

# SOXHLET AND MICROWAVE EXTRACTION IN DETERMINING THE BIOACCESSIBILITY OF PESTICIDES FROM SOIL AND MODEL SOLIDS

<sup>1</sup>B.K. Kramer and <sup>1,2</sup>P.B. Ryan

<sup>1</sup>Department of Chemistry, Emory University, Atlanta, GA 30322; Phone: (404) 727-9259; Fax: (404)727-8744. <sup>2</sup>Rollins School of Public Health, Emory University, Atlanta, GA 30322; Phone: (404) 727-3826; Fax: (404) 727-8744

## ABSTRACT

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The inadvertent ingestion of contaminated soil can be an important source of pesticide exposure, especially in young children. The actual level of a pesticide that is available for absorption into a biological system may be much lower than the overall contamination level due to the interaction of each chemical with the solid matrix. This bioaccessibility is dependent on characteristics of the chemical species as well as the soil type and residence time of the compound in the soil. In an effort to improve understanding of this phenomenon, we report a comparison of the recoveries of pesticides spiked into soil and two model solids using different analytical extraction techniques: Soxhlet extraction, microwave-assisted extraction with an organic solvent (MAE), and microwave extraction using water as a solvent (WME). The efficiency of each technique is compared to the maximum bioaccessible fraction determined by a physiologically based extraction test (PBET). Recoveries by Soxhlet and MAE are shown to be in good agreement with each other, but do not predict the bioaccessible fraction. Recoveries by WME, however, are in agreement with bioaccessible fractions of all pesticides and soil types studied. WME has the potential to be useful in bioavailability studies.

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

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## INTRODUCTION

Pesticide contamination of soil can be a major source of exposure, especially to children. The amount of a pesticide to which a person is exposed when contaminated soil is inadvertently consumed will depend not only on the amount of the pesticide that is in the soil, but also how much of the contaminant can be removed from the soil as it passes through the digestive system. Some compounds are so tightly integrated into the soil that they may stay bound and be excreted with the soil, never entering into the circulatory system where they could cause harmful effects. The amount of a particular compound that is available for extraction from the soil under physiological conditions depends on the chemical properties of the compound, the makeup of the soil, and how

long the contaminant has been in contact with the soil.

The quantity of a compound that is removed from the soil and then transferred into circulation is referred to as the bioavailable fraction (Ruby et al., 1996). Current techniques to determine bioavailability involve animal or bacterial models or complex models of the human digestive system (Koganti et al., 1998; Ruby et al., 1996; Sijm et al., 2000). There is a growing move to develop analytical techniques to mimic this process to determine quickly and easily the bioavailability of organic contaminants from environmental solids (Sijm et al., 2000).

In order to be bioavailable, contaminants must first be desorbed from the soil system under physiological conditions. Not all com-

pounds that are removed from the soil are necessarily absorbed into the biological system. The total amount of a compound that is desorbed from the soil, whether it is further absorbed into circulation or not, is defined as the bioaccessible fraction, sometimes referred to as the "mobile" fraction (Oomen et al., 2000). Bioaccessibility is a simpler quality to model than bioavailability since only desorption under physiological conditions is necessary. It is potentially an overestimate of bioavailability and can be seen as a conservative estimate of possible risk. The definition of bioaccessibility used in this work is the maximum fraction of contaminants in soil systems that can be recovered from a model of the physiological composition and digestion conditions of the human digestive tract. This test is based on the physiologically based extraction test (PBET) developed by Ruby et al. (1996) for the analysis of the bioaccessibility/bioavailability of lead and arsenic from soil.

Most chemical extraction methods explored in the past were studied in terms of their ability to recover all pesticides from a soil matrix. In this work, we examine the abilities of three simple analytical extraction methods to mimic the bioaccessibility determined by the more complex digestive system model. These methods are Soxhlet extraction, microwave-assisted extraction with an organic solvent (MAE), and microwave-assisted extraction with water as a solvent (WME).

Soxhlet extraction is a continuous solvent extraction method and is the standard technique used in most EPA methods (Smith, 1994). It is

a lengthy process, involving 24-hour extraction times and large volumes of organic solvent (up to 150 mL), and large sample sizes (up to 10 g). MAE is a similar extraction method which reduces the extraction process to a shorter duration using smaller samples and solvent amounts. MAE uses polar organic solvents in contact with solid samples heated in a microwave to extract organic contaminants (Barnabas et al., 1995). Extraction times can be as little as 3 minutes with sample sizes as small as 0.1 g and solvent volumes as small as 4 mL.

WME is a new technique that is based on the process of MAE. The sample size, solvent volume, and extraction time are the same as those of MAE. The difference is that water is used as the extraction solvent. Microwave extraction relies on the power of microwave energy to cause rapid rotation and concomitant heating in polar solvents (Barnabas et al., 1995). Water would be an ideal solvent for microwave heating since it is polar and does not result in any new disposal problems. Heated water, under pressure, can act as a better solvent for organic compounds than it would be able to under ambient conditions as its dielectric constant begins to decrease (Tödheide, 1972). WME may also be able to act as the best mimic of the bioaccessibility determined by digestive modeling as it places the soil system in a solvent environment similar to that of the bioaccessibility test. The chief differences between the two techniques are the temperature reached in the WME procedure over a short period of time and the digestive components contained in the gastric solution. These differ-

ences are less extreme than those that are present between Soxhlet or MAE and the bioaccessibility tests.

Different types of chemical compounds will behave differently when in the presence of soil as well as during different extraction procedures. In order to analyze the differences in the bioaccessibilities of different pesticides, five different compounds were studied, including two organochlorine pesticides, p,p'-DDT and chlordane, which have been banned in the US for nearly 20 years or more but are still found in soil and food samples as well as human tissue samples. Three organophosphorus pesticides, diazinon, malathion, and the recently restricted insecticide chlorpyrifos (Dursban) were also considered in this study. Each of these pesticides has different moieties that may interact more or less strongly with the different components of the soil.

Soil is a complex matrix of both organic and inorganic components. The specific portion of the soil to which a contaminant adsorbs will depend on the nature of both the compound and the matrix as well as the length of the contact time of the chemical with the soil. Non-volatile chemicals associate both reversibly and irreversibly to soil matrices (Bhandari et al., 1997; Chen et al., 2000; Kan et al., 2000; Kan et al., 1998; Kan et al., 1997). In order to study the effects of the organic component of the soil on the retention of the pesticides under investigation, model solids were chosen to distinguish between retention by organic and inorganic portions of the soil.

The main organic portion of soil is com-

posed of humic substances. These are substances that derive from the decomposition of plant and animal matter. Humic substances are complex compounds which cannot be defined as any particular class of compound and are instead classified operationally into three subdivisions: fulvic acid, humic acid, and humin. Fulvic acids are those humic substances which are soluble in acidic solution. Humic acids comprise the component that is insoluble in acid, but soluble in basic solution. The components that are not extractable in either basic or acidic solution make up the portion referred to as "humin" (Gaffney et al., 1996).

The first goal of the work presented here was to determine the bioaccessibility of several pesticides from soil and the two model solids that had been spiked and allowed to age in the laboratory. Secondly, the samples were analyzed by each of the three analytical methods previously discussed. Finally, the utility of each of these methods for determining bioaccessibility was determined by comparing the bioaccessible fractions determined to the amount recovered by each extraction method for each compound and soil type.

## **MATERIALS AND METHODS**

### ***Soil and Model Solid Preparation***

In an effort to study the portions of soil that interact most strongly with the different contaminants, two different model solids were studied as well as top soil. Pure inorganic sand was chosen to study the interaction of the contaminants with the inorganic portion of soil, while a humic acid-amended sand (HA-sand)

was created to study the interaction of the compounds with a “soil” that had a controlled organic component. Humic acid was chosen as the organic component for this research as it was the most readily available and is usually the larger organic component of soil. Soil was also analyzed to determine the ability of the HA-sand model to approximate soil.

Washed sea sand (Fisher Scientific, S-25-10) was first sieved to less than 150  $\mu\text{m}$  and then cleaned with 50%/50% v/v acetone:hexane (A/H) 5 times. Humic acid (sodium salt, Acros Organics, 12086-0010) was purified by first removing free fulvic acid in acidic solution, dissolving in basic solution, and then precipitating in acidic solution. Precipitated humic acid was collected by vacuum filtration and rinsed with  $\sim 0.1$  M HCl. To prepare 5 % by weight humic acid-amended sand, blank sand (95.0 g) was cleaned as described above, and then mixed with purified humic acid (5.0 g) and 150 mL deionized water and stirred with a mechanical stirrer for 24 hours. The humic acid-amended sand (HA-sand) was then allowed to dry in the hood and tested for the stability of HA on sand in several solvents (acetone, hexane, water, dichloromethane). HA-sand was aged in an amber glass jar at room temperature for approximately six months before being spiked with pesticides. Dry top soil (Organic Valley) was sieved to less than 150  $\mu\text{m}$  before spiking.

### ***Spiking***

Soil and the two model solids (sand and HA-sand) were prepared as above and then spiked with a custom pesticide solution

(ChemService, Inc., West Chester, PA) that consisted of a 1000  $\mu\text{g}/\text{mL}$  solution each of six compounds (diazinon, malathion, chlorpyrifos, *trans*- and *cis*-chlordane, and p,p'-DDT each > 98 % purity) in acetone. Approximately 50 g of each solid medium was placed in a 150 mL beaker to which was added 7 mL of standard solution to obtain  $\sim 145$   $\mu\text{g}/\text{g}$  of each pesticide in the sample medium. Approximately 60 mL of acetone (ACS reagent grade, Fisher Scientific, New Jersey) was added to thoroughly wet the medium. The solution was sealed with several layers of Parafilm<sup>®</sup> and stirred with a mechanical stirrer continuously for 8 or 9 hours and then intermittently to complete a 24-hour contact period. The Parafilm<sup>®</sup> was then removed to allow the solvent to evaporate with continued intermittent stirring.

### ***Aging***

Each spiked sample was aged at room temperature in a sealed amber glass jar for 12 weeks prior to analysis.

### ***Soxhlet Extraction***

A micro Soxhlet extractor (Ace Glass, Inc. 6776) was used for all Soxhlet extractions with a 10 x 50 mm single layer cellulose extraction thimble (Whatman International Ltd, 2800105). Extraction time was 24 hours with  $\sim 1.0$  g of the sample and 20 mL of solvent (A/H) with a cycle time of  $\sim 6$  minutes. Recovered solvent was diluted to 25 mL with acetone before analysis. All analyses were performed by direct injection into a GC-ECD.

### ***Microwave-Assisted Extraction, Organic (MAE)***

A microwave acid digestion bomb (45

mL capacity, Parr 4782) was used for all microwave extractions. The sample to be tested (0.10 – 0.15 g) was weighed into a Teflon cup. Solvent (A/H, 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 minutes. The sealed bomb was cooled outside of the microwave for 30 minutes. The Teflon cup was then opened and the supernatant was transferred to a glass vial by pipet. All analyses were performed by direct injection into a GC-ECD.

#### ***Microwave-Assisted Extraction, Aqueous (WME)***

WME was performed in the same manner as MAE with the exception that the solvent used for extraction was MilliQ® (Millipore, MilliQ Academic) distilled water (>18.2 Ω) instead of A/H. After the cooling period, the aqueous supernatant was transferred to a vial containing 2 mL of hexane. The vial was agitated for 1 minute to transfer the analytes into hexane, in which they are more soluble than in water. This hexane layer was directly injected into the GC-ECD for analysis.

#### ***Bioaccessibility Determination***

Bioaccessibility was determined using a physiologically based extraction test based on Ruby et al (1996). The procedures were adapted in order to analyze organic contaminants as well as to minimize the detection limits. In this method, a model of the digestive system was created by first subjecting a 0.40 g sample to 40 mL of an acidic gastric solution in a

separatory funnel in a 37 °C water bath with N<sub>2</sub> gas bubbling throughout to cause mixing. The gastric solution was prepared by acidifying 1 L of deionized water to pH 2 with 12 N HCl (ACS Reagent grade). To this solution was added 1.25 g pepsin (Acros Organics, New Jersey), 0.50 g citrate (99% purity, Acros Organics, New Jersey), 0.50 g malate (95% purity, Sigma, St. Louis, MO), 420 μL lactic acid (98 % purity, Sigma, St. Louis, MO), and 500 mL acetic acid (ACS reagent grade, Aldrich, Milwaukee, WI). After 1 hour in this solution, the sample was designated either as the “stomach” sample, in which case the aqueous supernatant was decanted and exhaustively exchanged into hexane, or the “total digestion” sample. To the total digestion sample was added a dialysis bag (MWCO 6000-8000, Fisher Scientific, Pittsburgh, PA) containing ~ 1 g sodium bicarbonate (99.7 % purity, Aldrich, Milwaukee, WI) and ~2 mL deionized water. The pH was monitored until the solution had reached neutrality, after which the dialysis bag was removed and ~ 70 mg bile salts (50%/50% w/w cholic acid sodium salt:deoxycholic acid sodium salt, Fluka, Switzerland) and ~20 mg pancreatin (porcine, Acros Organics, New Jersey) were added. This solution was kept at 37 °C in a hot water bath. Bubbling N<sub>2</sub> was continued to maintain mixing. After 3 hours, the supernatant was decanted and extracted exhaustively into hexane. The hexane extractions of the stomach sample and the total digestion sample were individually concentrated to 2 mL under a gentle stream of N<sub>2</sub> gas. These samples were then directly injected into the

GC-ECD for analysis.

The remaining solid from both bioaccessibility digestions as well as the WME extractions were further analyzed by MAE (as described above) in order to attempt to recover all of the contaminants that were spiked onto the samples. The WME samples were extracted twice more each while the digestion samples were extracted three more times each.

### Analysis

Samples were analyzed using a Hewlett Packard Model 5890 Series II GC with an autosampler, a DB5-MS column (30 m long; 0.25 mm i.d.; 0.25  $\mu$ m film thickness), and electron capture detector (ECD). Samples were analyzed by direct injection of 1  $\mu$ L 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 15 psi (He gas with N<sub>2</sub> make-up gas). Calibration curves were created for each pesticide using standard dilutions of the spiking solution.

## RESULTS

### Soxhlet Extraction

The micro-Soxhlet extraction was successful in recovering an average of 77 % of the OCPs (chlordane and DDT) that were spiked into all three solids with a standard deviation of 4 % and range of 72 to 88 % (Tables 1, 2, and 3; Figure 1). This recovery was independent of medium type or compound. The average recovery of the OPPs, however, was largely

**Table 1.** Average percent recovery by each technique from spiked sand.

Technique/ Pesticide	Mean Percent Recovery <sup>a</sup> (std dev)			
	Soxhlet <sup>b</sup>	MAE <sup>c</sup>	WME <sup>d</sup>	Maximum Bioaccessible <sup>e</sup>
Diazinon	21 (3)	19 (13)	9 (4)	10† (3)
Malathion	1 (2)	4 (9)	5 (5)	2‡ (2)
Chlorpyrifos	14 (0.7)	12 (1)	2 (0.6)	2† (0.5)
Chlordane-a	72 (2)	69 (3)	3 (0.4)	4‡ (2)
Chlordane-b	72 (2)	68 (3)	3 (0.4)	6‡ (4)
p,p'-DDT	73 (3)	77 (8)	10 (2)	17‡ (7)

<sup>a</sup> Percent recoveries as measured by fraction recovered of amount originally spiked onto sand.

<sup>b</sup> Each value represents an average of four, 24-hour mini-Soxhlet extractions with 50%/50% v/v Acetone:Hexane (A/H).

<sup>c</sup> Each value represents an average of five, 3-minute microwave extractions with A/H.

<sup>d</sup> Each value represents an average of five, 3-minute microwave extractions with MilliQ water.

<sup>e</sup> Each value represents either (†) the greater of either the average of three stomach digestions or the average of three total digestions, or (‡) the *overall* average of three stomach digestions *and* three total digestions, if the difference between the two averages was not significant at  $\alpha=0.05$ .

dependent on medium as well as compound. The average recovery of chlorpyrifos varied from a low of 14 % (s.d. 0.7 %) from sand to a high of 63 % (s.d. 7 %) from HA-sand, with the results from soil very similar to those of HA-sand. Malathion showed great variation in recovery increasing from 1 % (s.d. 2 %, not different from 0 %) from sand to 26 % (s.d. 6 %) from HA-sand to 57 % (s.d. 11 %) from soil. Diazinon recoveries did not vary significantly between media type, averaging 17 % over all media with a standard deviation of 6 %.

**MAE**

Microwave-assisted extraction with A/H gave recoveries that were in good agreement with those found by Soxhlet extraction. The

average recovery of OCP ranged from 68 to 107 %, with an average of 82 % and a standard deviation of 10 %. Only DDT recovery from soil differed greatly (107 % recovery), but the large standard deviation (37 %) kept the difference non-significant. Again, the recovery of chlorpyrifos varied between media types with a low recovery of 12 % (s.d. 1 %) from sand and high recoveries from HA-sand (72 %, s.d. 4 %) and soil (56 %, s.d. 7 %).

Malathion again had a range of recoveries dependent on media type with a low of 4 % (s.d. 9 %, not different from 0 %) from sand to 27 % (s.d. 2 %) from HA-sand to a high of 51 % (s.d. 17 %) from soil. Diazinon again did not show a media dependence, having average

**Table 2.** Average percent recovery by each technique from spiked HA-sand.

Technique/ Pesticide	Mean Percent Recovery <sup>a</sup> (std dev)			
	Soxhlet <sup>b</sup>	MAE <sup>c</sup>	WME <sup>d</sup>	Maximum Bioaccessible <sup>e</sup>
Diazinon	12 (6)	11 (6)	7 (2)	3‡ (3)
Malathion	12 (6)	11 (6)	7 (2)	3‡ (3)
Chlorpyrifos	26 (6)	27 (2)	7 (2)	11† (0.7)
Chlordane-a	63 (7)	72 (4)	20 (2)	6‡ (3)
Chlordane-b	76 (4)	83 (5)	19 (5)	8‡ (6)
p,p'-DDT	86 (4)	90 (8)	13 (2)	44† (2)

<sup>a</sup> Percent recoveries as measured by fraction recovered of amount originally spiked onto HA-sand.

<sup>b</sup> Each value represents an average of four, 24-hour mini-Soxhlet extractions with 50%/50% v/v Acetone:Hexane (A/H).

<sup>c</sup> Each value represents an average of five, 3-minute microwave extractions with A/H.

<sup>d</sup> Each value represents an average of five, 3-minute microwave extractions with MilliQ water.

<sup>e</sup> Each value represents either (†) the greater of either the average of three stomach digestions or the average of three total digestions, or (‡) the overall average of three stomach digestions and three total digestions if the difference between the two averages was not significant at  $\alpha=0.05$ .

recoveries ranging from 11 to 19 % with an average of 16 % (s.d. 10 %).

### WME

Microwave extraction with MilliQ® water as the extraction solvent gave low recoveries overall for all media and compounds. The average recoveries ranged from 2 to 24 % with a median recovery of only 8 %. The most obvious features were higher recoveries of OCPs and chlorpyrifos from HA-sand and a high recovery of malathion from soil; otherwise, the recoveries were very low (Figure 2).

### Bioaccessibility

Bioaccessibility was determined by finding the averages of three stomach extractions and three total digestion extractions for each medium. The extractability of each compound varied in both media type and digestion stage (Figure 3). In sand, the recovered fraction of

the OPPs was higher in the stomach stage of the digestion, while the recovered fraction of the OCPs was higher in the small intestine stage, as shown by higher recoveries from total digestion. In HA-sand, the recoverability of all OCPs increased in the total digestion when the humic acid was extracted by the neutral-basic conditions of the small intestine phase. The OPPs did not show this increase. In soil, all compounds were recovered in similar amounts from both phases. The maximum bioaccessibility was defined as the higher average of the stomach and total digestion for each compound in each medium, if the two recovered fractions were significantly different at  $\alpha=0.05$  in a two-sided t-test. If there was no significant difference, then the results of each type of digestion were pooled to determine maximum bioaccessibility.

**Table 3.** Average percent recovery by each technique from spiked soil.

Technique/ Pesticide	Mean Percent Recovery <sup>a</sup> (std dev)							
	Soxhlet <sup>b</sup>		MAE <sup>c</sup>		WME <sup>d</sup>		Maximum Bioaccessible <sup>e</sup>	
Diazinon	17	(10)	18	(13)	8	(3)	13‡	(4)
Malathion	57	(11)	51	(17)	24	(6)	21‡	(3)
Chlorpyrifos	59	(8)	56	(7)	8	(2)	13†	(2)
Chlordane-a	77	(5)	81	(12)	6	(2)	13†	(2)
Chlordane-b	76	(5)	81	(13)	6	(2)	13†	(2)
p,p'-DDT	88	(10)	107	(37)	10	(3)	10‡	(1)

<sup>a</sup> Percent recoveries as measured by fraction recovered of amount originally spiked onto soil.

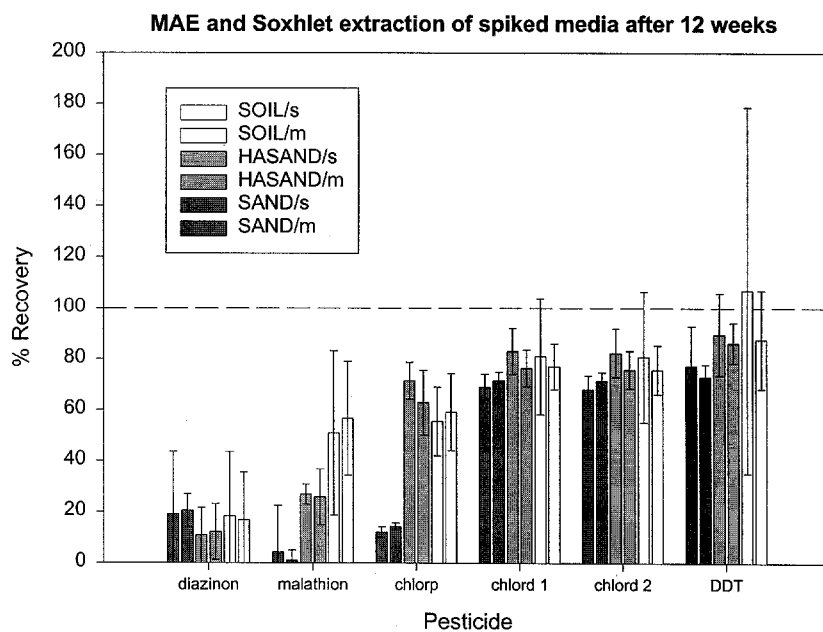
<sup>b</sup> Each value represents an average of four, 24-hour mini-Soxhlet extractions with 50%/50% v/v Acetone:Hexane (A/H).

<sup>c</sup> Each value represents an average of five, 3-minute microwave extractions with A/H.

<sup>d</sup> Each value represents an average of five, 3-minute microwave extractions with MilliQ water.

<sup>e</sup> Each value represents either (†) the greater of either the average of three stomach digestions or the average of three total digestions, or (‡) the *overall* average of three stomach digestions and three total digestions if the difference between the two averages was not significant at  $\alpha=0.05$ .





**Figure 1.** Comparison of Soxhlet and microwave extraction of sand, HA-sand, and soil 12 weeks after spiking. MAE extractions are the first of each pair identified by “/m” while Soxhlet extractions are identified by “/s”. Each peak represents the average of four extractions for Soxhlet or five extractions for MAE. Extraction conditions are described in the text. The error bars represent a 95% confidence interval for each case. The dashed line represents 100% recovery.

## DISCUSSION

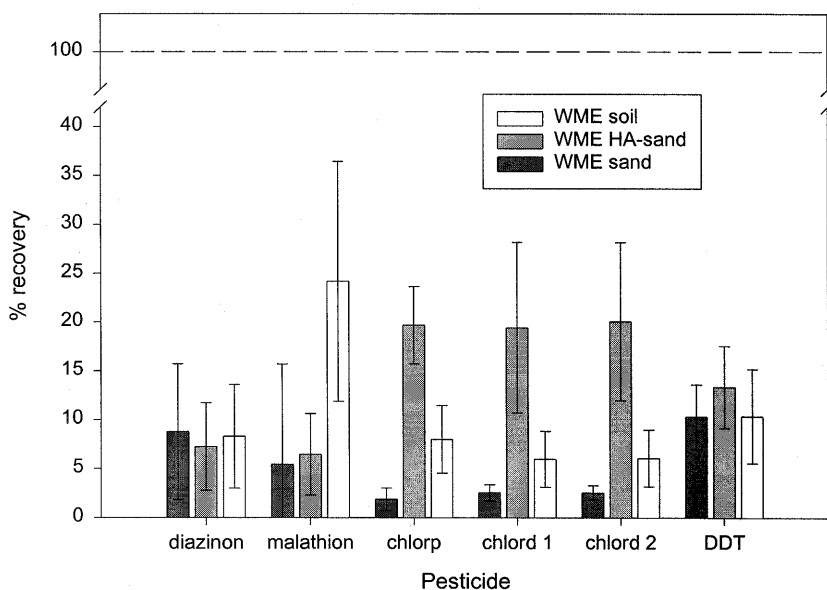
### *Soxhlet and MAE*

Study of the efficiency of Soxhlet extraction and MAE confirm their use as total extraction methods. Soxhlet is the standard extraction method used to determine the contamination level of organic compounds in soil (Smith, 1994). It is, however, a tedious and solvent- and sample-consuming process. Many different techniques have been proposed to replace Soxhlet extraction, some recovering more than twice the amount of a compound that is recovered by Soxhlet extraction (Barnabas et al., 1995); (Hawthorne et al., 1994; Reindl and Höfler, 1994). MAE is one such technique. In this work, a simple domestic microwave and an inexpensive extraction vessel were used. Although the equipment used allowed no control

of pressure and temperature, good reproducibility was found for extractions of all samples studied. In addition, the recoveries were all in good agreement with Soxhlet extraction (Tables 1, 2, and 3). Using a two-sided t-test for independent samples, no statistically significant differences ( $\alpha=0.05$ ) were found between recoveries by MAE and Soxhlet extraction for most compounds. The sole exception was the recovery of chlorpyrifos from sand.

The sample sizes, solvent volumes, and extraction times used for MAE in this work were generally less than those used in other MAE studies (Barnabas et al., 1995; Lopez-Avila et al., 1995), but were sufficient at achieving good recoveries as well as reproducible results. The lower recoveries by Soxhlet and MAE of the OPPs, especially from sand, may

### WME analysis of spiked media



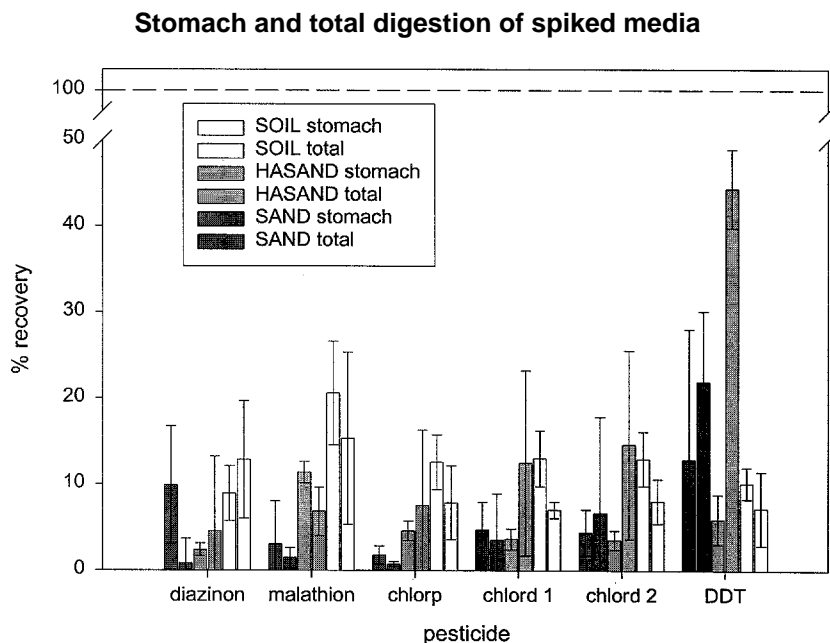
**Figure 2.** Comparison of aqueous microwave extraction (WME) of sand, HA-sand, and soil 12 weeks after spiking. Each peak represents the average of five extractions. Extraction conditions are described in the text. The error bars represent a 95% confidence interval for each case. The dashed line represents 100% recovery. Note change in scale.

be indicative of a kinetic preference for tight interactions of the compounds with the inorganic portion of the soil.

The poor recoveries of the OPPs could also be explained if the compounds were no longer present in the solids at all. If this were the case, the compounds might have never sorbed to the solids and been removed with the spiking solvent, or could have sorbed but subsequently decomposed. The methods used to spike the matrices reduce the possibility of the compounds being removed from solution before binding takes place. Since the spiking solvent was evaporated, rather than decanted, from the solids, the low volatility of the pesticides means that all of the spiked compounds remained with the matrix rather than with the spiking solvent, as is the case in some spiking methods which involve removing non-adsorbed

compounds immediately after spiking (Burgos et al., 1999; Kan et al., 1994). In the cases where non-adsorbed compounds are immediately washed off, there is no allowance for slow adsorption of contaminants into the matrix and only immediate adsorption is investigated. There is evidence, however, that adsorption of some contaminants, especially those migrating into the pore volume, may take place over an extended period of time (Chen et al., 2000; Nam and Alexander, 1993).

The possibility remains that the OPPs could have decomposed or biodegraded. If so, the decomposition products should show up in the GC analysis. At the sensitivities that are currently possible, no additional compounds were found. Further work is being conducted to determine whether these compounds have decomposed.



**Figure 3.** Comparison of stomach digestion and total digestion (stomach + small intestine) of sand, HA-sand, and soil 12 weeks after spiking. Each peak represents the average of three digestions. Digestion conditions are described in the text. The error bars represent a 95% confidence interval for each case. The dashed line represents 100% recovery. Note change in scale.

Soxhlet and MAE were not indicative of bioaccessibility determined by physiologically based tests. All Soxhlet-determined fractions were significantly different, at  $\alpha=0.05$ , from the bioaccessibilities determined, except for malathion from sand and diazinon from soil.

### WME

Recoveries by WME did not agree with those found by Soxhlet extraction. All recoveries except for malathion from sand and diazinon from HA-sand and soil were found to be significantly different at  $\alpha=0.05$  in a two-sided t-test. The ability of subcritical water to extract organic compounds from solid matrices has been explored in the past (Hageman et al., 1996; Lagadec et al., 2000; Yang et al., 1995; Yang et al., 1997). The conditions under which WME occurs may be similar to these subcritical

water conditions. As yet, however, our WME procedure has been unable to achieve the high recoveries of subcritical water extraction (SCWE). Its use appears to be more in bioaccessibility determinations.

Recoveries by WME are in good agreement with those determined by bioaccessibility tests (Tables 1, 2, and 3). Significant differences ( $\alpha=0.05$ , two-sided t-test) between recoveries by the two techniques were found for chlorpyrifos and chlordane in soil and all compounds except diazinon in HA-sand. No significant differences were found in the comparison of compounds extracted from sand. The differences found in recoveries of compounds from HA-sand may likely be due to the removal of HA from the solid matrix during the small intestine phase of the bioaccessibility

determination, as will be discussed subsequently. Even these differences, though statistically significant, are on a much smaller scale than those found between Soxhlet and the bioaccessibility determinations.

### **Bioaccessibility**

The maximum bioaccessibilities determined by the physiologically based digestion model were low in terms of recovery of total spiked pesticide. The total extraction techniques, MAE and Soxhlet, however, also had similarly low recoveries, especially for the OPPs. In a sample of unknown origin, the amount of a compound with which the soil was originally contaminated will not be known. In this case, the bioaccessibility would have to be stated in terms of the percentage of what is found by total extraction. In most cases, what is recovered by Soxhlet is considered to be all that is “extractable.” If Soxhlet is considered to recover 100 % of what is extractable, then the

bioaccessibilities found in this study can be recalculated to reflect a percentage of total extractable material (Table 4). When this is done, the trends in recovery appear to be reversed. The recovery of the OPPs is higher than that of the OCPs for most compounds in all three media. In field samples whose composition is unknown, while what percentage of the original contaminant that was present is accessible might be overestimated, the actual amount of the compound that is accessible would still be reported correctly.

The inconsistently high recovery of DDT from HA-sand in the maximum bioaccessibility determination (Tables 2 and 3), may be explained by considering the ability of the basic conditions of the small intestine portion of the digestive model to remove humic acid from the solid matrix. This desorption of the HA from the matrix is evident as the digestion solution takes on a brown color that is not present in the

**Table 4.** Average percent of Soxhlet recovery that is bioaccessible.

Medium/ Pesticide	Maximum Bioaccessible Fraction (Percent of Soxhlet) <sup>a</sup>		
	Sand	HA-sand	Soil
Diazinon	48	33	76
Malathion	-- <sup>b</sup>	42	37
Chlorpyrifos	14	13	22
Chlordane-a	7	17	17
Chlordane-b	10	20	17
p,p'-DDT	30	51	11

<sup>a</sup> Percent recoveries as measured by average maximum fraction recovered by bioaccessibility tests as a percentage of average recovery by Soxhlet extraction for each medium.

<sup>b</sup> Soxhlet recovery of malathion from sand was less than 1% and not significantly different from zero. The bioaccessible fraction determined was more than three times this amount, but not significantly different.

sand digestion and only slightly evident in the soil digestions. DDT may be removed along with the humic acid and dissolved into solution where it can be extracted into hexane for analysis. It might then be inferred that DDT is strongly associated with the organic or humic acid portion of the model solid. The OPPs are not highly recovered from this same system, indicating that they may be more tightly integrated into the inorganic portion of the solid.

Except for the higher recovery of DDT from the total digestion of HA-sand, the bioaccessibility determined of the OCPs (chlor-dane and DDT) was similar. The magnitude of the maximum bioaccessibilities was comparable across compound type in an individual spiked medium. These compounds are related chemically and could be expected to interact with the matrix in an equivalent way.

The OPPs behaved similarly across compound type in each matrix as well. The main trend seen with the bioaccessibilities of the OPPs was between matrix types. In diazinon, the maximum bioaccessible fraction was highest for spiked soil and lowest for spiked HA-sand. In the cases of malathion and chlorpyrifos, the bioaccessibility increased as the medium became more organic, from sand to HA-sand to soil.

The low bioaccessibilities of the OPPs with the inorganic sand may indicate an interaction between the phosphate groups and the inorganic portion of the sand. These interactions could be strong and reduce the ability of the digestive system to remove the compounds, lowering the bioaccessibility. The bioaccessibilities of malathion and chlorpyrifos

increased as more organic material was added to the medium. This indicates that these compounds may have stronger interactions that are favored when no organic portion is present, but, in general, favor a reversible interaction with the organic portion of the soil, behaving similarly to OCPs.

## **CONCLUSIONS AND FUTURE WORK**

The bioaccessibilities determined by the digestive system model of spiked OCP and OPP pesticides were generally low in soil, HA-sand, and sand. The percentages of pesticides that were accessible in comparison to the total extractable amount were higher for OPPs than OCPs, while the opposite was true when the comparison was made to the originally spiked amount. MAE and Soxhlet were unable to predict bioaccessibility, although they were in good agreement with each other. WME showed good potential as a possible model for bioaccessibility.

Future work in this field includes comparing the bioaccessibilities determined by the digestive model to bioavailabilities as determined by standard methods, including a bacterial toxicity model (Botsford, 1999). Further study of the mechanism of binding of both OCPs and OPPs to the different fractions of the soil system may help determine accessibility as well as predict the results of actual native contaminated samples. Eventually, these techniques will be applied to native contaminated samples to determine the usefulness of WME in determining bioaccessibility of organic contaminants in environmental solids.

## ACKNOWLEDGEMENTS

The authors would like to thank Dr. David MacIntosh at the University of Georgia for instrumentation during the preliminary portion of this research.

This publication was made possible by grant number 5 P42 ES-05947 from the National Institute of Environmental Health Sciences, NIH, with funding provided by EPA. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH, or EPA.

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