

# INITIAL INVESTIGATION OF ANALYTICAL EXTRACTION TECHNIQUES FOR THE DETERMINATION OF BIOAVAILABILITY OF PESTICIDES IN SOIL

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## ABSTRACT

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The inadvertent ingestion of contaminated soil can be an important source of pesticide exposure, especially in young children. Analytical extraction techniques explored in the past were designed to determine the total contamination level of environmental solids. The actual level of a pesticide that is available for absorption into the biological system may be much lower than the overall contamination level due to the interaction of each compound with the solid matrix. This bioavailability is dependent on the chemical species as well as the soil type. In an effort to gain understanding of this phenomenon, we report a comparison of the recoveries of pesticides from sand using different analytical extraction techniques. The techniques examined were Soxhlet extraction, microwave-assisted extraction (MAE), subcritical water extraction (SCWE), and aqueous microwave extraction (ME). Of five pesticides investigated, one showed a statistically significant difference in recovery between Soxhlet and MAE. For the two water extractions, three of five pesticides displayed statistically significant differences. Soxhlet and MAE had much higher average recoveries (74 % and 85 %, respectively) than the two water extractions, (ME (1 %) and SCWE (9 %)). Future comparisons of the results of these analytical extractions with data from physiological-based tests may lead to the development of a bioavailability-determination technique that will avoid the use of animals or complex models of biological systems.

**Key words:** *bioavailability, pesticides, soil, extraction*

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## INTRODUCTION

Individuals are in contact with soil through each of the established routes of exposure: ingestion, inhalation, and dermal absorption. Soil may contain numerous organic pollutants, including pesticides. The degree of harm caused by exposure to a pollutant in the soil depends on the ease with which the contaminant is released from the matrix under physiological conditions, that is, its bioavailability. Of concern is the potentially damaging quantity being absorbed by the human system. Current techniques to determine bioavailability involve animal models or complex models of the human digestive system (Ruby et al., 1996; Koganti et al., 1998). There is a need to develop analytical techniques to mimic this process to determine quickly and easily the bioavailability of organic contaminants from environmental solids.

The techniques that have been developed to date to extract organic contaminants from soil have been evaluated in terms of their ability to determine the aggregate concentration of these compounds accurately and completely. Currently there are several techniques in practice for the determination of the total pesticide content of soils including Soxhlet extraction and microwave-assisted extraction (MAE). Soxhlet extraction, a continuous solvent extraction method, is the standard technique used in most EPA methods (Smith, 1994). MAE uses polar organic solvents in

contact with solid samples heated in a microwave to extract organic contaminants (Barnabas et al., 1995).

Two new techniques not involving organic solvents are under investigation for pesticide concentration determination: subcritical water extraction (SCWE) and microwave extraction with water (ME). SCWE takes advantage of the lowered dielectric constant of water observed at temperatures and pressures somewhat below the critical point to extract organic contaminants from soil (Hageman et al., 1996). ME uses the microwave to create similar conditions. These techniques can be used for quantification in conjunction with solid-phase microextraction (SPME) (Zhang et al., 1994). SPME is an equilibrium technique that has been used for the extraction of organic analytes from water samples. This process involves the immersion of an organic-phase-coated silica fiber into an aqueous sample containing analyte. The analyte partitions between the aqueous medium and the coating. The fiber is then removed from the sample and placed in the injection port of a GC, where the analyte is thermally desorbed from the fiber coating and quantified.

Each of these techniques is expected to have a differing ability to recover pesticides from solid samples. Reported here is an initial comparison of the extraction efficiency of all four techniques in order to study these differences. Future work will compare these methods to an extraction technique adapted from that of Ruby et al. (1996) that uses a complex model of the human digestive system to determine bioavailability. Each technique will be individually optimized to the values determined by this physiologically based test.

## **MATERIALS AND METHODS**

### ***Sand***

Washed sea sand (Fisher Scientific, S-25-10) was first sieved to 150  $\mu\text{m}$  and then washed with 50%/50% v/v acetone:hexane (A/H) 5 times.

### ***Sand Spiking***

Dry, sieved sand (20.5 g) and 3.0 mL pesticide solution [1000  $\mu\text{g}/\text{mL}$  of 5 pesticides—malathion, diazinon, chlorpyrifos, chlordane (*trans* and *cis*) and p,p'-DDT] in acetone (Chem Service, Inc.) was added to a 150 mL amber jar. Approximately 50 mL acetone (HPLC grade, Mallinckrodt) was added in order to thoroughly wet the sand. A stir bar was added. The solution was stirred for 24 hours with the lid closed. The lid was then removed to allow the solvent to evaporate.

### ***Soxhlet Extraction/Concentration***

A single-step Soxhlet extractor/concentrator (Pyrex No. 3910) was used. Extraction times were 24 hours with 1.5 to 3.5 g of sand and ~150 mL solvent (A/H). The sample was concentrated to a chosen volume after extraction by closing the stopcock, stopping the solvent from

returning to the concentration tube. The samples were then diluted to 100 mL in a volumetric flask with acetone before analysis in order to be read reliably by the GC-ECD.

#### ***Microwave-Assisted Extraction***

A microwave acid digestion bomb (45 mL capacity, Parr 4782) was used for all microwave extractions. Spiked sand (0.10 – 0.15 g) was weighed into a Teflon cup. Solvent (A/H or deionized water, 4 mL) was added by pipet. The Teflon cup was sealed with a Teflon O-ring and lid. The assembly was placed in the bomb body and sealed. The bomb was heated in a microwave (1.52 kW, 900 W output, Sharp Carousel) for 3 min. The sealed bomb was cooled outside of the microwave for 30 min. The Teflon cup was opened and the supernatant was transferred to a glass vial by pipet. Organic samples were injected directly into a GC-ECD. Aqueous supernatant was analyzed by SPME as described below and by solvent exchange into hexane and direct injection.

#### ***Subcritical Water Extraction***

The technique was adapted from Hageman et al. (1996). The extraction vessel consisted of a 64-mm-long, 7-mm-i.d. stainless steel pipe with national pipe thread end caps (Cajon, SS-4-HLN-2.50 (pipe), SS-4-CP (end caps)). Each extraction vessel was constructed by sealing one end of the pipe with an end cap and one and one-half turns of Teflon tape. The cap tightened with a wrench. Sand (0.10 – 0.15 g) was weighed into the extraction vessel. Deionized water (3 mL) was added by pipet. The vessel was sealed with a second end cap and Teflon tape with wrench tightening. The vessel was placed in a pre-heated muffle furnace set to 200 °C. After 60 minutes extraction, the vessel was removed from the oven and immediately cooled under running tap water. The vessel was opened and the supernatant was removed. The supernatant was brought to 4 mL with deionized water and transferred by pipet to a glass vial. Analyte from 1 mL of supernatant was partitioned into 1 mL hexane and directly injected into GC-ECD.

#### ***Solid-Phase Microextraction***

Manual SPME extractor (7 µm PDMS coated fiber, Supelco) was used. Water samples (1 mL from microwave extractions) were placed in 2 mL amber glass vials with an 8 mm Teflon-coated stir bar and sealed with septum caps. The exposure time for the SPME fiber was 30 minutes. After being removed from the sample, the fiber was desorbed in the injection port of a GC-ECD for 10 minutes.

#### ***Gas Chromatography-Electron Capture Detector***

Samples were analyzed using a Hewlett Packard Model 5890 Series II GC with a DB5-MS column (30 m long; 0.25 mm i.d.; 0.25 µm film thickness) and electron capture detector (ECD). Organic solvent samples were analyzed by direct injection of 1 µL into an injection port held at 250 °C. The ECD was held at 200 °C. The GC oven temperature was held at 50 °C for 2 minutes and then ramped to 250 °C at 20 °C/min, where it was held for the remainder of the run with a column

head pressure of 10 psi (He gas with N<sub>2</sub> make-up gas). SPME samples were placed in the injection port which was held at 300 °C for 10 minutes to desorb the fiber while concentrating the sample at the head of the column. The oven program above was then followed.

## RESULTS

### *Spiked Sand*

Sand was used in this study in order to examine these techniques without the complication of variable organic content. The spiking procedures performed resulted in a concentration of ~ 150 µg each pesticide/g sand.

### *Soxhlet Extraction*

Soxhlet extraction was successful in recovering an overall average fraction of  $72 \pm 48$  % (95 % CI). In general, the variance within a pesticide and between pesticides was high (Table 1, Figure 1).

### *Microwave-Assisted Extraction (Organic Solvent)*

MAE with A/H was successful in recovering an overall average fraction of  $85 \pm 49$  %. The variance within each pesticide was lower than that of Soxhlet extraction. The variance between pesticides was similar to that of Soxhlet extraction.

### *Solid-Phase Microextraction (SPME)*

When aqueous standards of the five pesticides were exposed to the SPME fiber, ~ 2 % of the analytes (by weight) were adsorbed by the fiber at the highest concentrations. Because of this low recovery, aqueous samples were analyzed following solvent exchange with hexane.

### *Microwave Extraction (Water)*

The extraction efficiency of ME with water as a solvent was analyzed using solvent exchange with hexane and direct injection. Direct injection analysis of hexane samples gave the results presented in Figure 1, with an overall average recovery (excluding malathion) of  $2.5 \pm 4$  %. These results are not significantly different from zero at the 95 % level. Malathion gave no measurable quantities.

### *Subcritical Water Extraction*

Aqueous supernatants from SCWE were examined by solvent exchange with hexane and direct injection. The overall average recovery was  $8.6 \pm 6$  %.

### *Comparison of Techniques*

Comparison of the recoveries of the individual pesticides in Soxhlet and MAE yields a significant (95 % level) difference between the recovered fractions of diazinon, while the differences of the other pesticides are not significant. In a comparison of ME and SCWE, the differences in recoveries are significant for chlorpyrifos, chlordane, and p,p'-DDT. The differences in the recoveries of all pesticides are significant when comparing the organic solvent techniques to the aqueous methods.

## **DISCUSSION**

Although the recommended SPME fiber (7  $\mu\text{m}$  PDMS) and conditions (30 minutes extraction with agitation) for extracting semi-volatile compounds from aqueous solutions were used in this work, partitioning of the analytes into the fiber from the water samples was found to be very low. It is possible that these particular samples require a longer exposure time or that a different type of SPME fiber will be better suited for these analytes. These conditions must be examined before the SPME procedure can be integrated into the aqueous extraction techniques.

The procedures involved in microwave extraction include a 30-minute cooling time, necessary to lower the pressure in the extraction vessel. During this time the solvent returns to its ambient temperature and pressure while remaining in contact with the solid sample. When organic solvents are used, this extended cooling period does not affect the recovery of analyte as the semi-volatile compounds under investigation are highly soluble in the organic solvents at ambient temperatures and pressures. In the water analyses, however, the low recoveries may be due to this extended contact time during which the water cools. As the analytes have a low solubility in water at room temperature, they may re-partition into the sand.

The recoveries found using SCWE are also low with a large variance between the samples examined. They are, however, significantly higher than those obtained for ME with water. This may be due to the low contact time (<2 min) of the water with the sand after the water is cooled in SCWE, which does not allow for re-partitioning of the compounds into the solid sample.

Overall, the results of these four extraction techniques show that the recoveries of the methods involving organic solvents were substantially higher than those involving aqueous solvents. The sand used may be expected to have a low-binding coefficient with the organic compounds under investigation. The low recoveries of pesticides from sand using aqueous solvents suggest that even this low-binding coefficient is stronger than the partitioning of the compounds into water. These differences may play a significant role in determining the relative bioavailability of these compounds.

## **CONCLUSIONS AND FUTURE WORK**

Pesticides spiked into sand can be recovered appreciably using 50%/50% v/v acetone:hexane in both Soxhlet and microwave-assisted extraction. Microwave extraction using water as a solvent recovers very low fractions of pesticides spiked into sand. Subcritical water extraction at 200 °C recovers low fractions of pesticides from sand, but is more efficient than microwave extraction with water. Solid-phase microextraction must be studied to optimize its use to recover pesticides extracted into water. The ability of each of these techniques to recover pesticides from non-organic sand can be compared to future work using organic solids to demonstrate the effect of organic content on pesticide recovery. A physiologically based extraction test will be adapted from Ruby et al. (1996) to define a bioavailable fraction of pesticides from environmental solids. The use of each

of these techniques in bioavailability determinations may be possible since recoveries vary greatly between the different techniques, and a combination of the techniques can potentially be optimized to the bioavailable fraction of pesticides found in solid samples.

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#### **REFERENCES**

- Barnabas, I. J., J. R. Dean, et al., 1995. Extraction of Polycyclic Aromatic Hydrocarbons from Highly Contaminated Soils Using Microwave Energy. *Analyst* 120, 1897-1904.
- Hageman, K. J., L. Mazeas, et al., 1996. Coupled Subcritical Water Extraction with Solid-Phase Microextraction for Determining Semivolatile Organics in Environmental Solids. *Anal. Chem.* 68, 3892-3898.
- Koganti, A., D. A. Spina, et al., 1998. Studies on the Applicability of Biomarkers in Estimating the Systemic Bioavailability of Polynuclear Aromatic Hydrocarbons from Manufactured Gas Plant Tar-Contaminated Soils. *Environ. Sci. Technol.* 32, 3104-3112.
- Ruby, M. V., A. Davis, et al., 1996. Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test. *Environ. Sci. Technol.* 30, 422-430.
- Smith, R.-K., 1994. *Handbook of Environmental Analysis*, Genium Publishing. Schnectady, New York, p. C-4.
- Zhang, Z., M. J. Yang, et al., 1994. Solid-Phase Microextraction. *Anal. Chem.* 66, 844 A-853 A.

**Table 1.** Recovered fractions of each pesticide using different extraction techniques.

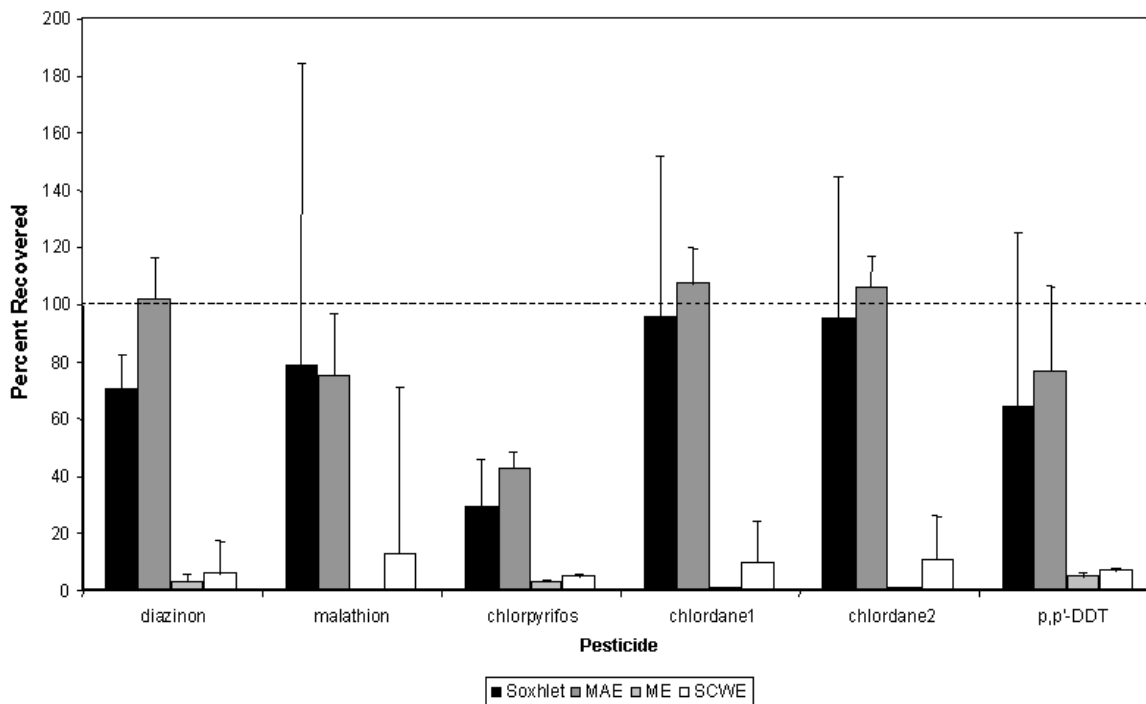
Pesticide	Mean Percent Recovery (std dev)			
	Soxhlet <sup>a</sup>	MAE <sup>b</sup>	ME <sup>c</sup>	SCWE <sup>c</sup>
Diazinon	71 (6)	102 (8)	3.23 (1)	5.9 (6)
Malathion	79 (54)	75 (11)	0 (0.2)	13.2 (30)
Chlorpyrifos	30 (8)	43 (3)	3.29 (0.3)	4.8 (0.5)
Chlordane-1	96 (29)	107 (6)	0.70 (0.2)	10.2 (7)
Chlordane-2	95 (26)	106 (6)	0.98 (0.3)	11.0 (8)
p,p'-DDT	64 (31)	76 (15)	4.91 (0.8)	6.8 (0.6)

<sup>a</sup>In Soxhlet extractions, each mean represents an average of n=3 extractions, each averaged from triplicate injections on the GC-ECD.

<sup>b</sup>For MAE, each mean represents an average of n=5 extractions, each averaged from 3 injections, except for p,p'-DDT where n=4.

<sup>c</sup>For both ME and SCWE, each mean represents an average of n=5 extractions, each averaged from duplicate injections.

### Pesticide Recovery by Technique



**Figure 1.** Average percent recoveries for each pesticide by each technique as determined by GC-ECD analysis. For all techniques n=5 except for Soxhlet, where n=3 for all compounds and MAE, where n=4 for p,p'-DDT and n=5 for all other compounds. Each value was averaged from 3 injections for Soxhlet and MAE while only two injections were performed for SCWE and ME. Error bars represent a 95% confidence interval.