Degradation of Pesticides in Nursery Recycling Pond Waters

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Recycling or collection ponds are often used in outdoor container nursery production to capture and recycle runoff water and fertilizers. Waters in recycling ponds generally have high concentrations of nutrients, pesticides, and dissolved organic matter, as well as elevated salinity and turbidity. Little is known about pesticide degradation behavior in the unique environment of nursery recycling ponds. In this study, degradation of four commonly used pesticides diazinon, chlorpyrifos, chlorothalonil, and pendimethalin in waters from two nursery recycling ponds was investigated at an initial pesticide concentration of 50 μg/L. Results showed that the persistence of diazinon and chlorpyrifos appeared to be prolonged in recycling pond waters as compared to surface streamwaters, possibly due to decreased contribution from biotic transformation, while degradation of chlorothalonil and pendimethalin was enhanced. Activation energies of biotic degradation of all four pesticides were lower than abiotic degradation, indicating that microbial transformation was less affected by temperature than chemical transformation. Overall, the pesticide degradation capacity of recycling ponds was better buffered against temperature changes than that of surface streamwaters.

KEYWORDS: Nursery recycling pond; pesticide degradation; water quality

INTRODUCTION

Container nursery production represents an extremely intensive agricultural setting. Generally, one hectare of land may host 80000—600000 containers. Various pesticides, including insecticides, nematicides, fungicides, algaecides, and herbicides, are applied to sustain healthy plant growth. The intensive use of pesticides in nursery production causes concern for their environmental behavior and fate, such as their distribution, movement, persistence, runoff risk to downstream water bodies, and leaching potential to groundwater (*1*, *2*).

In outdoor container nursery production, recycling or collection ponds are increasingly used to capture and recycle runoff water and fertilizers (dissolved in the runoff water) and to reduce runoff water to downstream water bodies. Recycling ponds are often mentioned as the primary method of eliminating potential problems that arise from container nursery runoff (*3*). During overhead irrigation, pesticides are washed away from plants and/or potting mixes (*4*, *5*) and carried to the recycling ponds, where accumulation and dissipation processes may take place.

Because of continuous reuses, waters in nursery recycling ponds are constantly exposed to various pesticides. These waters often have high concentrations of nutrients and other applied chemicals, as well as elevated salinity and turbidity. The persistence of pesticides in recycling pond environments is speculated to be different from that in natural water because of the differences in physical, chemical, and biological conditions. Pesticide dissipation is an extensively studied topic, but most of the previous studies focused on deionized or natural waters, and little is known about the recycling pond environment. Knowledge of pesticide persistence in recycling ponds is critical for preventing phytotoxicity of pesticides during water reuse and to assess their impacts to the environment. It is therefore imperative to investigate the persistence of pesticides in recycling ponds, especially for the compounds that are heavily used in outdoor nursery production and are of high toxicity to aquatic organisms, such as diazinon (insecticide), chlorpyrifos (insecticide and nematicide), chlorothalonil (fungicide), and pendimethalin (herbicide). All of the four pesticides are among the top pesticides that are heavily used in outdoor container nursery production in California (*6*).

The objectives of this study were to evaluate the persistence of four commonly used pesticides in water from nursery recycling ponds and to investigate the effects of water characteristics, microbial activity, and temperature on the degradation of these pesticides.

MATERIALS AND METHODS

Chemicals. Standards of diazinon {*O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl]ester, 98% purity}, chlorpyrifos {*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate, 98% purity}, chlorothalonil (2,5,6-trichloroisophthalonitrile, 98% purity), and pen-

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Table 1. Physical and Chemical Properties of the Two Water Samples from Nursery Recycling Ponds

sampling sites	TOC (mg/L)	TSS (mg/L)	pH	EC (mS/cm)	cations (mg/L)				anions (mg/L)		
					Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	NO_3^-	SO_4^{2-}
nursery A	34.4	61.5	6.9	3.20	239.8	75.4	81.2	92.9	181.8	820.5	443.9
nursery B	13.9	37.5	7.3	1.11	82.9	23.8	51.7	22.8	32.2	45.7	231.5

dimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine, 99.2% purity] were purchased from Chem Service (West Chester, PA). Organic solvents (methylene chloride, acetone, and hexane) used for extraction were of pesticide grade and, for other uses, were of gas chromatography (GC) resolution grade (Fisher Scientific, Pittsburgh, PA). Sodium sulfate used in pesticide analysis was of pesticide grade.

Water Sampling. Two water samples were collected from two representative commercial nurseries in southern California. The first sample was collected from the recycling pond of a nursery (nursery A) in Irvine, CA, and the other sample was from the recycling pond of a nursery (nursery B) in Ventura, CA. Nursery A is a large-scale nursery with 100 hectares of plant production area, and its recycling pond has a capacity of about 7500 m^3. Nursery B is a small-scale nursery with 16 hectares of plant production area and a recycling pond of about 300 m^3.

The water samples were collected manually by immersing and filling 4 L precleaned glass bottles at approximately 5 cm below the surface. Samples were transported to the laboratory in Riverside (CA) within 4 h from the time of sampling and were kept at 4 °C in the dark. Prior to use, samples from the same nursery (approximately 20 L in total) were combined and homogenized manually in a glass tank and were allowed to settle overnight to remove large particles. Sterile water samples were prepared by autoclaving a 4 L aliquot for 1 h at 121 °C and 0.09 MPa. Sterilization treatment was used to remove microbial activity in the water samples.

The total organic carbon (TOC), total suspended solids (TSS), electricity conductivity (EC), pH, major cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+), and anions (NO_3^-, Cl^-, and SO_4^{2-}) of the two water samples were determined, and the results are listed in **Table 1**. The TOC was measured on a Shimadzu TOC Analyzer (Shimadzu, Kyoto, Japan). The TSS was determined by weighing after filtration through a 0.7 μm glass fiber filter (Whatman, Florham Park, NJ). Major cations (Ca^{2+}, Mg^{2+}, Na^+, and K^+) were determined by inductively coupled plasma using an Optima 3000 DV Spectrophotometer (Perkin-Elmer, Norwalk, CT). Major anions (Cl^-, NO_3^-, and SO_4^{2-}) were determined on a Dionex 500 ion chromatography with an Ion Pac AS11-HC column (Dionex, Sunnyvale, CA).

Degradation Experiments. Degradation of pesticides in water samples was performed by measuring the residue pesticide concentration at different time points into incubation. Before the degradation experiments, the two water samples were analyzed for background concentrations of the four pesticides, and no significant amounts were found. A total of four variables were considered, including waters from the two different nurseries, type of pesticides, biological activity (sterile or nonsterile), and temperature (10 or 22 ± 2 °C). Briefly, 4 L of the sterile or nonsterile water in a precleaned glass bottle was spiked with 200 μL of acetone solution containing 200 μg of each of the four pesticides, and the treated samples were thoroughly mixed. Thus, the initial concentration was 50 μg/L for each pesticide. Aliquots of 100 mL were transferred to 120 mL wide-mouth brown glass bottles, and the bottles were loosely covered with aluminum foil to exclude sunlight. The sample bottles were kept at room temperature (22 ± 2 °C) or in an incubator at 10 ± 0.5 °C in the dark. The amount of water in the sample bottles was checked by weighing every week and maintained by adding deionized water when necessary. After 0, 3, 7, 14, 28, 56, and 90 days (for diazinon, chlorpyrifos, and pendimethalin) or 0, 0.5, 1, 2, 3, 7, and 14 days (for chlorothalonil) of incubation, triplicate sample bottles were removed from each treatment and analyzed for pesticide concentration.

Pesticide Analysis. Pesticides in the nursery water samples were analyzed by a procedure including extraction by organic solvent, cleanup by Florisil, and determination by GC. For each sample, all of the water (100 mL) in the sample bottle was transferred to a 250 mL separatory

funnel. The bottle was rinsed twice each with 20 mL of methylene chloride, which was transferred to the separatory funnel. The mixture in the separatory funnel was then vigorously shaken in a head-over-head mode for 2 min and was allowed to settle for 30 min to separate the organic solvent phase and the aqueous phase. The solvent phase was collected, and the remaining aqueous phase was extracted one more time with 40 mL of fresh methylene chloride. The solvent phases were combined, dehydrated with 35 g of anhydrous sodium sulfate, and evaporated to dryness on a vacuumed rotary evaporator at 38 °C. The extract was recovered in 2 mL of hexane and was transferred onto the top of a PrepSep 1-g Florisil column (Fisher) that was pre-eluted with 6 mL of hexane. The Florisil column was eluted with 6 mL of an acetone−hexane mixture (20:80, v/v). The cleaned extract was concentrated to 1.0 mL, and an aliquot was injected into an Agilent 6890N GC equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Wilmington, DE) for analysis. The GC conditions were as follows: the inlet at 270 °C and operated on a pulsed splitless mode with an injection volume of 1 μL; oven initially set at 50 °C with a hold of 1.4 min, then climbed to 280 °C at 25 °C/min with a final hold of 2 min; microelectron capture detector (for chlorothalonil, chlorpyrifos, and pendimethalin) at 290 °C; and nitrogen phosphorus detector (for diazinon) at 290 °C. The retention time was 10.08 min for diazinon, 10.13 min for chlorothalonil, 10.91 min for chlorpyrifos, and 11.18 min for pendimethalin. The method detection limits calculated as a signal-to-noise ratio of 10:1 were 0.01 μg/L for chlorothalonil, 0.03 μg/L for chlorpyrifos and pendimethalin, and 0.1 μg/L for diazinon. The method recoveries were 92.8 ± 3.8% for diazinon, 98.7 ± 4.1% for chlorpyrifos, 89.2 ± 6.9% for chlorothalonil, and 94.3 ± 2.6% for pendimethalin.

RESULTS AND DISCUSSION

Pesticide concentrations measured after different time intervals of incubation are plotted in **Figure 1** for the water samples from nursery A and in **Figure 2** for the water samples from nursery B. The disappearance of pesticides was fitted to a first-order kinetics plot to estimate the rate constants k (days^{-1}) and the half-life $t_{1/2}$ (days) (**Table 2**). The constant k was obtained as the slope of the linear regression of ln of the concentration vs incubation time, and $t_{1/2}$ was calculated by the formula $t_{1/2}$ = ln2/k. As shown in **Table 2**, most of the correlation coefficients (r^2) are >0.95, suggesting that degradation of pesticides in the nursery pond waters followed first-order kinetics, which has been observed in other waters, such as deionized waters (*7*), estuarine waters (*8*), and surface stream-waters (*9*). Relatively poor fits were found for the degradation of pendimethalin in nonsterile waters from nursery A (**Figure 1d**), where pesticide degradation appeared to abruptly accelerate after 3 weeks of incubation.

Degradation of pesticides in water may be influenced by both pesticide properties and environmental conditions. The degradative forces include abiotic chemical transformation (such as surface-catalyzed hydrolysis), microbial degradation, and photocatalytic reactions (*10*). Photolysis of pesticides was suppressed in this study by the experimental setup. The chemical structure of a pesticide is usually the dominant factor for its persistence since it determines the chemical stability for abiotic transformation and the accessibility for microbial metabolism. Under different temperature and sterilization treatments, the persistence of the four pesticides in the recycling pond waters generally

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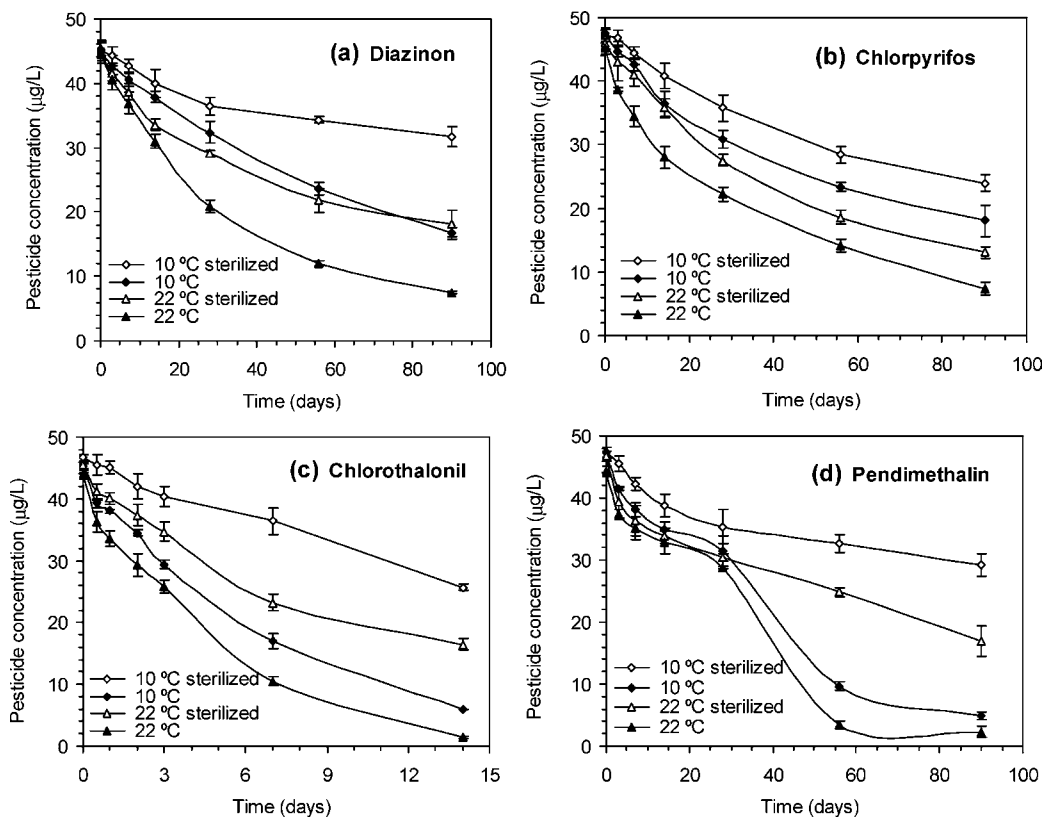
Figure 1. Degradation of diazinon, chlorpyrifos, chlorothalonil, and pendimethalin in water from the recycling pond of nursery A. Error bars are standard deviations of three replicates.
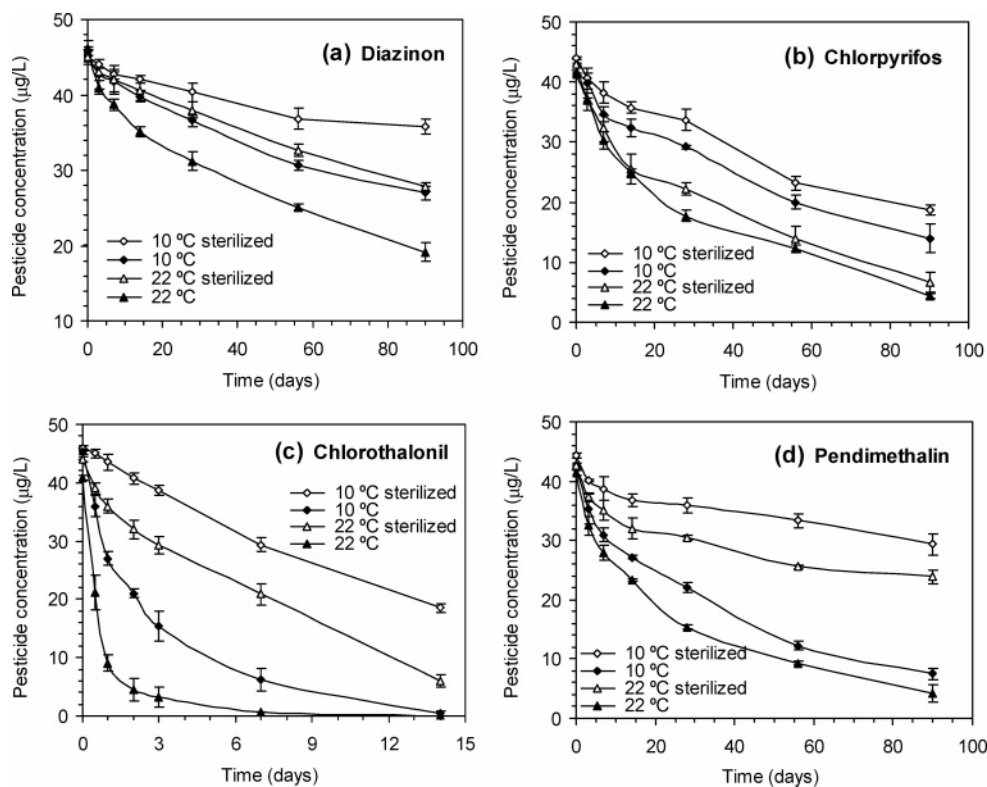
Figure 2. Degradation of diazinon, chlorpyrifos, chlorothalonil, and pendimethalin in water from the recycling pond of nursery B. Error bars are standard deviations of three replicates.

followed the order of diazinon > pendimethalin > chlorpyrifos > chlorothalonil, with the exception that chlorpyrifos was slightly more persistent than diazinon in the nonsterile water samples from nursery A (**Table 2**). In addition to molecular structure, the rate of pesticide degradation in this study could

also be influenced by water characteristics that influence the environment of chemical reaction and the population and activity of microbial species and temperature that affects both chemical reaction rate and enzyme activity. The $t_{1/2}$ and other obtained values are evaluated with respect to these factors.

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Table 2. First-Order Half-Lives (Days^{-1}) of the Four Pesticides in Nursery Recycling Pond Waters^a

pesticide	10 °C		22 °C	
	nonsterile	sterile	nonsterile	sterile
		nursery A		
diazinon	63.1 (0.98)	178.3 (0.91)	34.4 (0.98)	69.5 (0.95)
chlorpyrifos	68.1 (0.97)	92.7 (0.98)	39.4 (0.98)	51.8 (0.98)
chlorothalonil	4.9 (0.99)	16.7 (0.98)	3.5 (0.98)	9.6 (0.97)
pendimethalin	26.9 (0.92)	137.5 (0.89)	16.4 (0.87)	71.4 (0.89)
		nursery B		
diazinon	122.9 (0.98)	268.5 (0.92)	76.6 (0.95)	134.4 (0.99)
chlorpyrifos	58.7 (0.99)	73.6 (0.98)	29.7 (0.97)	35.7 (0.98)
chlorothalonil	2.9 (0.98)	10.6 (0.99)	1.5 (0.96)	5.2 (0.97)
pendimethalin	37.4 (0.98)	183.4 (0.89)	29.1 (0.98)	121.0 (0.86)

^{a} Values in parentheses are correlation coefficients (r^2, $n = 7$).

Water Characteristics. As compared to natural surface stream or lake waters, the most apparent characteristic of nursery recycling pond waters is their high salinity. Waters from nursery A and nursery B had an EC of 3.2 and 1.1 mS cm^{-1}, respectively (**Table 1**). The salts mainly originate from the runoff fertilizers during nursery operation. As a result, these waters contain high concentrations of nutrients, such as nitrate and potassium. For example, the pond water of nursery A had a nitrate concentration of 820.5 mg/L and a potassium concentration of 92.9 mg/L. High salinity can inhibit microbial activity, but the nutrients might stimulate the growth of microorganisms. Another characteristic of nursery recycling pond waters is their relatively high levels of TOC and TSS (**Table 1**), as a result of continuous recycling. The unique physical, chemical, and biological characteristics of recycling pond waters were expected to affect pesticide behaviors.

Degradation of the two organophosphate compounds, diazinon and chlorpyrifos, appeared to be slower in the nursery recycling pond waters than in natural surface waters. Literature-cited half-lives of diazinon in waters are highly variable, ranging from 6.3 to 185 days (*9, 11, 12*), depending on pH and temperature. At 22 °C, the $t_{1/2}$ of diazinon in recycling pond waters was 34.4 days for the nursery A samples and 76.6 days for the nursery B samples (**Table 2**). Under similar temperature conditions, the $t_{1/2}$ of diazinon was reported to be 6.3−14.0 days in surface streamwaters (pH 8.02−8.86) from a coastal watershed (*9*) and 8.2 days in estuarine waters with a pH of 7.8 (*8*). Similarly, the $t_{1/2}$ for chlorpyrifos was estimated to be 39.4 days for the nursery A samples and 29.7 days for the nursery B samples (**Table 2**), which was longer than the previously reported $t_{1/2}$ of 5.5−9.2 days for surface streamwaters (*9*) and 7.4 days for estuarine waters (*8*). The longer half-lives in nursery recycling pond waters are likely due to the decreased contribution from microbial transformation (see discussion later) and the relatively low pH (6.9 in nursery A and 7.3 in nursery B) as compared to the surface streamwaters and the estuarine waters. Lower pH has been shown to prolong the persistence of chlorpyrifos by slowing down its hydrolysis (*13*).

In contrast to the two organophosphate pesticides, significant enhancement of degradation was observed for chlorothalonil and pendimethalin in the nursery recycling pond waters. Literature-cited half-lives for chlorothalonil degradation were 8 days in an estuarine water (*14*) and 47.8 days in distilled water (*15*). Under similar pH and temperature conditions, chlorothalonil exhibited a much faster degradation in the recycling pond waters, with $t_{1/2}$ of 3.5 days in the nursery A samples and 1.5 days in the nursery B samples (**Table 2**). Enhanced degradation was also obvious for pendimethalin, which had $t_{1/2}$
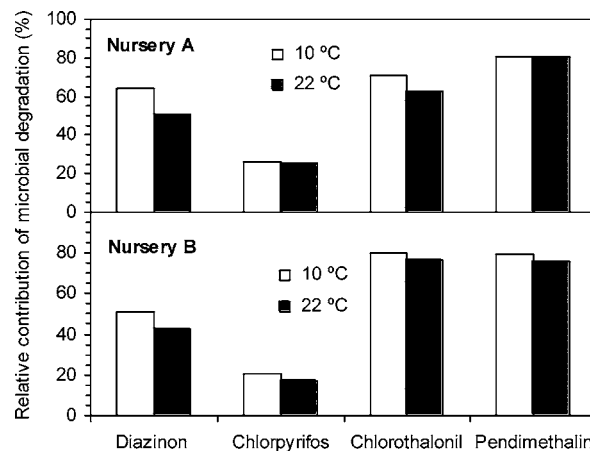
Figure 3. Relative contribution (%) of microbial transformation to the total degradation of the four pesticides in nursery recycling pond waters.

values of 16.4 days in the nursery A samples and 29.1 days in the nursery B samples (**Table 2**), much shorter than the previously reported $t_{1/2}$ of >64 days in surface waters (*16*).

Microbial Activity. The role of microorganisms in pesticide degradation was studied by evaluating the rate of pesticide degradation in the absence of microbial activity. Sterilization increased persistence of all four pesticides in water samples from both nurseries (**Figures 1** and **2** and **Table 2**), suggesting that both abiotic and biotic transformations contributed to the dissipation of these compounds. Because pesticide dissipation followed first-order kinetics, the overall rate constant k (days^{-1}) can be written as the sum of the rate constant for abiotic degradation (k_c [days^{-1}]) and that of biotic degradation (k_b [days^{-1}]) (*9*):

$$k = k_c + k_b$$

The biotic rate constant k_b can be calculated from the difference between that of the nonsterile waters (k) and sterile waters (k_c). The relative contribution of microbial degradation to the overall pesticide dissipation was then calculated as the percentage of k_b in k and plotted in **Figure 3**. It is clear from **Figure 3** that the contribution of microbial degradation differed considerably with pesticide type but did not vary greatly with respect to different nursery waters and temperature.

The average contribution of biotic transformation was 73% for chlorothalonil and 79% for pendimethalin (**Figure 3**), suggesting that degradation of chlorothalonil and pendimethalin in both nursery waters was largely a result of microbial activity. It has been reported that microbial degradation contributed significantly to the dissipation of chlorothalonil in estuarine waters (*14*) and was the primary pathway for degradation of chlorothalonil in soils (*17−19*). No previous information is available on the contribution of microbial degradation to pendimethalin dissipation in water, but microorganisms were found to play an important role in its degradation in soil (*20−22*).

Organophosphate compounds are known to degrade via both abiotic and biotic pathways in water (*23, 24*). Several studies have attempted to investigate the relative importance of abitoic vs microbial degradations of chlorpyrifos and diazinon in water and water/sediment systems, and the results are highly variable. Degradation of chlorpyrifos was found to be primarily abiotic in water samples from a freshwater marsh (*25*) or an estuary (*14*) but was found to be primarily biotic in seawater/salt marsh sediment suspensions (*26*) and in surface streamwaters (*9*). Microbial degradation of diazinon was found to play a major

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Table 3. Activation Energy (kJ/mol) Estimated for Temperature Dependence of Pesticide Degradation in Waters from Nursery Recycling Ponds

pesticide	E_a apparent	E_a abiotic	E_a biotic
	nursery A		
diazinon	35.2	54.5	21.0
chlorpyrifos	32.9	33.6	30.8
chlorothalonil	18.3	32.9	11.2
pendimethalin	28.4	38.3	25.7
	nursery B		
diazinon	28.7	37.6	18.7
chlorpyrifos	39.4	41.9	28.3
chlorothalonil	21.3	41.4	14.9
pendimethalin	14.6	24.2	13.4

role in surface streamwaters (*9*) but only a secondary role in freshwater marsh waters (*25*). In the two nursery recycling pond waters, the contribution of biodegradation was estimated to be about 52% for diazinon and 22% for chlorpyrifos (**Figure 3**). As compared to the surface streamwaters where >75% of diazinon or >60% of chlorpyrifos was degraded biotically (*9*), the role of microorganisms in nursery pond waters appeared to be partially inhibited, possibly due to the high salinity of the water samples. Extremely high salinity such as those in seawaters has been shown to prolong the persistence of diazinon and chlorpyrifos significantly (*7, 9*).

Temperature. Temperature can be an important factor in modulating environmental degradation rates of pesticides due to its acceleration of both abiotic chemical reactions and microbial activity. Significant effects of temperature have been observed in many studies (e.g., *7, 9, 12*). For example, the $t_{1/2}$ of diazinon increased from 14 days at 21 °C to 45 days at 6 °C (*12*). In this study, the effect of temperature was evaluated by comparing the degradation half-lives at 10 and 22 °C. Persistence of the pesticides significantly increased at 10 °C when compared to 22 °C, in both sterile and nonsterile waters (**Table 2**). Overall, the half-lives of pesticides increased by 0.29—1.6-fold over the 12 °C temperature difference. The greatest increase in $t_{1/2}$ was observed for diazinon in the sterilized waters from nursery A, where $t_{1/2}$ increased by 1.6-fold, from 63.1 days at 22 °C to 178.3 days at 10 °C. Degradation of pendimethalin in nonsterile water samples from nursery B showed the least effect from temperature, where $t_{1/2}$ only increased by 0.29-fold when temperature decreased from 22 to 10 °C.

The degree of temperature effect on degradation kinetics depends on the type of pesticides, water characteristics, and sterilization treatment. To quantitatively evaluate the effect of temperature on degradation rate, the activation energy E_a (kJ/mol) of pesticide degradation was calculated for each treatment (**Table 3**). The values of E_a were obtained from the rate constants by using the Arrhenius equation:

$$k = A \, e^{-E_a/RT}$$

where T is temperature in degrees Kelvin (K), R is the universal gas constant with a value of 8.314×10^{-3} kJ/mol/K, and A is a constant. Apparent E_a, abiotic E_a, and biotic E_a were calculated by using k, k_c, and k_b at 10 and 22 °C, respectively. The values of E_a demonstrate the influence of temperature on degradation: The greater the E_a value is, the greater dependence on temperature, i.e., the degradation kinetics will change more significantly with temperature variation (*7*). Two conclusions can be drawn from the calculated E_a values (**Table 3**). First, E_a values depended mainly on pesticide chemical structure, but they also varied between nurseries. Apparently, the influence of temper-

ature on pesticide degradation was also affected by the different water characteristics of the two nurseries; second, temperature change had a greater effect on abiotic degradation than on biotic degradation, as the values of abiotic E_a were constantly higher than those of biotic E_a. This can be explained by the fact that temperature does not affect the growth and activity of micro-organisms very much as long as it is in a preferred range. For abiotic chemical degradation, the general rule is that the reaction rate always increases by 1—2 times with every 10 °C increase in temperature.

The activation energies for degradation of the two organo-phosphate pesticides in surface streamwaters have been reported, with values ranging from 36.5 to 93.5 kJ/mol for diazinon and from 56.0 to 77.0 kJ/mol for chlorpyrifos (*9*). The apparent E_a values obtained in this study (**Table 3**) are around one-third to half of these previously reported values, suggesting that temperature change had a greater effect on degradation of these two pesticides in surface streamwaters than in the nursery recycling pond waters or that the pesticide degradation capacity of recycling ponds was better buffered against temperature changes than that of surface streamwaters.

In summary, degradation of pesticides in nursery recycling pond waters under different temperature and sterilization treatment conditions generally followed first-order kinetics. Persistence of pesticides was mainly determined by their chemical structures but also affected by water characteristics, microbial activity, and temperature. Microbial degradation was the dominant dissipation pathway for chlorothalonil and pendimethalin, while abiotic and biotic transformations were equally important for the degradation of diazinon and chlorpyrifos. The half-lives of diazinon and chlorpyrifos appeared to be prolonged in recycling pond waters as compared to surface streamwaters, possibly due to decreased contribution from biotic transformation. In contrast, degradation of chlorothalonil and pendimethalin appeared to be enhanced. The rate of abiotic degradation of these pesticides was affected by temperature more significantly than that of biotic degradation.

The results obtained in this study will be helpful for understanding pesticide behaviors in nursery recycling ponds. The relatively long persistence of diazinon and chlorpyrifos suggests that the recycling water should be retained on-site for a longer time before it is discharged. For the same reason, if an overflow occurs due to, e.g., a heavy rainstorm, an increased pesticide load may be expected in the runoff. The short persistence of chlorothalonil implies that the potential for this herbicide to cause phytotoxicity to plants when the recycled water is used for irrigation, if any, will be negligible. However, it should be mentioned that the half-lives obtained in this study are likely longer than real values under outdoor conditions, since photolysis can also be an important degradation pathway for these pesticides (*22, 26—28*). In addition, to fully evaluate the persistence of pesticides in nursery recycling pond environment, degradation kinetics in sediments and partition of pesticide between the solution and the sorbed phases must be also considered.

ABBREVIATION USED

GC, gas chromatography; TOC, total organic carbon; TSS, total suspended solids; EC, electrical conductivity.

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