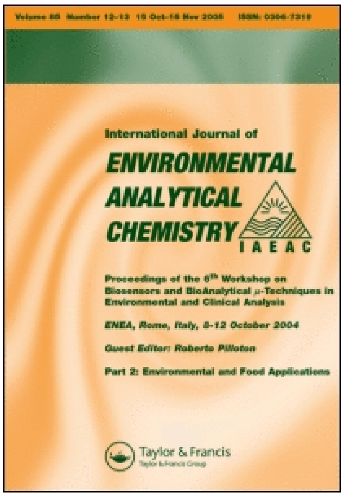

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A CHEMICAL TEST FOR DETERMINING BIOLOGICAL AVAILABILITY OF AGED CHEMICALS IN SOIL

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Aging is one of several processes that are known to affect exposure of chemicals to organisms by decreasing the available fraction of chemical contaminants in soil. This phenomenon has important implications in the assessment of the hazards of chemicals and regulations for soil cleanup. Passive sampling devices (PSDs) are potentially direct chemical indicators for assessing bioavailability of pesticides (and other chemicals). PSDs consist of lipophilic material within a semi-permeable membrane, similar to biological systems. In this study, a pesticide mixture was aged in soil for up to eight months. Earthworms and PSDs were placed in soil and chemical uptake into both was determined over time. Uptake rates into PSDs and maximum concentrations were observed to positively correlate with uptake rates and maximum concentrations in earthworms for both of the soil types studied (sandy loam, silt loam). These results indicate that PSDs may be used as a surrogate for earthworms and provide a chemical technique for assessing the availability of aged chemical residues in soil.

Keywords: Bioavailability; organochlorine pesticides; DDT; aging

INTRODUCTION

At present, risk assessments and regulatory monitoring at contaminated sites are mostly determined from soil residue data based on rigorous chemical extractions using organic solvents [1]. These extraction methods are intended to extract as much of the chemical as possible from soil. Use of these analytical data for hazard assessment of chemicals and regulations for site remediation has been rationalized with the assumption that all of the chemical existing in soil is available to organisms and the chemical concentrations in biota are equivalent to chemical

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concentrations in their external environment (soil). However, chemical residues in biota may not simply relate to the chemical concentrations in soil because the soil matrix (pH, % organic carbon, moisture, and texture) may substantially change the portion of chemical available to organisms (bioavailability).

Accumulating scientific evidence suggests that organic contaminants are less available to soil organisms (e.g. earthworms and bacteria) as residence time of the compounds in soil increases [2, 3]. This phenomenon is referred to as "aging" or sequestration. Because "aging" could play significant roles in the fate of organic contaminants and subsequent risk assessments, there is a need to understand bioavailability in chemical fate and toxicological studies in order to identify the true potential risk to wildlife and humans.

One approach to determine bioavailability of a chemical to organisms is the use of passive sampling devices (PSDs), which are monitoring devices based on passive diffusion. PSDs, often referred to as semipermeable membrane devices (SMPDs), have been utilized to monitor water quality (for example, [4]). Due to the similarities of PSDs to biological systems (hydrophobic depots covered with a semipermeable membrane), their potential as biological surrogates has attracted interest among scientists. In fact, several scientists have investigated their practical use as biological surrogates in aquatic environments and have indicated their potential to predict the bioavailability of organic contaminants [5, 6]. On the contrary, research to investigate their use in terrestrial environments is relatively new and few studies have been conducted to evaluate their application in this matrix [7]. Because of the potential importance of bioavailability in risk assessment, this study was designed as an evaluation of a chemical assay using PSDs to predict the bioavailability of aged organochlorine (OC) pesticides to earthworms.

EXPERIMENTAL

Chemical application and aging procedure

A certified organochlorine pesticide mixture (AccuStandard, Inc., purity ≥ 98%) used for this study consisted of the following chemicals: aldrin, DDT, dieldrin, endrin, heptachlor, and lindane. Two soil types used for the experiments were collected from the surface at sites near Ropesville, Terry County, Texas (sandy loam, pH 8.3, 1.3% OC), and Stamford, Harlan County, Nebraska (silt loam, pH 7.0, 2.5% OC). Before chemical application, soils were prepared by sieving through a 4-mm sieve and moistened to adjust moisture contents to about 30% of water holding capacity. The pesticide mixture (in an acetone solution) was spiked into the non-sterile soils and the soils were mixed thoroughly to distribute the chemicals evenly and to allow the solvent to evaporate. Measured chemical concentrations in the soils were approximately 0.5 μg/g for lindane, heptachlor and aldrin, and 1.0 μg/g for dieldrin, endrin, and DDT after spiking [8]. The soils were stored in plastic containers (Sterilite®, Townsend, MA) at room temperature to prevent moisture loss. At the beginning of each earthworm/PSD chemical uptake test, the soils were moistened again to adjust their moisture contents to around 30% of water holding capacity.

Chemical uptake into earthworms

Adult earthworms (*Lumbricus terrestris*) were purchased locally and analyzed for OC pesticide residues before the experiments. At the end of each aging period (0 day (unaged), 42 days, and 250 days (227 days for experiment in silt loam soil)) a 300 g subsample of each soil sample was transferred to a 470-mL glass jar (Qorpak®, Pittsburgh, PA). At the beginning of each experiment, three active earthworms (approximate total weight of 10 g) were placed on the surface of the soil in each jar. A Teflon®-lined lid covered the top of each jar. The lid was removed regularly to exchange air. The worms were kept in the dark up to 36 days at 16–21°C. Earthworms were removed from the jars on days 5, 8, 12, 18, and 36, and placed on wet filter paper for 24 hours to depurate the gut contents. Worms were cleaned with distilled water to remove external soil particles and frozen (-10°C) until extraction.

Chemical uptake into PSDs

PSDs were constructed as described by Johnson et al. [7]. One gram (1.0000 g ± 0.001) of octadecyl sorbent (C_{18}) was placed into a 30-mL polyethylene sampling bag (Whirl-Pack® bags, Nasco, Fort Atkinson, WI) and heat-sealed. The final dimensions of sampling bags are the following: the nominal film thickness ≈ 63.5 to 71.1 μm, height and width ≈ 5.5 × 7.0 cm, and surface area ≈ 77 cm^2. In a procedure similar to that of the earthworm chemical uptake test, a subsample of soil (300 g) was transferred into 470-mL glass jars at the end of each aging period. A PSD was buried in the center of each jar. The sample jar was sealed with a Teflon®-lined lid during the experiment in order to retain soil moisture. PSDs were collected from duplicate jars on days 3, 6, 12, 18, and 36. PSD samples were transferred to clean jars and frozen (-10°C) until extraction.

Chemical extraction

Earthworms

Worms were cut into small pieces and mixed with anhydrous sodium sulfate to remove water. Chemicals were extracted from worms using hexane:acetone (3:1 v:v) and an orbital shaker. The extractant volume was reduced to a final volume of 5 mL using a gentle flow of nitrogen gas, filtered (0.45-μm Acrodisc® syringe filter), transferred to a GC vial, and stored (-10°C) until analysis. Extraction recoveries for the chemicals were ≥ 77%.

PSDs

Chemicals were extracted from PSD samples in a way similar to that used for Soild Phase Extraction (SPE). The C_{18} sorbent was poured into a glass SPE column and chemicals were eluted from the sorbent with hexane:acetone (1:1 v:v). Excess water was removed by adding anhydrous sodium sulfate when necessary. Extractant volume was reduced to 2 mL using a gentle flow of nitrogen gas. Samples were transferred to GC vials and stored (-10°C) until analysis. Extraction recoveries for the chemicals were ≥ 94%.

Soil

Five subsamples of soil of approximately 1 g each were collected at the beginning of each PSD/earthworm chemical uptake test. Soil samples were mixed with anhydrous sodium sulfate to remove water and placed into cellulose extraction thimbles (Whatman®, Maidstone, England). Soil samples were Soxhlet-extracted for 24 hours with hexane:acetone (3:1 v:v) and the extractant concentrated to near dryness by vacuum rotary evaporation. Concentrated samples were brought to the exact volume of 2 mL, transferred to GC vials, and stored (-10°C) until analysis. Extraction recoveries for the chemicals were ≥ 91%.

Instrumental methods

Chemicals were analyzed using a Hewlett-Packard Model 6890 gas chromatograph (GC) equipped with an ^{63}Ni electron capture detector (ECD). Details of the analytical methods are provided elsewhere [8]. Detection limits (based on DDT) were 10 ng/g and 75 ng/g for the PSD and soil samples, and the earthworm samples, respectively. Each analyte was individually quantitated. A four-point standard curve was constructed from constant volume (2 μL) injections of calibration standards. Computer-generated peak areas were used to measure sample concentrations in an external standard method.

RESULTS

Chemical uptake experiments

In order to investigate the effects of aging on the uptake of the organochlorine compounds from soil into PSDs and earthworms, the initial data points (Day 3 for PSDs and Day 5 for earthworms) were used to determine "initial rates" of uptake. It was assumed that these data points were within the linear region of the uptake curves, and the calculated rates were used for comparative purposes among chemicals and soil types. Comparisons of these values were made between chemicals aged for different periods and between sandy loam soil and silt loam soil for each uptake experiment [8]. In addition, a maximum uptake concentration for each chemical/soil combination was determined for both PSDs and earthworms [8]. The maximum concentration represents chemical distribution between earthworm/soil and PSD/soil at an apparent equilibrium [9]. Attempts were made to correlate chemical uptake into the PSDs with chemical uptake into the earthworms in order to develop a chemical test or surrogate for determining biological availability. Initial chemical uptake rates and maximum concentrations in both PSDs and earthworms were used in the correlations.

Chemical uptake into PSDs versus chemical uptake into earthworms

A relatively good positive correlation between initial chemical uptake rate in PSDs and initial chemical uptake rate in earthworms was obtained for the sandy loam soil despite the use of three different aging periods. A linear relationship between initial chemical uptake rate in the PSDs and initial chemical uptake rate in earthworms was observed (Figure 1A). The data were evaluated in two clusters based on the initial chemical concentrations in the soils (recall the initial soil concentrations of lindane, heptachlor, and aldrin were approximately 0.5 ppm while dieldrin, endrin, and DDT were approximately 1 ppm). The correlation coefficient (r) for the chemicals at the higher concentration was good ($r = 0.72$; $p \leq 0.05$); whereas, the correlation for uptake rates of the chemicals at the lower concentration was poor ($r = 0.30$). Similarly, two clusters (based on initial concentrations) were observed for the correlation between maximum chemical concentration in the PSDs and maximum chemical concentration in the earthworms (Figure 1B). In the same way as the initial uptake rates. the correlation for chemicals at the higher concentration was good ($r = 0.87$; $p \leq 0.05$) while the correlation for those at the lower concentration was poor ($r = 0.17$).

For the silt loam soil, different results were observed in the relationship between chemical uptake in PSDs and chemical uptake in earthworms. Neverthe-

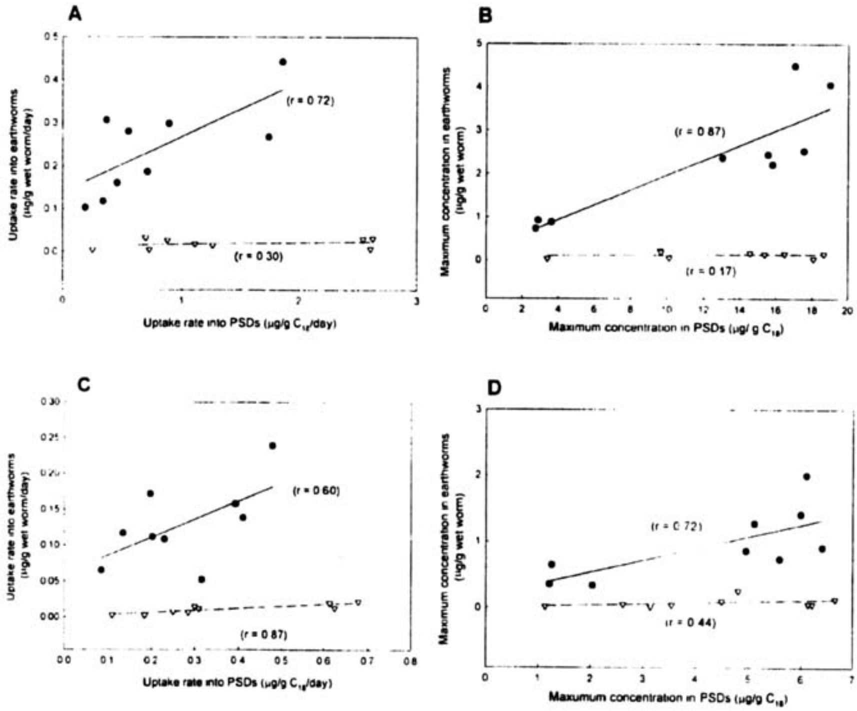

FIGURE 1 A, C) Correlation of the initial chemical uptake rate from soil into PSDs versus the initial uptake rate from soil into earthworms in studies on the availability of aged pesticide residues in soil (A: Sandy Loam, C: Silt Loam). B, D) Correlation on the maximum chemical concentration in PSDs from soil versus the maximum chemical concentration in earthworms from soil in studies on the availability of aged pesticide residues in soil (B: Sandy Loam, C: Silt Loam) • High initial concentration chemicals (dieldrin, endrin, DDT) ∇ Low initial concentration chemicals (lindane, heptachlor, aldrin)

less, a similar pattern was observed based on the initial concentrations; again, the data from silt loam soil were in two clusters. A good positive correlation between chemical uptake rate in PSDs and chemical uptake rate in earthworms was obtained for the chemicals at the lower concentration ($r = 0.87$; $p \leq 0.05$); however, the slope of the correlation curve was extremely small (Figure 1C). The correlation coefficient (r) for the chemicals at the higher concentration was good ($r = 0.60$; $p \leq 0.10$). For the maximum concentrations (Figure 1D), two clusters of data were also obtained. The correlation coefficient (r) for the chemicals at the higher concentration was good ($r = 0.72$; $p \leq 0.05$); however, the correlation for the chemicals at the lower concentration was poor ($r = 0.44$).

Maximum concentrations in PSDs and maximum concentrations in earthworms for the chemicals at higher concentrations in soil (dieldrin, endrin, and

DDT) were plotted for both the sandy loam and silt loam soil (Figure 2). This combined data set yielded an excellent correlation ($r = 0.91$; $p \leq 0.001$).

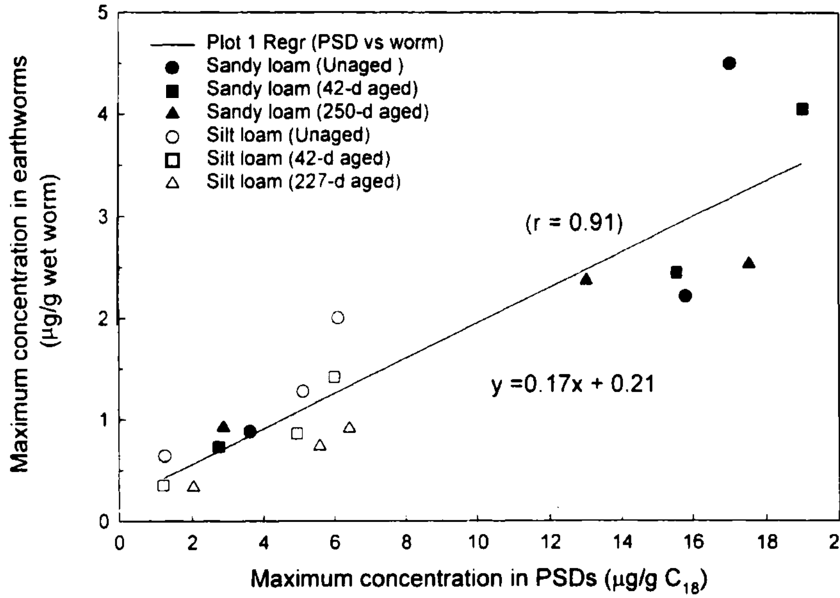

FIGURE 2 Correlation of the maximum chemical concentration in PSDs from sandy loam and silt loam soils versus the maximum chemical concentration in earthworms from sandy loam and silt loam soils. Data present are for the higher concentration chemicals (dieldrin, endrin, DDT) in studies on the availability of aged pesticides residues in soil

DISCUSSION

Overall, PSDs behaved more sensitively to differences in soil type and aging than earthworms. The PSD/soil chemical accumulation ratio was three to ten times higher than the earthworm/soil biological accumulation ratio (whole body basis). This high accumulation ratio has been observed in previous studies investigating the relationship between semipermeable membrane devices (SPMDs) and biological systems in aquatic environments [6, 10]. The difference in chemical uptake into the PSD appears to depend on the vapor pressure or water solubility of the chemical. This observation indicates that the more volatile the chemical, the more chemical in the vapor phase and the more readily the chemicals accumulate in the PSD, especially at the soil moisture level in this study. Generally speaking, chemical uptake rates in PSDs decreased with an increase in aging.

Increase diffusion path or tight sorption in the internal soil particles due to aging may have prevented the chemicals from diffusing into the external vapor phase.

In contrast to what was found for the initial uptake rate data, there was a positive correlation between the maximum concentrations in earthworms and the maximum concentrations in PSDs. Correlation coefficients for those chemicals that had a similar accumulation pattern into earthworms and PSDs (dieldrin, endrin, and DDT) were good (r values were 0.87 and 0.72 for sandy loam soil and silt loam soil, respectively). Nevertheless, this was not the case for the chemicals at lower concentrations in the test soils: aldrin, heptachlor, and lindane.

Related to the initial chemical concentration, two clusters were observed for initial uptake and maximum concentrations in both soil types. All chemicals were taken up into the PSDs readily regardless of their initial concentrations. The uptake rates ranged from 0.25 to 2.62 μg/g/day and 0.11 to 0.68 μg/g/day for sandy loam and silt loam soil, respectively. On the other hand, chemical uptake into earthworms was different depending upon the initial concentrations of the chemicals, resulting in the formation of the two apparent clusters.

The worms did not appear to accumulate lindane, aldrin, and heptachlor as fast as they accumulated dieldrin, endrin, and DDT. If first-order kinetics can be applied to the first several days of chemical uptake into earthworms, the difference in initial concentration of the test chemicals should not affect the uptake rate because the rate should be concentration independent. Therefore, the clusters might indicate that there are some differences in earthworm behavior toward the chemicals spiked into the soil at different concentrations. Taking into consideration that individual chemicals in the pesticide mixture were not highly concentrated in the soil, the earthworms might have readily eliminated the chemicals without accumulating them into their bodies. In fact, aldrin and dieldrin have nearly the same bioconcentration factor for earthworms (log BCFs are 3.56–4.88 and 3.65–4.69 for aldrin and dieldrin, respectively, [11]). This suggests that if there is enough chemical source available to earthworms and the uptake process exceeds the elimination process, they should accumulate nearly the same amount of chemical in their bodies. It was observed that earthworms accumulated lindane to a lesser extent when it was applied to soil with DDT, indicating that the presence of other pesticides might have played a role in reducing uptake into the worms [12].

In this study, an excellent positive correlation between maximum concentrations in PSDs and maximum concentrations in earthworms (chemicals at the higher concentration in soil) was obtained for both soil types. The correlation coefficient (r) obtained for the combined data was 0.91. This observation indicates that a direct linear relationship existed between maximum chemical concentration in PSDs and maximum chemical concentrations in earthworms, despite multiple chemicals (3), soil types (2), and aging periods (3). This observation provides strong evidence that PSDs are useful in predicting the biological availability of aged chemicals in soil.

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