

# Extraction of Tricyclazole from Soil and Sediment with Subcritical Water

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The use of subcritical water to extract tricyclazole from soils and sediments was examined. Extraction efficiency and kinetics were determined as a function of temperature, sample age, sample matrix, sample size, and flow rate. Extraction temperature was the most influential experimental factor affecting extraction efficiency and kinetics, with increasing temperature (up to 150 °C) yielding faster and higher efficiency extractions. Higher extraction temperatures were also important for quantitative recovery of tricyclazole from aged samples. Extraction at 50 °C yielded 97% recoveries from samples aged 1 day but only 30% recoveries for samples aged 202 days, whereas extraction at 150 °C yielded recoveries of 85–100% that were independent of incubation time and sample matrix, with the exception of one sediment that contained a large amount of organic matter. Sample extracts from subcritical water extraction were generally a pale yellow color, contrasted with a dark brown color from organic solvent extractions of the same matrixes. Less sample cleanup was therefore required prior to analysis, with the total time for the extraction and analysis of a single sample being approximately 2 h. Subcritical water extraction is an effective technique for the rapid and quantitative extraction of tricyclazole from soils and sediments.

**Keywords:** *Tricyclazole; subcritical water extraction; extraction kinetics; desorption*

## INTRODUCTION

Tricyclazole [5-methyl-1,2,4-triazolo[3,4-*b*]benzothiazole] is a fungicide used to control *Pyricularia oryzae* (rice blast) in transplanted and direct-seeded rice by flat drench, root soak, or foliar applications (Froyd et al., 1976; Abdullah and Amin, 1982; Singh and Dodan, 1995; Dubey, 1997; Saifulla et al., 1995); the current maximum application rate is 450 g/ha. It is readily absorbed by plant roots and is translocated to the plant leaves, where it provides residual disease control. Tricyclazole is nonvolatile (vapor pressure  $2 \times 10^{-7}$  Torr at 25 °C) and relatively water-soluble (reported as 700  $\mu\text{g/mL}$ , *Farm Chemicals Handbook*, 1997, and 1600  $\mu\text{g/mL}$ , *The Pesticide Manual*, 1991, both at 25 °C), with a reported  $K_{ow}$  of 25 (*The Pesticide Manual*, 1991). The  $pK_a$  of tricyclazole (with protonation on the nitrogen) is 1.6 (Weber, 1982), indicating that tricyclazole will exist in the neutral form in typical environmental compartments.

An effective extraction method is required to characterize the fate of tricyclazole in soil and sediment environments. Two soil extraction methods that achieved quantitative extraction on freshly spiked samples have been developed (Yunxiang et al., 1990; Tsukioka, 1988), but these methods were not validated on aged samples and also required numerous sample cleanup steps before chromatographic analysis. Methods used to extract tricyclazole from other solid matrixes such as rice grain and straw include refluxing in 4 N  $\text{H}_2\text{SO}_4$  (Koons et al., 1985) or organic solvent extraction (Tsumura et al., 1996). These methods require a substantial amount of sample cleanup to remove interfering co-extractants, and recoveries typically are not quantitative. Our

preliminary extraction method development work using 90:10 acetone:1.0 N HCl (at 50 °C) yielded tricyclazole recoveries of only 73–83% from soil samples aged 12 days. Also, the sample extracts were dark brown in color, suggesting that extensive sample cleanup would be required before chromatographic analysis.

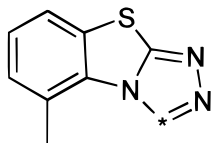
Understanding the factors controlling the adsorption of tricyclazole on soils is important for designing effective soil extraction methods. Tricyclazole is strongly adsorbed to soils, with sorption coefficients ( $K_d$ ) ranging from 4 to 96 mL/g (Cook, 1998) that increase with time after application to soil. Other work has suggested that adsorption of tricyclazole is dependent primarily upon the bulk clay content, cation exchange capacity (CEC), and illite and montmorillonite content, but is not as significantly dependent on soil organic matter (Roldan et al., 1993). Similar trends were observed in another study (Weber, 1982), wherein it was noted that adsorption increased as pH decreased, and that the significance of organic matter increased with decreasing pH.

Because tricyclazole is stable to hydrolysis at pH 3, 6, and 9 at 52 °C (Saunders, 1976), we believed that subcritical water extraction (Yang et al., 1995, 1997; Hartonen et al., 1997; Pawlowski and Poole, 1998; Field and Reed, 1999; Crescenzi et al., 1999; di Corcia et al., 1999; Lou et al., 2000) might be an effective alternative to conventional extraction methods. The solubility of many organic compounds in water has been shown to increase significantly with increasing temperature (Miller and Hawthorne, 1998; Miller et al., 1998). In addition to an increase in the solute vapor pressure, the physical properties of water change with increasing temperature. The predominant influence of increasing temperature on the solvation properties of water is to reduce the water's cohesion and capacity for hydrogen-bond interactions (Pawlowski and Poole, 1999); the dielectric constant is also reduced from 79 at 25 °C to 44 at 150 °C (Akerlof and Oshry, 1950). Unlike the nonpolar

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**Table 1. Physical and Chemical Characteristics of Soils Used in This Study**

| property                      | soils      |                  |            |         | sediments |            |
|-------------------------------|------------|------------------|------------|---------|-----------|------------|
|                               | Hanford    | Crosby-Brookston | Ottobiano  | Greggio | Rivalta   | Mergozzo   |
| source (location)             | California | Indiana          | Italy      | Italy   | Italy     | Italy      |
| pH                            | 7.3        | 6.3              | 5.2        | 5.2     | 7.5       | 7.1        |
| CEC (mequiv/100 g)            | 9.2        | 12.3             | 4.1        | 6.4     | 16.7      | 2.0        |
| organic matter (%)            | 1.25       | 2.82             | 1.30       | 2.38    | 9.35      | 0.54       |
| moisture holding capacity (%) | 42.6       | 46.9             | 71.2       | 54.6    | 53.4      | 6.9        |
| textural class                | loam       | clay loam        | sandy loam | loam    | silt loam | loamy sand |
| sand (%)                      | 41.2       | 31.2             | 71.2       | 45.2    | 24.0      | 84.0       |
| silt (%)                      | 38.0       | 38.0             | 22.4       | 34.4    | 54.0      | 14.0       |
| clay (%)                      | 20.8       | 30.8             | 6.4        | 20.4    | 22.0      | 2.0        |

**Figure 1.** Structure of tricyclazole. Location of the radiolabeled carbon atom is noted with an asterisk.

organic compounds examined by other researchers, in which the increase in water solubility with increasing extraction temperature was postulated to have an effect on increasing the extraction efficiency, this effect was unlikely to be important for tricyclazole because of its high aqueous solubility at ambient temperature. However, an increase in the rate of desorption was still possible because of the higher temperature of the system.

We examined the overall recoveries of tricyclazole from soil and sediment using subcritical water extraction, as well as the effect of temperature, incubation time, soil type, sample size, and flow rate on the extraction recovery. Extraction recovery experiments were performed on aged samples rather than freshly spiked samples to more accurately represent the occurrence of tricyclazole in the environment.

#### EXPERIMENTAL PROCEDURES

**Soils and Sediments.** Tricyclazole was applied to four aerobic soil test systems, one flooded (anaerobic) soil test system, and two flooded (aerobic) sediment systems. Physical properties of the soils and sediments are given in Table 1. The soils exhibited a wide range of physical and chemical properties and are representative of soils in typical use regions for tricyclazole. Sediment samples were collected from the River Mincio near Rivalta sul Mincio, Lombardy, Italy, and from a small lake near Mergozzo, Piemonte, Italy. These sediments were designated Rivalta and Mergozzo in the context of this study. Aerobic soil test systems were prepared by adding approximately 30 g (dry weight) of the Hanford, Crosby-Brookston, Ottobiano, and Greggio soils to individual two-chamber biometers; 100 mL of 0.1 N NaOH was added to the biometer side flask for the collection of evolved  $^{14}\text{CO}_2$ . Anaerobic test systems containing the Ottobiano soil were prepared as above, flooded with 30 mL of water (giving an approximate water column depth of 1 cm), purged with nitrogen (to induce anaerobic conditions), and sealed before being placed into the incubator. The two sediments used for the aerobic sediment test systems were also prepared in two-chamber biometer flasks, using approximately 10 g (dry weight) of sediment and 100 mL of water.

**Test Materials, Sample Treatment, and Incubation.** Radiolabeled tricyclazole was used as the test material for all experiments; the structure of tricyclazole and the location of the radiolabeled carbon atom are shown in Figure 1. The radiochemical purity of the test material was 98.7% and the specific activity was 30.5 mCi/mmol. Aerobic and anaerobic soil samples were treated with  $^{14}\text{C}$ -tricyclazole at a rate of 0.53  $\mu\text{g/g}$  (dry weight); aerobic sediment samples were treated at a rate of 0.35  $\mu\text{g/mL}$ . Samples were incubated from 0 to 202 days

after treatment (DAT) in darkened incubators set at 20 °C (a subset of samples was incubated for 1 d in a darkened incubator set at 25 °C); all samples except the flooded Ottobiano soil samples were connected to an oxygen supply to maintain aerobic conditions. Tricyclazole was applied to the aqueous layer in the aerobic and anaerobic aquatic systems.

**Extraction Methods.** Subcritical water extractions were performed using an ISCO (Lincoln, NE) SFX 220 supercritical fluid extraction system. Disposable 10-mL plastic extraction cells with 2.0  $\mu\text{m}$  frits were used; each cell was reused approximately 50 times before it exhibited signs of wear and was replaced. HPLC-grade water was used as received (no purging) for all subcritical water extractions. Pressure was maintained using fixed-diameter (15 cm  $\times$  50  $\mu\text{m}$  i.d.) fused silica restrictors (Polymicro Technologies, Phoenix, AZ); restrictor heaters were not used. The extraction pressure was varied (typically from 75 to 300 atm) to maintain a flow rate of approximately 1 mL/min. The effluent from the restrictor was collected in glass test tubes or vials (typically 20–40 mL) at room temperature without a cosolvent. Samples used to determine extraction kinetics were collected as timed fractions, typically 5 min. Approximately 10 g of soil or sediment was extracted for up to 60 min, at temperatures ranging from 50 to 150 °C (the upper temperature limit of the ISCO SFX 220). Soil and sediment samples were extracted without any pre-treatment or preparation. The amount of tricyclazole in each fraction was determined by transferring the fraction to a tared vial, weighing the vial with the extraction fluid, and converting to volume using the density of water (after cooling to room temperature). The mass of tricyclazole extracted was then determined from the concentration measured by liquid scintillation counting (LSC) of a known aliquot volume and the sample volume.

**Analytical Methods.** Samples were analyzed by reverse-phase HPLC using an Alltech (Deerfield, IL) Spherisorb ODS (4.6  $\times$  150 mm) column on a Hewlett-Packard 1090 liquid chromatograph. An isocratic mobile phase of 65:35 methanol–water was used for elution. The column effluent was collected in 0.5-min fractions, which were mixed with scintillation cocktail and analyzed for  $^{14}\text{C}$  by LSC.

**Liquid Scintillation Counting.** Radioactive material in solution was quantified by a liquid scintillation spectrometer. All LSC measurements were performed on a Packard (Meriden, CT) 2500TR or 2000CA liquid scintillation counter; correction for chemiluminescence was accomplished using the scintillation counter's on-board logic. Ultima Gold XR scintillation cocktail (Packard, Meriden, CT) was added to each sample before counting; samples were generally counted for 3–5 min.

#### RESULTS AND DISCUSSION

**Hydrolysis and Recovery.** To determine whether subcritical water would be a viable extraction technique, it was necessary to determine whether tricyclazole would be stable under the conditions of the extraction. Previous work (Saunders, 1976) indicated that tricyclazole was stable to hydrolysis at pH 3, 6, and 9 for 32 days at 52 °C, but suggested that there was a small amount of degradation at 100 °C. To confirm that tricyclazole would not hydrolytically degrade over the duration of a typical extraction experiment, tricyclazole

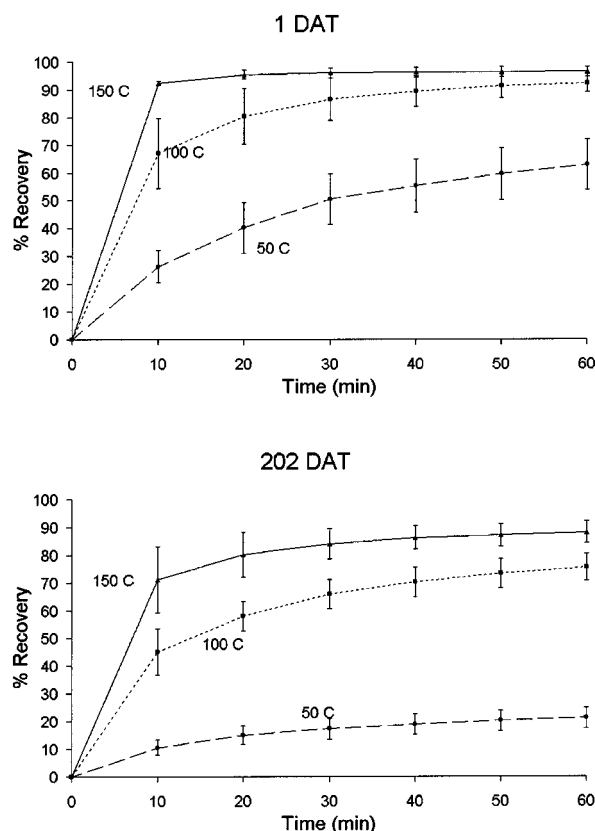
in pH 4, 7, and 10 buffer solutions was heated at 150 °C for 2 h. Each sample was analyzed by HPLC before and after heating to determine the amount of hydrolytic degradation. Approximately 2, 16, and 12% of the initial amount of tricyclazole degraded over the 2 h reaction time at pH 4, 7, and 10, respectively. Assuming pseudo-first-order degradation kinetics, less than 4% of tricyclazole would degrade over a 30-min extraction period. (Note that these off-line hydrolysis experiments were performed at a different pressure than the dynamic extractions used in the remainder of this study; it is possible that the hydrolysis reaction could be affected by pressure as well as temperature.) These results indicate that for hydrolysis not to be a significant artifact in the analytical process, the bulk of extractable tricyclazole must be removed from the sample matrix in less than 30 min. Analysis of samples from experiments designed to determine the effect of sample matrix and incubation time indicated that all of the extractable material was tricyclazole, and therefore hydrolysis was not significant over the duration of the extraction (note that these extractions had a duration of 30 to 60 min). Extractions at 150 °C generally showed that most of the tricyclazole was extracted in the first 10-min of the extraction, decreasing the worst-case degradation of tricyclazole due to hydrolysis to <1%.

The recovery of  $^{14}\text{C}$  tricyclazole spiked into an empty cell and extracted at 150 °C and 1 mL/min was  $106 \pm 4\%$ , indicating that collection at ambient temperature without a cosolvent provided quantitative recovery of tricyclazole. This result is not surprising given the low vapor pressure and high water solubility of tricyclazole at 25 °C.

**Effect of Extraction Temperature and Sample Incubation Time.** The effect of temperature on the extraction kinetics and overall recovery was examined using the Hanford loam soil. Samples were incubated for either 1 or 202 days to provide aged residues and extracted at a constant flow rate (1 mL/min) at 50, 100, and 150 °C. The effluent was collected in 10-min increments (approximately 10 mL) for a total of 60 min; aliquots of each fraction were analyzed for  $^{14}\text{C}$  by LSC.

Both the rate of extraction and total extraction recovery were a strong function of temperature (Figure 2). The total amount of tricyclazole recovered after a 60-min extraction was 63, 92, and 97% from a 1 DAT sample at 50, 100, and 150 °C, respectively. Extraction of tricyclazole was essentially complete within 20 min at 150 °C, with 96% of the tricyclazole removed; only an additional 1% was recovered over the following 40 min. The rate of extraction decreased with decreasing extraction temperature, and the extraction did not reach completion at either 100 or 50 °C at the end of 60 min.

Similar trends were observed from samples aged for 202 days, although the overall recoveries were slightly lower. In this data set, recovery was defined as the amount of tricyclazole as a percentage of that originally applied to the soil. Because these soil and sediment systems were not sterile, some microbial degradation of tricyclazole and subsequent incorporation of the radiolabeled carbon atom into soil organic matter is expected to have occurred. Each aged sample was analyzed for tricyclazole and extractable degradation products by reversed-phase HPLC; however, no degradation products (only tricyclazole) were observed in any sample. First-order degradation half-lives of tricyclazole in the soils used in this work ranged from 240 to 840



**Figure 2.** Effect of temperature on extraction kinetics and efficiency of tricyclazole from soil as a function of incubation time. Data are the average and standard deviation of three replicates from each temperature/incubation time pair.

days under aerobic conditions and from 120 to 465 days in the two aerobic Italian sediments maintained flooded under aerobic conditions; the half-life was 290 days in the Ottobiano sediment maintained under anaerobic conditions (Cook, 1998; Kennard, 1998). The use of radiolabeled test material allowed us to directly determine the amount of  $^{14}\text{C}$  that was nonextractable (by combustion of the extracted soil sample, conversion of nonextracted  $^{14}\text{C}$  to  $^{14}\text{CO}_2$ , and measurement by LSC). However, it was not possible to determine whether the nonextracted residue was tricyclazole or a product of microbial degradation and subsequent incorporation into the soil organic matter. Therefore, calculation of extraction recoveries based on the percentage of total radiocarbon in the soil may underestimate the actual extraction efficiency. Further extraction of other samples with conventional organic solvents yielded negligible amounts of tricyclazole; thus, we conclude that recoveries of tricyclazole (not  $^{14}\text{C}$ ) were quantitative at 150 °C for the 202-DAT samples.

These results suggest that the extraction of tricyclazole from soil is dependent on the desorption of tricyclazole from the soil matrix, rather than on an increase in solubility. The rate of this process is obviously enhanced by increasing temperature (ten Hulscher and Cornelissen, 1996). An alternative explanation is that the rate-limiting step in the extraction could be solubilization of soil organic matter (1.25% in the Hanford soil) followed by release of tricyclazole into the solution phase as the organic matter that it is associated with dissolves into solution. The hot water (100 °C) extractable components of soil have been shown to be principally carbohydrates and proteinaceous species such as amino acids and amides, rather than the more stable,



**Table 2. Predicted Total Extraction Recoveries as a Function of Temperature and Incubation Time**

| DAT | temp (°C) | <i>a</i> | <i>b</i> | <i>c</i> | <i>d</i> | <i>r</i> <sup>2</sup> | recovery (%) |           | extraction completion % |
|-----|-----------|----------|----------|----------|----------|-----------------------|--------------|-----------|-------------------------|
|     |           |          |          |          |          |                       | actual       | predicted |                         |
| 1   | 50        | 47.2     | 0.066    | 46.6     | 0.007    | 1.000                 | 63.0         | 93.9      | 67.1                    |
| 1   | 100       | 51.9     | 0.273    | 41.7     | 0.059    | 1.000                 | 92.4         | 93.5      | 98.8                    |
| 1   | 150       | 94.1     | 0.356    | 2.7      | 0.044    | 1.000                 | 96.6         | 96.8      | 99.8                    |
| 202 | 50        | 11.5     | 0.122    | 15.1     | 0.017    | 1.000                 | 21.2         | 26.6      | 79.6                    |
| 202 | 100       | 28.0     | 0.318    | 50.8     | 0.045    | 1.000                 | 75.5         | 78.8      | 95.9                    |
| 202 | 150       | 62.6     | 0.316    | 26.4     | 0.055    | 1.000                 | 88.2         | 89.1      | 99.0                    |

<sup>a</sup> The fitted parameters to the two-compartment exponential rise are *a*, *b*, *c*, and *d*. The actual amount extracted at *t* = 60 min and the predicted total amount extracted at *t* = ∞ are given, as are the percent to completion of the extraction at *t* = 60 min (actual recovery at *t* = 60 divided by predicted final recovery at *t* = ∞).

humified organic matter that is more closely incorporated with the mineral fraction of the soil (Leinweber et al., 1995). The color of the sample extracts ranged from clear (at 50 °C) to pale yellow (at 150 °C), as opposed to the typical dark brown color of organic solvent soil extracts, suggesting that the organic material extracted was principally composed of these labile fractions rather than the more highly conjugated humified soil organic matter. However, studies investigating parameters controlling adsorption of tricyclazole to soil (Weber, 1982; Roldan, 1993) suggest that sorption is principally determined by the clay fraction of soil and that organic matter was only significant at low pH values. It seems likely that the increase in desorption kinetics is the dominant mechanism for the increase in extraction rate with temperature. However, at the present time, we have insufficient data to unambiguously determine the rate-limiting step in the extraction of tricyclazole.

Because the extractions did not appear to have reached completion at the end of 60 min at 50 and 100 °C, we estimated the amount of tricyclazole that would have been extracted had the extraction time been infinite. We fitted the observed extraction rate data to a two-compartment exponential rise to a maximum function of the type

$$y = a(1 - e^{-bt}) + c(1 - e^{-dt})$$

where *y* = the amount of tricyclazole remaining at time *t* and *a*, *b*, *c*, and *d* are fitted constants. A two-compartment model was used to simulate release of tricyclazole from separate fast-desorbing (labile) and slow-desorbing (recalcitrant) pools of material in the soil (Brusseau and Rao, 1989; Cornelissen et al., 1997). We set *t* = ∞ and solved for *y* to estimate the total amount of extractable tricyclazole at each temperature; results are summarized in Table 2.

The effect of incubation time is shown most clearly by comparing the predicted extractable amount of tricyclazole at each temperature between 1 and 202 DAT. For example, at 1 DAT the ratio of the predicted amount of tricyclazole extractable at 50–150 °C was 0.97. This ratio indicates that the difference in observed extraction recoveries (63.0 vs 96.6%) was due to length of time of the extraction, and that 97% of the material extractable at 150 °C could have been extracted at 50 °C had the extraction time been long enough. Conversely, at 202 DAT this ratio was 0.30, indicating that only 30% of the material extractable at 150 °C could have been extracted at 50 °C if the extraction time had been increased to infinity. The difference in recovery at long incubation times was therefore the result of an increase in the strength of the interaction with the matrix, requiring higher temperatures to provide enough

energy for desorption of bound tricyclazole. This conclusion supports our two-compartment model of tricyclazole in soil, in which the tricyclazole in the labile compartment (easily extractable) is assumed to be in a rapid equilibrium with the soil solution phase and also capable of moving into the recalcitrant pool (which can be considered to encompass both high energy binding sites in the soil matrix and soil micropores inaccessible to solvent molecules). Over time, the amount of tricyclazole in the labile pool is depleted by biodegradation in the soil solution and also by movement into the recalcitrant pool. Increasing the extraction temperature allows sequentially larger fractions of molecules in the recalcitrant pool to overcome the desorption energy required for release from the recalcitrant pool. Other models, such as those used to describe extraction kinetics from supercritical fluid extraction (Pawliszyn, 1993; Langenfeld et al., 1995; Clifford et al., 1995), could also be used; similar results would be expected, because in each case the extractions had progressed through the initial slow release and intermediate rapid extraction phase to a slow final phase.

**Effect of Soil Type.** The effect of soil type and incubation time on the extraction kinetics and overall recovery was examined using the six soils and sediments described in Table 1 at incubation times of 60–120 DAT. Samples were extracted at a flow rate of 1 mL/min and a temperature of 150 °C for 30 min. The total amount of radioactivity present in each soil sample was determined by oxidation of the soil organic matter to CO<sub>2</sub>, and analysis of the evolved <sup>14</sup>CO<sub>2</sub> content of the soil by LSC. The total extractable tricyclazole was determined by following the subcritical water extraction with extraction using 25 mL of 0.5 N NaOH at 50 °C for 5 h. Samples collected at 100 DAT from the two sediments maintained under aerobic conditions were further extracted using 25 mL of methanol. The NaOH extracts were not characterized chromatographically because the amount of tricyclazole was below the quantitation limit of our analytical method, or, as in the case of the Rivalta sediment, it was a gelatinous extract (due to the high organic matter content of the sediment) that could not be adequately cleaned up to give meaningful chromatographic data. Nevertheless, to be conservative in our estimates of extraction efficiency, we assumed that all of the extractable radiocarbon was tricyclazole.

With the exception of the Rivalta sediment, extraction efficiencies (expressed as a percentage of the total extractable tricyclazole) ranged from 85 to 100% across all time points and across all soil and sediment types (Table 3). Overall, recoveries of tricyclazole did not change with incubation time, averaging 95.9 ± 4.3% at 60 DAT and 95.3 ± 5.5% at 120 DAT. The difference in extractability between the Ottobiano soil maintained

**Table 3. Extraction Efficiency of Tricyclazole Using Subcritical Water Extraction as a Function of Soil and Sediment Type and Incubation Time**

| soil or sediment      | extraction efficiency <sup>a</sup> (%) |                 |                   |
|-----------------------|--|-----------------|-------------------|
|                       | 0 DAT                                  | 60 DAT          | 120 DAT           |
| Hanford               | 98.1                                   | 88.3            | 95.4              |
| Crosby–Brookston      | 98.2                                   | 98.3            | 97.7              |
| Ottobiano (aerobic)   | 96.7                                   | 98.7            | 98.2              |
| Greggio               | 95.8                                   | 97.7            | 97.1              |
| Ottobiano (anaerobic) | 94.7                                   | 96.7            | 84.4              |
| Rivalta               | 27.8                                   | NA <sup>b</sup> | 44.4 <sup>c</sup> |
| Mergozzo              | 97.4                                   | NA <sup>b</sup> | 98.9 <sup>c</sup> |
| av                    | 96.8 <sup>d</sup>                      | 95.9            | 95.3 <sup>d</sup> |
| SD                    | 1.4 <sup>d</sup>                       | 4.3             | 5.5 <sup>d</sup>  |

<sup>a</sup> Results are expressed as a percentage of the total extractable tricyclazole. <sup>b</sup> Samples not collected and analyzed at these points. <sup>c</sup> Samples collected at 100 DAT. <sup>d</sup> Average and standard deviation exclude results from the Rivalta sediment.

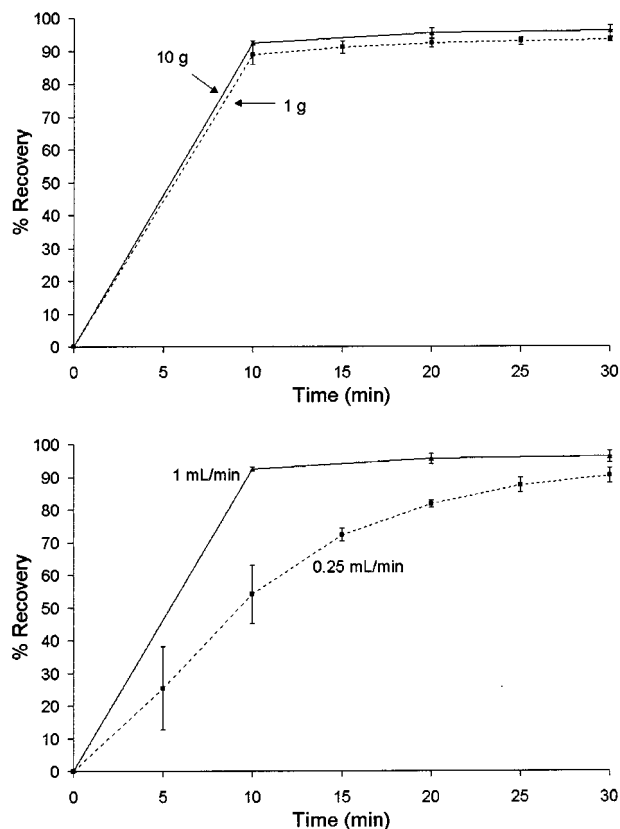
aerobically and that maintained under anaerobic conditions suggests that the redox state may have some influence on the extractability of tricyclazole, although there is not enough data to determine a trend.

The Rivalta sediment possessed distinctly different physical properties than the other soils and sediments. A particular difference was its large amount of organic matter (9.34%). The Rivalta sediment also had the highest values for pH and cation exchange capacity of any of the soils or sediments studied, but these values were not substantially outside the range of the other soils and sediments. The bulk clay content of the Rivalta sediment lies in the middle of the range; we do not have detailed mineralogy on the clay fraction to indicate whether differences exist in the types of clays present in each soil or sediment. Although previous work (Weber, 1982; Roldan et al., 1993) indicated that sorption of tricyclazole was dependent on the clay fraction of soil to a greater extent than the organic fraction, this trend is not observed in the extraction data. The low recoveries from this sediment at 0 DAT suggest that organic matter content is an important parameter controlling the desorption of tricyclazole.

**Effect of Sample Size and Flow Rate.** The effect of flow rate on extraction rates using supercritical fluid extraction has been used to determine whether the extraction is limited by analyte solubility, by chromatographic retention of the analyte on the matrix, or by the kinetics of the initial desorption from the matrix (Hawthorne et al., 1995); similar experiments using subcritical water instead of supercritical fluids should yield the same information.

The effect of sample size and extraction flow rate on the extraction kinetics and overall recovery was examined on the Hanford loam soil. The soil was treated with tricyclazole and incubated for 1 day at 25 °C prior to extraction. To determine the effect of sample size, the amount extracted was varied between 1 and 10 g. These samples were extracted at a flow rate of 1 mL/min and a temperature of 150 °C. To determine the effect of flow rate, the flow rate was varied between 0.25 and 1 mL/min. These samples (10 g) were also extracted at a temperature of 150 °C. For both sample sets, the effluent from the restrictor was collected in 10-min increments for a total of 30 min; aliquots of each fraction were analyzed for <sup>14</sup>C by LSC.

Changing the sample size from 1 to 10 g had no impact on either the rate or total recovery of tricyclazole from soil (Figure 3), suggesting that solubility was not a limiting factor in the rate of extraction. Recoveries



**Figure 3.** Effect of sample size (top) and flow rate (bottom) on extraction kinetics and efficiency of tricyclazole extraction from soil. Data are the average and standard deviation of three replicates at each set of conditions.

were  $93.5 \pm 0.6\%$  and  $96.6 \pm 1.8\%$  from the 1 and 10 g samples, respectively, and the kinetic profiles of each set of conditions were identical. These results are consistent with the known solubility data of tricyclazole; for a 10-g sample containing tricyclazole at a concentration of  $0.5 \mu\text{g/g}$ , the initial mass of tricyclazole in the extraction cell was only  $5 \mu\text{g}$ . Given that the solubility of tricyclazole at 25 °C is approximately  $700 \mu\text{g/mL}$  (which may be significantly higher at 150 °C; see Miller et al., 1998), it was unlikely that solubility in the extraction solvent was limiting.

Decreasing the flow rate from 1 to 0.25 mL/min decreased the rate of the extraction, with only  $54.0 \pm 9.0\%$  versus  $92.4 \pm 0.6\%$  of the tricyclazole extracted after 10 min, but the overall extraction efficiency was similar at 30 min ( $90.2 \pm 2.3\%$  at 0.25 mL/min versus  $96.6 \pm 1.8\%$  at 1 mL/min; see Figure 3). If the rate of extraction was not limited by solubility of tricyclazole, as is suggested by the lack of change in the extraction kinetics with a change in sample load, a decrease in flow rate should not have affected the rate of extraction. However, the slower rate of extraction may simply be an artifact of the slower flow rate of extraction fluid flushing the void volume of the extraction cell at a slower rate. We estimate that the void volume of the cell after filling with 10 g of soil was approximately 5 mL; therefore, the void volume of the cell was flushed approximately 2 times in 10 min at 1 mL/min but only 0.5 times in 10 min at 0.25 mL/min.

**Conclusions.** Subcritical water is a simple, effective technique for extracting tricyclazole from soils and sediments. Extraction efficiency and kinetics were influenced most by temperature, with increasing tem-

perature giving faster extractions and higher recoveries for aged samples. Higher extraction temperatures were also important for quantitative recovery of tricyclazole from aged samples. The extraction recoveries ranged from 85 to 100% for aged residues across a variety of soil and sediment types, with the exception of one sediment containing a large amount of organic matter. The total analysis time required using subcritical water extraction was a few hours, and no sample preparation steps were required between extraction and chromatographic analysis with radiochemical detection. Sample extracts from subcritical water extraction were generally a pale yellow color, as opposed to a dark brown color from conventional organic solvent extracts. It is likely that some chromatographic cleanup would still be required for the analysis of nonradiolabeled tricyclazole. Because the sample extracts from subcritical water extraction were in an aqueous solution, cleanup with liquid-liquid partitioning or solid-phase extraction could proceed immediately without the need for removal of organic solvent from the extract.

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