

## TECHNICAL NOTE: EVALUATION OF EXTRACTION METHODOLOGIES FOR THE DETERMINATION OF AN ORGANOCHLORINE PESTICIDE RESIDUE IN VEGETATION

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Numerous extraction methodologies are used to quantify pesticide levels in vegetation. Sample availability, resource use, efficiency, time consumption, space allocation, and cost vary considerably among the commonly employed techniques. A study was conducted to compare the efficiency of microwave assisted extraction (MAE), blender homogenized extraction (BE), Soxhlet extraction (SE), the QuEChERS (“Quick, Easy, Cheap, Effective, Rugged, and Safe”) method, and a simple oven assisted extraction (OAE), to recover *p,p'*-DDE from the tissues of *Cucurbita pepo*. A hot-solvent soak of stem or root tissues in a 2-propanol/hexane mixture, OAE yields recoveries that are statistically equivalent to the other procedures. The method recovered  $1800 \pm 190 \text{ ng g}^{-1}$  and  $8100 \pm 900 \text{ ng g}^{-1}$  ( $\text{BCF} = 87 \pm 9.7$ ) *p,p'*-DDE from stem and root tissue, respectively. Recoveries for the other methods ranged from  $1400\text{--}2200 \text{ ng g}^{-1}$  for the stems and  $3600\text{--}7200 \text{ ng g}^{-1}$  for the roots. Statistical analyses for stem and root extraction indicate that there is no significant difference among the variances of each method. Given its simplicity, precision, and efficiency, OAE appears to be suitable for the extraction of an organic pollutant such as *p,p'*-DDE from plant tissues and for use in phytotechnology development and risk assessment.

**KEY WORDS:** phytoextraction, method, DDE

## INTRODUCTION

Plants have been previously evaluated as a viable remediation strategy for organochlorine contamination (Anderson and Walton, 1995; Kelsey and White, 2005; Wang *et al.*, 2004). For example, the bioconcentration of weathered *p,p'*-DDE has been studied among several *Cucurbita* species; the stems and roots of zucchini/pumpkin (*Cucurbita pepo* ssp *pepo*) cultivars have been shown to accumulate contaminant concentrations 5–20 times that in the soil, resulting in over 2% pollutant removal. These findings have been previously conducted and validated in both field and laboratory settings (White *et al.*, 2003a,b).

A number of multi-residue methods have been developed for the extraction of pesticides and other hydrophobic chemicals from vegetation. Although varied, many of these

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techniques are labor intensive and time consuming, require large amounts of petroleum-based solvents, and utilize expensive equipment (Luke *et al.*, 1975; Okumura *et al.*, 1991; Pylypiw, 1993). Soxhlet extraction (SE) with a variety of solvents has been widely implemented as specified by the US EPA Method 3540 (USEPA, 1986). This technique involves the closed circulation of a refluxing hot solvent condensate, and has been widely used for homogenized soil, animal, and plant matrices using common solvents such as *n*-hexanes, acetone, or dichloromethane (Tang *et al.*, 1998; Morrison *et al.*, 2000; Diagne *et al.*, 2002). Despite its time- and resource-intensive nature, SE is used extensively.

Organic solvents can also be used in combination with blending or homogenization methods to extract organic pesticides from vegetation. For example, Pylypiw (1993) proposed a method that involves a liquid-liquid partitioning system between the water miscible 2-propanol (100 mL) that is used to blend 100 g of plant vegetation with 200 mL of petroleum ether. Although these strategies achieve desirable extraction efficiencies, there is a variety of problems with their implementation. The use of petroleum-derived solvent can be wasteful and inefficient, blending necessitates the use of large sample mass for efficient laceration, and sample processing is limited by cost, space, and availability of specialized blenders and homogenizers. Furthermore, there are safety concerns associated with such techniques, including the risk of explosion.

Microwave assisted extraction (MAE) has been previously applied according to the EPA Method 3546 (USEPA, 1986). MAE involves the use of a chemical industrial grade microwave irradiation system to heat a solvent mixture under pressurized conditions in a closed vessel to achieve a rigorous and targeted extraction of pesticides from vegetation matrices. This method has been successfully implemented on a multi-tissue and multi-residue scale as described by Pylypiw *et al.* (1997) to achieve less solvent (~15 mL), sample (~10 g), and time (~10 min) consumption relative to other methods. Disadvantages of MAE are its small sample batch size and reliance on costly industrial grade microwaves and their necessary accessories.

The QuEChERS method was published by workers of the U.S. Department of Agriculture Eastern Regional Research Center (Anastassiades, 2003a, b) to address many of the resource- and labor-intensive drawbacks of the other analytical procedures now in use. This method has been adopted by members of the U.S. Food and Drug Administration (Schenck and Hobbs, 2004), Committee of European Normalization (CEN) (CEN, 2006), and the Commission of the European Communities (CEC, 2006). QuEChERS was adopted "First Action" as AOAC International Official Method 2007.01. This method calls for agitation of plant samples in acetonitrile following the use of salts such as magnesium sulfate to induce an exothermic mass partition of pesticides from the aqueous to the organic phase. This method simultaneously dehydrates the aqueous phase and cleans the sample matrix via the dispersive primary-secondary amine bound silica (PSA) solid phase extraction (SPE), and reconstitutes the analytes in an organic solvent (Lehotay, 2004). However, Okihashi *et al.* (2005) identified two drawbacks to this method. First, shaking as performed in the QuEChERS method is insufficient for the extraction of organic pesticides, such as *o,p'*-DDE. Second, batch clean up of samples is insufficient as proposed in the original method (shown in Table 1).

The goal of the current study was to systematically compare commonly employed extraction techniques for the removal of a hydrophobic chemical from plant tissue. Root and stem tissue from zucchini cultivars (*Cucurbita pepo*) grown in a *p,p'*-DDE-contaminated field site were subjected to chemical extraction using microwave assisted extraction (MAE), blender extraction (BE), Soxhlet extraction (SE) and the QuEChERS method (all methods

**Table 1** Summary of vegetation-bound organochlorine pesticide extraction techniques

Method <sup>a</sup>	Description	Duration	Sample capacity	Solvent volume	Waste
Oven Assisted Extraction (OAE)	10 g sample in co-solvent is extracted at 65°C for 4.5 h and cleaned via separatory extraction (water & Na <sub>2</sub> SO <sub>4</sub> )	4.6 h	Batch (>200)	5 mL 2-propanol 10 mL <i>n</i> -hexanes	Saturated Na <sub>2</sub> SO <sub>4</sub> 2-propanol Water
Microwave Assisted Extraction (MAE)	10 g sample and co-solvents are heated via microwave energy for 27 min. Extract is isolated via solvent exchange, pre-concentration, column chromatography, and solvent reduction	>24 h	12	26 mL <i>n</i> -hexanes 39 mL acetone >300 mL petroleum ether 12 mL diethyl ether	Granular Na <sub>2</sub> SO <sub>4</sub> <i>n</i> -hexanes Acetone Petroleum ether Diethyl ether Florisil
Blender Extraction (BE)	25 g sample and co-solvents are homogenized for 6 min. Cleaned via separatory extraction (water & Na <sub>2</sub> SO <sub>4</sub> )	4.4 h	1	20 mL 2-propanol 52 mL petroleum ether 0.5 mL diethyl ether	Granular Na <sub>2</sub> SO <sub>4</sub> Saturated Na <sub>2</sub> SO <sub>4</sub> 2-propanol Water
Soxhlet Extraction (SE)	10 g sample in a cellulose cartridge is extracted with continuously circulating hot solvent via reflux for 16 h. Extract is isolated via solvent exchange, pre-concentration, column chromatography, and solvent reduction	~48 h	1	133 mL <i>n</i> -hexanes 133 mL acetone >300 mL petroleum ether 12 mL diethyl ether	Granular Na <sub>2</sub> SO <sub>4</sub> Florisil cartridges <i>n</i> -hexanes Acetone Petroleum ether Diethyl ether Florisil
QuEChERS	15 g sample, solvents, and solid reagents are mechanically agitated. Extract is isolated by vortex and centrifuge	50 min	6–12	15 mL acetonitrile 2 mL toluene	Granular Na <sub>2</sub> SO <sub>4</sub> MgSO <sub>4</sub> NaC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> PSA silica

<sup>a</sup>Oven-assisted extraction (proposed); microwave assisted extraction (US EPA Method 3546); blender extraction (US EPA Pylypiw Method, 1993); soxhlet extraction (US EPA Method 3540); and QuEChERS (J AOAC Int, Method 2007.01).

summarized in Table 1). *Cucurbita pepo* were used since these plants have been found to accumulate organochlorine pesticides such as DDE, chlordane, and PCBs in their aerial tissues by an order of magnitude greater than other plant species (White *et al.*, 2006). Efficiency and reproducibility of these techniques were compared to a newly devised oven assisted extraction (OAE) method that maximizes the number of batch samples extracted at one time, minimizes sample mass use, reduces resource consumption, and simplifies sample preparation.

## MATERIALS AND METHODS

### Field Site and Vegetation Source

Tissue samples used in this study were acquired from plants grown at the Connecticut Agricultural Experiment Station's Lockwood farm (Hamden, CT, USA). The soil is a fine sandy loam (56% sand, 36% silt, 8% clay) with 1.4% organic carbon and a pH of 5.8. It contains residues of *p,p'*-DDE at 150–1200 ng g<sup>-1</sup> (dry weight) (White, 2002) from historical DDT application. The field site was covered with 1000 m<sup>2</sup> of black polyethylene plastic sheeting to minimize weed growth and water loss. Thirty cm<sup>2</sup> squares were cut at 3.0 m<sup>2</sup> intervals. Six cultivars were grown in 2007. As part of a separate and ongoing study, three cultivars of *Cucurbita pepo* ssp *pepo* ("Gold Rush"/"Raven"/"Costata Romanesco") that accumulated *p,p'*-DDE and three cultivars of *Cucurbita pepo* ssp *ovifera* ("Zyphyr"/"Yellow Crook"/"Patty Pan") that do not accumulate *p,p'*-DDE were acquired from Johnny's Selected Seeds (Albion, ME, USA) (White *et al.*, 2003a). During the 2007 growing season, all possible F1 hybrids were created by manual cross pollination. Seeds from the cross-pollinated plants were collected, and during the 2008 growing season, 18 mounds of the F1 hybrids were planted (four plants per mound, one replicate mound for each F1 hybrid). These plants (F1 hybrids) were the tissues utilized in the current study. Plants were destructively harvested in mid-August 2008; stems of the zucchini were cut at ground level. Root mass was carefully excavated by turning over a 1.0 × 1.0 × 0.25 m section of soil. Stem and root samples were washed thoroughly with tap water and were chopped finely with a food cutter (Hobart Model 84145, Corp., Troy, OH, USA). The stems and roots of the 18 mounds of vegetation were composited and homogenized by hand. Vegetation was combined and homogenized to minimize variation that could occur due to heterogeneous pollutant concentration in soil and obscured the central question of this work regarding differences in efficiency among extraction procedures.

### Soil DDE Extraction

The soil *p,p'*-DDE content was determined as described previously (White *et al.*, 2003). Briefly, soil cores were collected from replicate mounds prior to planting. Sieved and air-dried soil samples (3.0 g) were extracted with 15 mL of *n*-hexanes (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) and 1 μg of *o,p'*-DDE as an internal standard (in 100 μL of 2,2,4-trimethylpentane) at 65°C for 5 h. A portion of the supernatant was passed through a glass microfiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV, USA) prior to collection in a chromatography vial. This method of soil extraction has previously been validated through comparison with microwave assisted extraction (MAE) (White, 2002).

### Vegetation Extraction: Oven Assisted Extraction (OAE)

Quadruplicate 10-g samples of roots or stems were weighed into 35 mL Teflon<sup>®</sup>-lined screw-cap vials that were amended with 5 mL 2-propanol, 10 mL *n*-hexanes (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA), and 1  $\mu$ g of *o,p'*-DDE (in 100  $\mu$ L 2,2,4-trimethylpentane) as an internal standard. The vials were placed into a standard incubator oven at 65°C for 4.5 h. After a cooling period of 5 min, the extracts were decanted through a funnel lined with glass wool and collected in 500 mL glass separatory funnels with Teflon<sup>®</sup> stopcocks. An additional 15 mL of 1:2 v/v 2-propanol/hexane was used to rinse the culture tubes; the rinsate was then added to the separatory funnels. Extracts were amended with 100 mL of reverse osmosis (RO) water and 10 mL saturated sodium sulfate, the extracts were then shaken rigorously for 5 s. After phase separation (~2 min), this step was repeated with 50 mL RO H<sub>2</sub>O and 10 mL saturated sodium sulfate. Hexane extracts were collected in 35-mL amber Teflon<sup>®</sup> screw-cap vials containing 5 g granular anhydrous sodium sulfate. A portion of the extracts was passed through a glass microfiber filter (0.45  $\mu$ m, Laboratory Science Inc., Sparks, NV, USA), and stored in chromatography vials at -4°C prior to analysis.

### Microwave Assisted Extraction (MAE)

The procedure by US EPA Method 3546 (USEPA, 1986) with modifications proposed by Pylypiw *et al.* (1997) and Fish and Revesz (1996) was followed. Quadruplicate 10-g samples of roots or stems were weighed into microwave PFA-Teflon<sup>®</sup> vessel liners. Vegetation was amended with 50 mL of 2:3 v/v hexanes/acetone (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) containing 1  $\mu$ g of *o,p'*-DDE (internal standard, in 100  $\mu$ L of 2,2,4-trimethylpentane). After securely covering and capping the irradiation vessels, a rupture membrane was installed in the vent fitting which was secured to the vessels. The mixtures were allowed to equilibrate for 45 min and were subsequently irradiated in a CEM MES-1000 microwave solvent extraction system (CEM Corporation, Mathews, NC, USA) using the following program: 100% power, 7 min ramp to 120°C; 20 min hold time. After extraction, the liquid phase was decanted into Kuderna-Danish flasks fitted with 10 mL concentrator tubes containing a boiling chip. The remaining vegetation in the extraction vessels were rinsed twice with 15 mL portions of 2:3 v/v hexanes/acetone, and the solvents were combined with the original extracts. A Snyder column was fitted to each Kuderna-Danish flask and the samples were reduced to less than 10 mL in a 95°C water bath. Twenty-five milliliters of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) was added through the Snyder columns and the volumes were again reduced to 10 mL. This solvent exchange process was repeated two additional times, resulting in a final volume of 10 mL for each sample. The extracts were purified through chromatography columns with PTFE stopcocks packed with dry (12 cm) florisil followed by 2-cm sodium sulfate. Columns were pre-eluted with 50 mL petroleum ether. Kuderna-Danish flasks fitted with labeled 10 mL concentrator tubes containing several boiling stones were placed beneath the columns to capture the eluate. After loading MAE extracts to the columns, 200 mL of 6% diethyl ether in petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) were used to elute vegetation extracts into Kuderna-Danish flasks. Petroleum ether extracts were again concentrated in a hot water bath to a final volume of less than 10 mL. A portion of this eluate was taken for analysis.

### Blender Extraction (BE)

The BE method used for extraction of *p,p'*-DDE residues from vegetation was adapted from Pylypiw (1993). Quadruplicate 25-gram portions of roots or stems were weighed into one-quart blender containers with 25 mL of 2-propanol (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) and 1  $\mu\text{g}$  of *o,p'*-DDE as an internal standard (in 2,2,4-trimethylpentane). The sample was blended at low speed for 30 s in an explosion-proof blender (Fisher Scientific, Springfield, NJ, USA). Then, 50 mL of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) was added to each of the containers. The sample was blended on low speed for an additional 5 min. After settling for 30 s, the samples were decanted into funnels packed with glass wool, and the extracts were collected in 500 mL glass separatory funnels with Teflon<sup>®</sup> stopcocks. After complete draining of the solids (approximately 20 min), 100 mL of RO H<sub>2</sub>O and 10 mL of saturated sodium sulfate solution were added separately to each funnel. The funnels were capped, shaken gently for 5 s, and allowed to sit for 20 min to enable phase separation. The aqueous phase was drawn off and the ether was rinsed three additional times with RO water and saturated sodium sulfate. Final extracts (approximately 35–40 mL) were then amended with 10 g of anhydrous sodium sulfate; the extracts were allowed to settle for at least 3 h. A 1-mL portion of each petroleum ether extract was then filtered through a florisil cartridge (Alltech, Deerfield, IL, USA). Following preconditioning with 1 mL of petroleum ether, each florisil cartridge (200 mg) was amended with 1 mL of vegetation extract, followed by 6 mL of 6% diethyl ether in petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA). Extracts were collected in 8 mL glass vials and reduced to approximately 1 mL under nitrogen flow.

To investigate the impact of drying on *p,p'*-DDE extraction, the blender extraction method was also applied to root and stem samples that had been previously air or freeze-dried. For air drying, four stem and four root samples (25 g wet weight) were placed on individual sections of aluminum foil and were stored in a fume hood for 24 h at 22  $\pm$  2°C. For freeze drying, four stem and four root samples (25 g wet weight) were added to pre-labeled 500-mL whirlpak bags and were placed in a freeze dryer (Labconco Freezone 6, Labconco, Kansas City, MO, USA) for 48 h. The entire mass of air or freeze dried vegetation was then solvent extracted by the standard blender method as described above.

### Soxhlet Extraction (SE)

Two hundred and fifty mL of 1:1 v/v acetone/hexanes containing 100  $\mu\text{L}$  of 10  $\mu\text{g}/\text{mL}$  *o,p'*-DDE (in 2,2,4-trimethylpentane) internal standard and 3 boiling stones were added to 500 mL round bottom flasks. Triplicate samples of roots and stems ground with granular anhydrous sodium sulfate were placed in pre-extracted cellulose thimbles and extracted for 16 h as specified by the US EPA Method 3540 (USEPA, 1986). Soxhlet refluxing progressed at a rheostat heat setting, which yielded 4–5 cycles hour<sup>-1</sup> (approximately 105°C). At completion, the condensers were allowed to quench volatilization and cool the extracts. Extracts were dried over approximately 15 g anhydrous granular sodium sulfate for 2 h (covered with septa), and were decanted into Kuderna-Danish flasks fitted with 10 mL concentrator tubes containing a boiling chip. Soxhlet round-bottom vessels were rinsed twice with 15 mL portions of 1:1 hexane/acetone, and the solvent was combined with the original extracts. Snyder columns were fitted to the flasks and the solvents were reduced to less than 10 mL in a 95°C water bath. Twenty-five milliliters of petroleum ether

(Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA) were added through the Snyder columns and the volume of the extracts was again reduced to 10 mL. This solvent exchange process was repeated two additional times yielding a final volume of 10 mL. The extracts were purified through florisil as specified by US EPA Method 3640C prior to analysis (USEPA, 1986).

### QuEChERS Extraction

The QuEChERS (“Quick, Easy, Cheap, Effective, Rugged, and Safe”) method of Anastassiades *et al.* (2003a) was used to extract *p,p'*-DDE residues from the roots and stems. Briefly, quadruplicate 15-g samples of roots or stems were placed into graduated 50 mL centrifuge tubes amended with 1  $\mu$ g of *o,p'*-DDE internal standard (in 100  $\mu$ L of 2,2,4-trimethylpentane), 6 g magnesium sulfate, 1.5 g sodium acetate, and 15 mL acetonitrile (all chemicals purchased as Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ, USA). Each sample was tightly capped, hand shaken to disperse reagents, agitated on a Burrell Wrist-Action<sup>®</sup> Shaker for 30 min (Burrell Scientific, Pittsburgh, PA, USA), and finally centrifuged at 3000 *g* for 10 min. Ten milliliters of the extracts were transferred to 15 mL disposable plastic centrifuge tubes containing 1.5 g magnesium sulfate, 500 mg PSA (primary/secondary amine), and 2 mL toluene (all reagents purchased from J.T. Baker, Phillipsburg, NJ, USA; PSA bonded silica was purchased from Supelco, Bellefonte, PA, USA). Extracts were vortexed for 30 s and were centrifuged at 3000 *g* for 10 min. Ten milliliters of the toluene extract was decanted into concentrator tubes and the volumes were reduced under nitrogen at 50°C to less than 2 mL. These samples were stored in chromatography vials prior to analysis.

### DDE Quantitation

The *p,p'*-DDE content in the soil or tissue extracts was determined on a Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with a <sup>63</sup>Ni micro-electron capture detector ( $\mu$ -ECD) according to USEPA Method 80810A with specific modifications. An SPB-1 column (30 m  $\times$  0.53 mm  $\times$  0.5  $\mu$ m) (Supelco, Bellefonte, PA, USA) was used; the GC program was 175°C initial temperature ramped at 3.5°C/min to 225°C, then ramped at 25°C/min to 250°C with a hold time of 4.71 min. The injection port was maintained at 250°C and a 2  $\mu$ L splitless injection was used. Hydrogen was the carrier gas, and the makeup gas was 5% CH<sub>4</sub> in Ar at 60 mL/min. The  $\mu$ -ECD was maintained at 325°C. Crystalline *p,p'*-DDE and *o,p'*-DDE were acquired from the EPA National Pesticide Standard Repository (Fort Meade, MD, USA). Portions of *p,p'*-DDE were transferred to 2,2,4-trimethylpentane and calibration standards were prepared at 10–500 ng/mL. One hundred ng/mL *o,p'*-DDE was added to each calibration level as an internal standard. Concentrations of *p,p'*-DDE in the various tissues and soil extracts were determined by internal standard calibration at multiple intervals over the total run time of all samples. All six-point calibration solutions differed by <3% for *o,p'*-DDE and <5% for *p,p'*-DDE analytes between each set of calibration curves. Method blanks and spiked standards were analyzed. To monitor procedural performance and matrix effects, spike-recovery assessment of the internal standard (*o,p'*-DDE) peak areas showed an 82.1–91.7% recovery for plant and 81.9–97.2% recovery for soil extracts. These recoveries ranged within the accepted limits set by USEPA Method 80810A.

The identity of individual peaks (from the  $\mu$ -ECD) was confirmed on an Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with an Agilent 5973 mass selective detector (MSD). The column (30 m  $\times$  250  $\mu$ m  $\times$  0.25  $\mu$ m) contained a MDN-12 film (Supelco, Bellefonte, PA, USA) and the GC program was 80°C initial temperature, held for 1 min, then ramped at 15°C/min to 350°C and held for 10 min. The total run time was 27 min. A 2- $\mu$ L splitless injection was used, the injection port was maintained at 300°C and the MS detector was maintained at 280°C.

### Statistical Analysis

All soils and vegetation were extracted in quadruplicate. Significance was evaluated by performing a one way analysis of variance (ANOVA) on tissue concentrations relative to each extraction technique. To compare variability or reproducibility between extraction methods, Bartlett's test of homogeneity of variances and the subsequent mean normalized residuals were plotted as previously described in Pylypiw *et al.* (1997). Root and stem bioconcentration factors (BCFs; dry weight ratio of *p,p'*-DDE in the tissue to that in the soil) were analyzed statistically by Kruskal-Wallis one way analysis of variance (ANOVA) followed by a Tukey multiple comparison analysis ( $p < 0.05$ ). Translocation factors (TFs; stem BCF divided by root BCF) were also calculated.

### RESULTS AND DISCUSSION

This study used *p,p'*-DDE, one of the most recalcitrant and ubiquitous insecticides, and is the contaminant which is found in more than 450 of 1,613 National Priorities List (NPL) for long-term U.S. EPA federal clean-up sites (ATSDR, 2002). The simpler and less resource-intensive OAE method was compared to other standard extraction techniques for *p,p'*-DDE residues in vegetation. The data in Table 2 indicate that for stem and root tissue, there were no statistically significant differences in *p,p'*-DDE extraction among methods ( $p < 0.05$ ). The average *p,p'*-DDE concentration in roots and stems extracted with the OAE

**Table 2** Mean tissue *p,p'*-DDE concentrations and variances for each extraction method

Extraction method	Root (ng g <sup>-1</sup> ) <sup>a</sup>	Stem (ng g <sup>-1</sup> ) <sup>a</sup>	Root variance <sup>b</sup>	Stem variance <sup>b</sup>
Oven assisted extraction	7800 (600) A <sup>c</sup>	1800 (190) CD	5.5 E <sup>d</sup>	4.5 F
Microwave assisted extraction	6700 (460) AB	1900 (93) CD	5.4 E	3.9 F
Blender extraction				
Wet	6700 (1100) AB	1500 (158.67) C	6.1 E	4.4 F
Air dried	5300 (1400) AB	1400 (280) C	6.3 E	4.9 F
Freeze dried	3600 (240) B	2200 (340) D	5.0 E	5.1 F
Soxhlet extraction	5800 (340) AB	1700 (210) CD	5.1 E	4.6 F
QuEChERS extraction	7300 (1300) AB	1900 (240) CD	6.2 E	4.8 F

<sup>a</sup>Values are the mean *p,p'*-DDE concentrations with standard deviations in parentheses (n = 4) based on vegetation dry mass. <sup>b</sup>Values are log transformed variances (log  $s^2$ ) used to determine relative variability of pollutant extracted via each technique. <sup>c</sup>Different capital letters within columns are significantly different ( $p < 0.05$ ), one way analysis of variance (ANOVA) followed by a Tukey multiple comparison analysis. <sup>d</sup>Different capital letters within columns are significantly different ( $p < 0.05$ ) variances, Bartlett's test for homogeneity of variance.

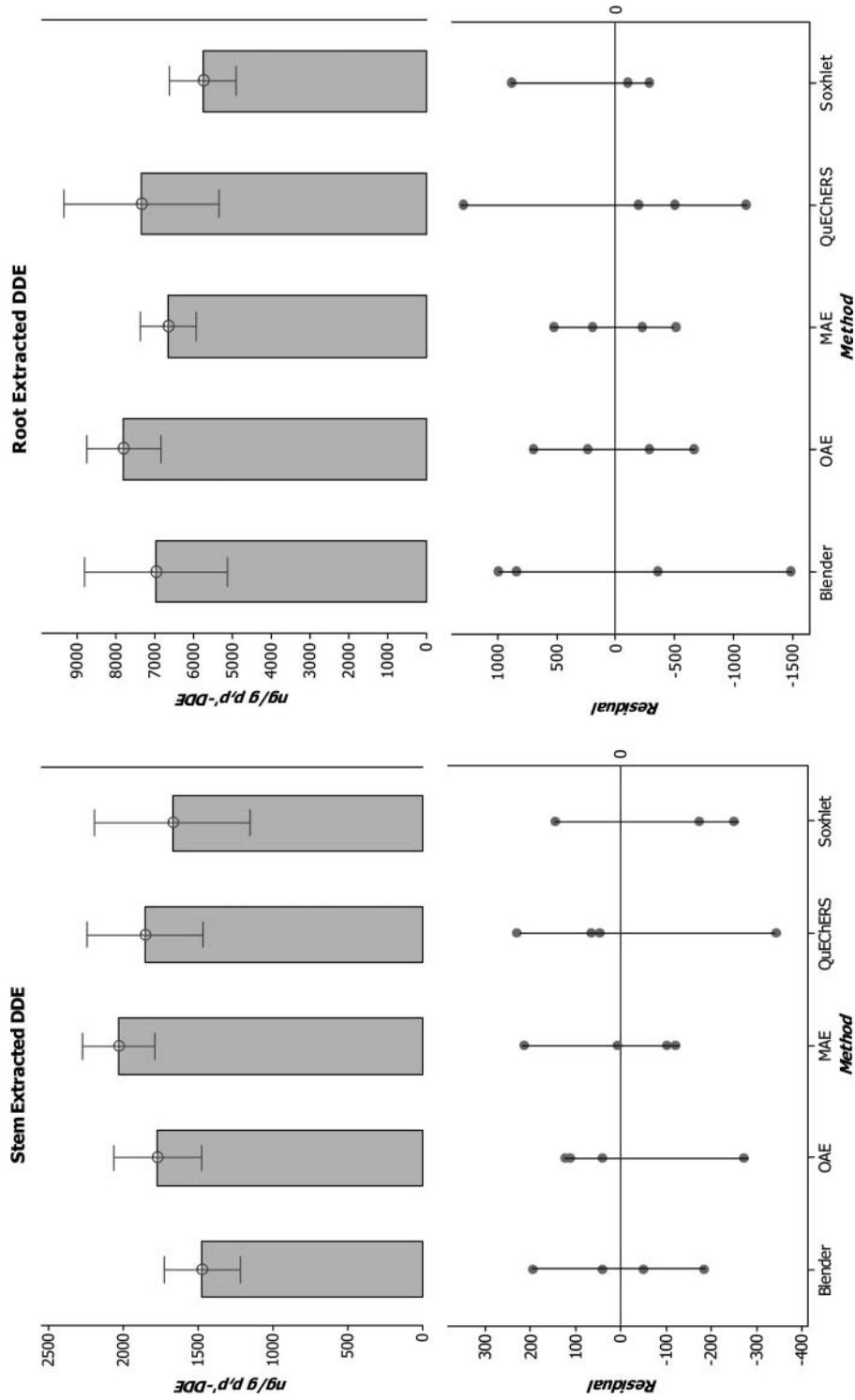
are 7800 and 1770 ng g<sup>-1</sup>, respectively, and average values for the other four techniques are 6625 and 2100 ng g<sup>-1</sup> respectively.

Although not the central objective of this study, work was conducted to determine the effects of drying method applied prior to the extraction of *p,p'*-DDE from tissues, as these methods of sample storage can alter total pollutant concentration. Since differences among extraction methods could obscure the effect of different storage techniques, only one method (BE) was used here. Data for air-drying were statistically consistent with concentrations established via the standard wet BE method. However, Table 2 indicates tissue-specific results, where freeze drying led to a slight but statistically significant increase in *p,p'*-DDE concentration in the stems. Conversely, freeze-dried roots contained significantly less *p,p'*-DDE than those from a standard wet extraction. For example, OAE of wet roots yielded a 2-fold greater concentration of the pesticide than that from the blender extraction of the same freeze-dried tissues.

To assess reproducibility of the methods, variances of each technique were statistically evaluated using Bartlett's test for homogeneity of variances. For stem ( $\chi^2 = 2.76$ ;  $p = 0.838$ ) and root extraction ( $\chi^2 = 11.11$ ;  $p = 0.085$ ), variances between groups were not significantly different, suggesting that OAE has similar robustness of reproducibility compared to other standard methods. To visualize the homogeneity of variances among the extraction techniques, and to illustrate the difference between the individual value and the average value for each technique, mean-normalized residuals following Bartlett's test were plotted (Figure 1). Although differences in variance among methods were not observed for any one tissue, residual plots indicate that tissue-specific variances are significantly different across all methods. In a previous study evaluating the application of MAE to a multi-residue extraction of multiple matrices, Pylypiw *et al.* (1997) found that extraction variability was affected by sample matrix complexity. However, the fact that OAE uses less solvent for extraction does not hinder its reproducibility. A similar conclusion can be extended to the QuEChERS method, which, in its simplicity and conservative consumption of resources, performed equally well relative to more solvent-demanding methods such as Soxhlet and blender extraction as shown in Table 1.

Using the OAE method, which involves partitioning of organic residues from plant tissues into the relatively non-polar *n*-hexanes using 2-propanol as a mediating solvent, thermal digestion is required for extraction of finely cut vegetation. Saturated sodium sulfate is employed to promote additional partitioning of organic residues into the organic solvent in the subsequent separatory work-up. Furthermore, OAE promotes extraction with heat (~65°C). This temperature is slightly below the boiling points of both *n*-hexanes and 2-propanol, allowing for higher permeation and penetration without loss of solvent and analytes in the process.

From a risk assessment perspective, adequate extraction methodology is required to accurately predict the potential for pollutant compartment partitioning and accumulation that may result in toxicity and food-chain contamination. As is shown in Table 3, BCFs among methods for stems and roots were not significantly different, suggesting that OAE is an adequate extraction method to predict the uptake of a compound such as *p,p'*-DDE ( $p < 0.05$ ). Contaminant translocation as calculated from OAE data were also comparable to those derived from the other extraction methods in the current study (TF =  $0.23 \pm 0.03$ ) and a previous study conducted by White *et al.* (2007) involving *C. pepo* grown at the same site (TF = 0.26). Additionally, oven assisted extraction does not break down or degrade vegetation tissue to any extent (seen with BE, MAE, and Soxhlet), an undesirable process which can lead to solvation of polyprotic organic material. Specialized clean-up



**Figure 1** Comparison of extraction efficiency of *p,p'*-DDE between methods from two tissues of field grown *Cucurbita pepo*. Plot of mean-normalized residuals from Bartlett's test for homogeneity of variance shows statistical equality in variation between methods.

**Table 3** *p,p'*-DDE bioaccumulation and translocation potentials predicted by five extraction methods

Extraction method	Root BCF <sup>a</sup>	Stem BCF <sup>a</sup>	TF <sup>b</sup>
Oven assisted extraction	83.9 (6.4) A <sup>c</sup>	19.1 (2.0) CD <sup>d</sup>	0.23 (0.03)
Microwave assisted extraction	71.6 (4.9) AB	20.0 (1.0) CD	0.28 (0.02)
Blender extraction			
Wet	74.9 (12.4) AB	15.8 (1.7) C	0.21 (0.04)
Air dry	57.7 (14.8) AB	15.4 (3.0) C	
Freeze dried	38.8 (2.6) B	23.4 (3.6) D	
Soxhlet extraction	61.9 (3.7) AB	18.0 (2.2) CD	0.29 (0.04)
QuEChERS extraction	79.0 (13.5) AB	19.9 (2.6) CD	0.25 (0.05)

<sup>a</sup>Average tissue-to-soil (dry mass) concentration (92.9 ng/g) ratio for *p,p'*-DDE. Standard deviations in parentheses ( $n = 4$ ). <sup>b</sup>Translocation factor (TF), ratio of stem BCF to root BCF. <sup>c</sup>Values followed by different capital letters within column are significantly different ( $p < 0.05$ ), Kruskal-Wallis one way analysis of variance (ANOVA) followed by a Tukey Multiple comparison analysis. <sup>d</sup>Values followed by different capital letters within column are significantly different ( $p < 0.05$ ), Kruskal-Wallis one way analysis of variance (ANOVA) followed by a Tukey multiple comparison analysis.

procedures to remove pigmentation, phospholipids, glycolipids, sulfolipids, waxes, fats, or other contaminants, which may adversely affect quantitative analytical instrumentation, may be required when using the more exhaustive methods (Tadeo, 2008).

A variety of protocols are recommended by national regulatory agencies for the quantitative extraction of organic pollutants. Although successful, many immediate and ultimate limitations often prevent their full-scale adoption. The OAE technique is a reasonable alternative to other cumbersome methods because it yields comparable extraction efficiencies for a hydrophobic compound such as *p,p'*-DDE, requires a relatively small investment of time and other resources, and it has a high capacity to handle large numbers of samples simultaneously. More extensive research should be conducted to assess the extent to which a larger spectrum of organic pollutants at varying concentrations can be extracted by OAE from a variety of plant tissues.

## ACKNOWLEDGMENTS

Funding for the research was provided by the Program in Environmental Science and the Department of Chemistry at Muhlenberg College. We thank Joseph Hawthorne, Terri Arsenault, and William Berger of the Connecticut Agricultural Experiment Station for technical assistance.

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