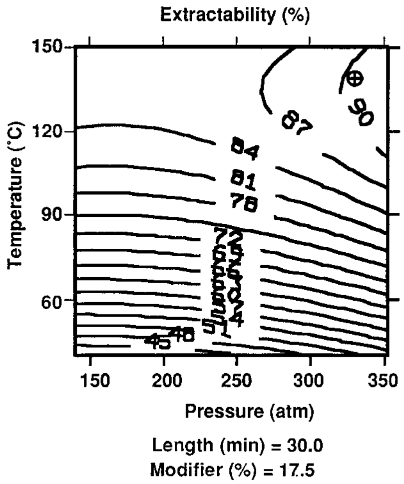
FIGURE 6. SFE extractability of aged atrazine from Alliston soil with multivariate optimization scheme (effect of pressure and temperature).

polar contaminants (*10*). Under certain conditions both a supercritical phase (modified CO_2) and a subcritical phase (modifier, i.e., water) may coexist, enhancing extractability of aged residues, probably associated with a potential swelling process as well as changing polarity of the modified supercritical fluid in the extraction.

Surfactant as a modifier to supercritical fluid has not been reported to date. Interestingly, modifier containing surfactant Triton X-100 yielded better extraction rates and efficiencies for aged soils in these studies with one exception, bensulfuron-methyl in the aged samples. This result was presumably related to the particular sorption mechanism associated with bensulfuron-methyl (unpublished research).

Multivariate optimization schemes were performed for field aged atrazine, diuron, and bensulfuron-methyl samples (Table 2, i.e., atrazine). Four independent variables were experimented for the optimization of extraction. Temperatures seemed to have the most significant effects on the recovery for field aged residues based on the *t*-test. Recovery increased to 86% (diuron) while elevating temperature to 100 °C and to 88%, (atrazine, Figure 6) with a temperature increased to 150 °C. However, elevated temperature would not be applicable for a thermally labile compound such as bensulfuron-methyl.

For field aged residues, the SFE extraction seemed to relate mostly to mass transfer or diffusion-controlled processes. Therefore, solubility of an analyte in the supercritical fluid appeared not to be an important parameter for better extraction efficiency. Thus, the extraction rate would be expected to be determined by mass transfer, which can be a rather slow process. Using initial static and followed by dynamic extraction, more than 80% of aged atrazine residues were extracted from Alliston soil within 25−30 min (Figure 6). It was observed that the pressure during SFE of atrazine appeared to be important (Figure 7) in maintaining the SC-CO_2 density required for better extractability at an elevated temperature (150 °C). This was not the case for bensulfuron-methyl since a relatively low temperature was applied to the SFE process. Elevation of temperature was crucial to recovering the "bound atrazine". Addition of modifier was also significant to the extraction. Among the tested modifiers, the use of surfactant yielded the highest recovery (especially when temperature was increased to 150 °C), while other



Pressure = 330		Temperat = 140
Value	Low Limit	High Limit
90	84	97

FIGURE 7. SFE Extractability of aged atrazine from alliston soil with 2-D optimization contour plot (effect of pressure and temperature).

modifiers (such as acetonitrile and methanol) were not as effective.

It is possible that in the static method the long exposure to solvent allows the matrix to swell, thus improving the penetration of carbon dioxide into its interstices and increasing analyte recovery. In the dynamic method, continual exposure of analyte to fresh solvent enhances partitioning of the analyte into the mobile phase (*4*). As with other SFE operating criteria, selection should be based on actual performance.

Prewetting the matrix sample with modifier and proceeding with a static extraction followed by dynamic extraction yielded faster extraction rates than either repeated static or dynamic extraction only (work in process). This is probably due to the matrix being allowed to swell during the initial static step, resulting in better exposure to supercritical fluid and modifier, especially in the case of extracting native or bound residues, also reported by Fahmy et al. (*11*). Studies also indicate that the mode of SFE (either dynamic or repeated static) and length of extraction are crucial in recovering bound residues, while pressure is no longer an important parameter for the extraction. Mass transfer or slow diffusion processes appeared to be determining factors for the extraction.

Acknowledgments

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