Bull. Environ. Contam. Toxicol. (2006) 76:712–719

DOI: 10.1007/s00128-006-0978-y

Bulletin of Environmental Contamination and Toxicology

Effects of Temperature and Microorganisms on Malathion Transformation in River Water

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Received: 16 September 2005/Accepted: 10 February 2006

Malathion [S-1, 2-bis (ethoxycarbonyl) ethyl O, O-dimethyl phosphorodithioate; CAS# 121-75-5; $C_{10}H_{12}O_6PS_2$] is one of the most widely used organophosphate insecticides in the United States and throughout the world. It has been used to control sucking and chewing insects on field crops, fruits, vegetables, stored grain and live-stock, and also extensively used to prevent mosquitoes, flies, household insects, animal parasites and head/body lice (Barlas 1996; Chembers 1992; Rettich 1980). Malathion is a slightly toxic compound and is listed as EPA toxicity class III. Malathion has been shown in animal testing to affect the central nervous system, immune system, adrenal glands, liver, and blood.

Malathion's widespread use makes potential for human exposure high. Malathion is slightly soluble in water with solubility of 130 mg/L (Kidd and James 1991). It may pose a risk to groundwater or surface water contamination in situations which are less conducive to its degradation. In some US states, malathion was detected in groundwater and surface water (Hind and Evans 1988; US EPA 1988). Therefore, the persistence of malathion in the natural environment is of concern. Many research workers have studied the transformation of malathion in water (Howard 1991; US EPA 2000; HSDB 2000; Guerrant et al. 1970; Wolfe et al. 1975; Wolfe et al. 1977). Depending on the water system which the investigators used, the results obtained varied greatly among the reports. For example, the half-life of malathion transformation in water was reported to range from 1.5 days to months or even years. Furthermore, to the best of our knowledge, most of the previous works were conducted under controlled pH, which may not reflect its realistic fate in the natural environment.

The objective of this research was to determine the effects of two processes, hydrolysis and biodegradation, on malathion transformation in the natural water. Without adding chemicals into water systems for the control of pH, the obtained kinetic data of malathion transformation in aqueous solutions should reflect the realistic fate of malathion in the natural water. The effects of microorganisms (particularly, fungi and bacteria in water), matters dissolved in water and temperature on malathion transformation were investigated in this study.

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MATERIALS AND METHODS

Malathion of pesticide grade (99.2%) was purchased from Chem Service, Inc (West Chester, Philadelphia, USA). Methylene chloride of GC/MS grade (99.9%) was obtained from Fisher Scientific, Inc (Houston, Texas, USA). The river water was sampled from the reservoir near Jackson, Mississippi. The physical and chemical parameters tested by a PASTEL UV spectrophotometer (SECOMAN, France) were as follows: COD = 106 ± 2 mg/L, BOD = 23.4 ± 0.5 mg/L, TOC = 27.4 ± 0.5 mg/L, nitrates < 1.0 mg/L and surfactants < 1 mg/L. The pH of the river water was 6.9 ± 0.5.

Malathion was weighed to 0.05 g and transferred into a 1000 mL Pyrex® flask. Then 500 mL water (sterile distilled water, non-sterile or sterile river water) was added into the flask to obtain an aqueous solution of malathion of final concentration 100 mg/L (0.3 mM). The flask was immediately covered with aluminum foil to protect the solution from light and then placed into a Standard Multi-Tube Vortexer (VWR Scientific Product, Inc.) for shaking at the speed of 400 RPM for 10 minutes. The resulting solution was finally placed into an Orbit Environ Shaker (Lab-Line Instruments, Inc) for continuous shaking at a speed of 100 RPM at a specifically controlled temperature. Sterile water (sterile either distilled water or river water) was obtained by placing the water samples in an autoclave (SterilMatic, Market Forge Industries, Inc.) at 121.1°C for 15 minutes.

A water sample of 10 mL was taken from the aqueous solution at a specific period of time and extracted with methylene chloride (20 mL) in a 250 mL separatory funnel. The two liquids in the funnel were vigorously shaken by hand for at least 100 cycles (1 to 2 minutes). The mixed liquids were settled for at least 10 minutes. The organic phase (or extract at the bottom) was collected. The procedure was repeated for the second and the third extractions and the extract from each extraction was collected into the same flask. After the third extraction was completed, the combined extract in the flask was shaken to make sure it was mixed well. The total volume of the combined extract was measured with a graduated cylinder. In addition, 1 mL of the extract was transferred into a 1.5 mL vial with cap for the determination of malathion concentration by GC/MS. The recovery efficiency of malathion with the extraction technique was 102.7±13.8%.

A Hewlett Packard (HP) 5890 series II gas chromatograph equipped with 5972 series Mass Selective Detector and Agilent 6890 series auto injector was used to determine the concentration of malathion. A DB-5 capillary column (0.25 mm I.D. x 30 m) was obtained from J & W Scientific (Folsom, CA). Flow rate of the mobile phase (helium) was set at 0.8 mL/min. The injection volume was 1μL and the splitless model was employed. The operation temperature of the column was programmed as follow: 50°C (2 minutes) - 180°C (1 minute) - 240°C (8 minutes). The temperature ramp for the range from 50°C to 180°C was 15°C/min and the second one from 180°C to 240°C was 12°C/min. The temperatures of injection port and detector (transfer line) were set at 250°C and 300°C, respectively. Mass signals were detected by the MS detector with scan model. Quantitative analysis of malathion in methylene chloride was carried out by the external standard

method. The concentration was determined on the basis of peak area and results were reported based on four replicate samples.

Statistical software SPSS (version 11.0) was applied to analyze and compare the kinetic rates of malathion transformation in the sterile distilled water and sterile river water at 25°C and 37°C, respectively. Differences (t-test) at $p < 0.05$ are considered significant.

RESULTS AND DISCUSSION

The transformation of malathion with initial concentrations of 0.3 mM in aqueous solutions at 25°C was investigated under three conditions: sterile distilled water, sterile river water and non-sterile river water. The retention time of malathion in the GC column was 16.41±0.01 minutes. The changes of malathion concentration in aqueous solutions were expressed as the ratios of the concentration at a specific time (C_t) to its initial concentration (C_o). The results are shown in Fig.1. It indicates that malathion transformation in the non-sterile river water was the fastest, with half-life of less than two days. The transformation in the sterile distilled water was the slowest with half-life of about ten days. It took about eight days for malathion to be transformed by 50% in the sterile river water.

At 37°C, malathion transformation in the sterile distilled water, sterile river water and non-sterile river water followed a similar pattern to that at 25°C. As shown in Fig. 2, over 50% of malathion was transformed in the non-sterile river water in one day. As for the sterile river water, it took about two days to transform 50% of the malathion. The transformation in the sterile distilled water took longer time.

The sterile distilled water should contain no microorganisms and, theoretically, malathion in the distilled water was transformed at neutral pH. Sterile river water still has dissolved organic and inorganic compounds as well as dead microorganism cells. The change of malathion concentration in the sterile river water vs sterile distilled water would reflect the effect of the matter dissolved in water on malathion transformation. In the non-sterile river water, experiments were carried out within 2 hours from the collection time of river water. The river water was considered initially fresh and contained microorganisms and dissolved matter. The major microorganisms included fungi and bacteria. They could be further divided into aerobic and anaerobic fungi or bacteria. Under the operation conditions, no additional oxygen was supplied for aerobic microorganisms during the experimental periods. The effect of aerobic microorganisms on malathion transformation was more significant during the initial period. After this short period, biodegradation was assumed to be dominated by the anaerobic microorganisms. For the transformation at 25°C, there was no significant difference among these three aqueous solutions within the first 24 hrs. In agreement with the previous report (HSDB 2000), biodegradation was not significant until one day later. At 37°C, however, biodegradation quickly became a significant transformation factor in less than one day.

As shown in Fig. 3, the pH decreased during malathion transformation in aqueous

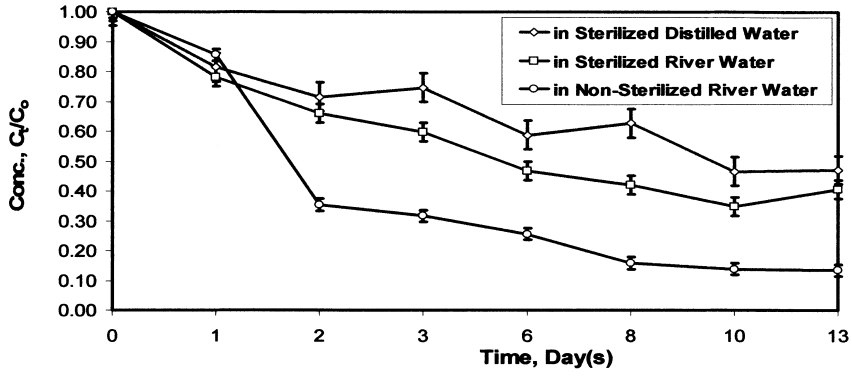

Figure 1. Changes of concentration during malathion transformation at 25°C.

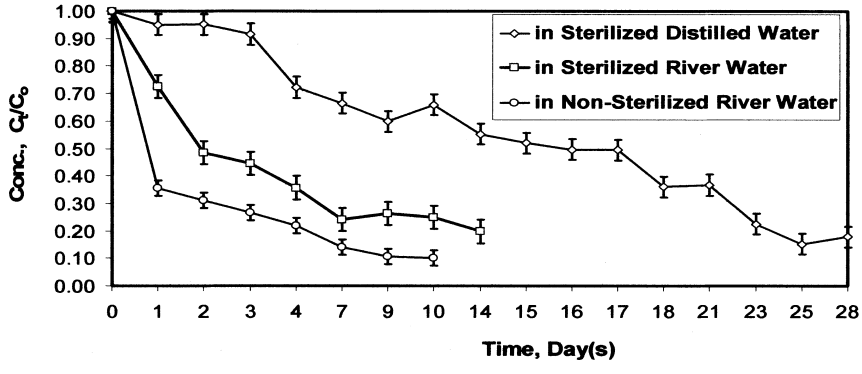

Figure 2. Changes of concentration during malathion transformation at 37°C.

solutions at 25°C. When over 50% of malathion was transformed, the pH value decreased by less than 0.5 unit in the non-sterile river water and over 1 unit in the sterile river water. As shown in Fig. 4, the pH change was less drastic at 37°C than that at 25°C when 50% of malathion was transformed in either the non-sterile river water or the sterile river water. It was reported that during the transformation of malathion in water, the major metabolites of malathion included malathion α- and β- mono- carboxylic and malathion dicarboxylic acid (Lalah and Wandiga 2002; Howard 1991). Without buffering the aqueous solutions, the pH of the water containing malathion would be below 7 and would become lower and lower while transformation of malathion proceeded. Fig. 3 and Fig. 4 confirm the trend.

However, the rate of pH change decreased when the pH value approached 4, which indicated that the transformation rate became slower at pH below 4 (Wolfe et al. 1977). If malathion transformation in water is first order with respect to malathion concentration, the differential equation of the kinetics can be expressed as: $- dC_t/dt = kC_t$. Its integration form is $\{-LN [C_t/C_o]\} = k\ t$. C_o and C_t are the concentrations of malathion in water at the time t (= 0) and t (=t), respectively. k is the rate constant. The half-life is expressed as: $t_{1/2} = 0.693/k$. The first order rate

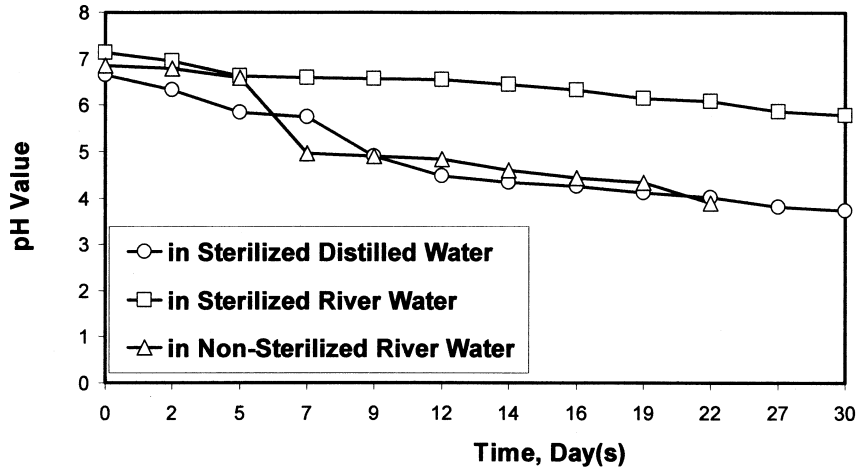

Figure 3. pH change during malathion transformation in water at 25°C.

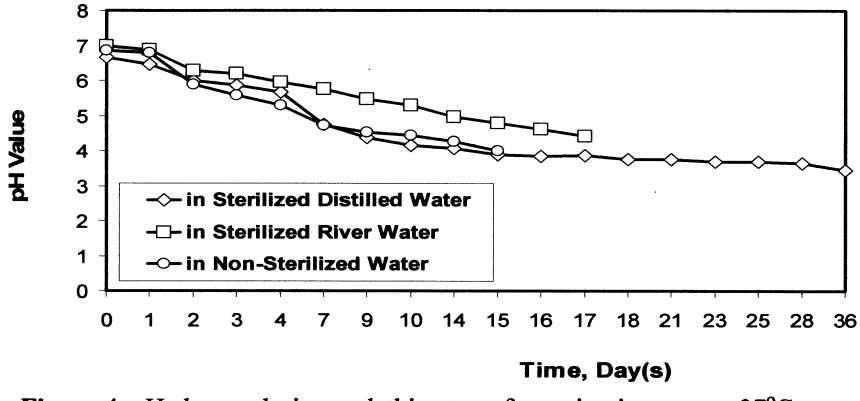

Figure 4. pH change during malathion transformation in water at 37°C.

constant can be calculated according to the integration equation. In addition, the rate constant can be obtained by plotting $[C_t/C_o]$ against time on a semi-logarithm scale. By this method, the rate constant is the slope of the straight line.

As shown in Fig. 5, the plot of $[C_t/C_o]$ vs time on a semi-logarithm scale yielded straight lines for malathion transformation at 25°C in the sterile distilled water and sterile river water, respectively. It indicates that malathion transformation can be described by first order kinetics. The calculated rate constants were 0.051±0.009 day^{-1} in the sterile distilled water and 0.072±0.010 day^{-1} in the sterile river water, respectively. Similarly, Fig. 6 indicates that malathion transformation at 37°C in the sterile distilled water and sterile river water may be assumed first order. The first order rate constants in the sterile distilled water and in the sterile river water were calculated as 0.055±0.012 day^{-1} and 0.109±0.019 day^{-1}, respectively. At 25°C, the rate of malathion transformation in the sterile distilled water was

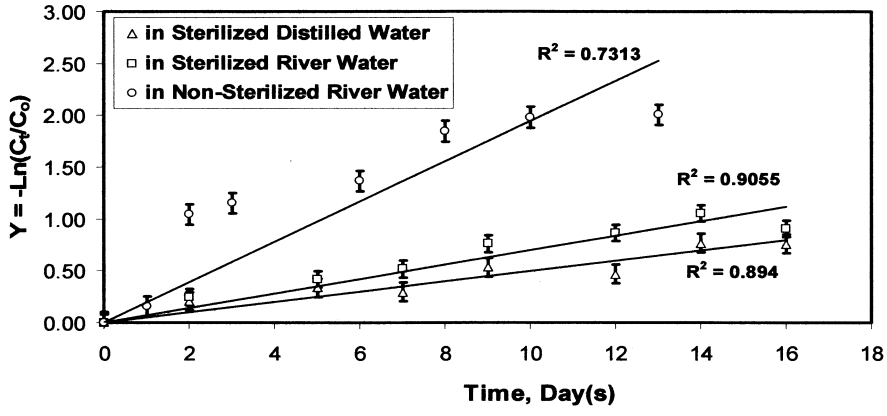

Figure 5. Kinetic orders of malathion transformation in water at 25°C.

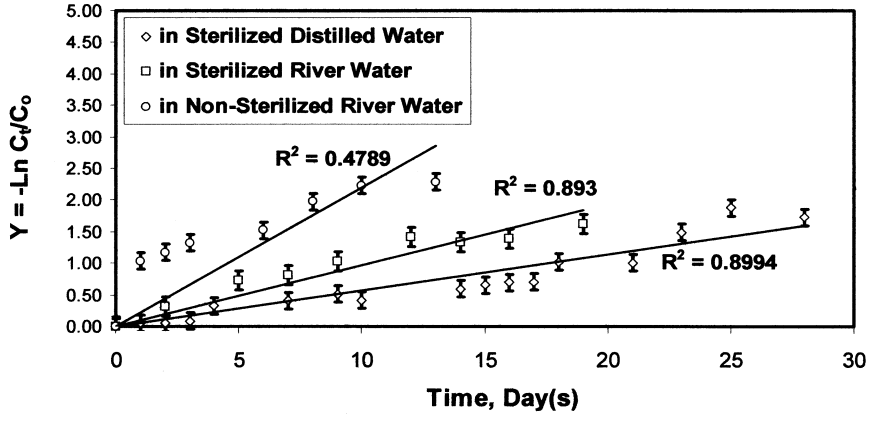

Figure 6. Kinetic orders of malathion transformation in water at 37°C.

significantly different from that in the sterile river water (t-test; $p < 0.05$). Similarly, the transformation rate of malathion in the sterile distilled water at 37°C was significantly different from that in the sterile river water (t-test; $p < 0.05$). Fig. 5 and Fig. 6 also indicated that, at 25°C and at 37°C, malathion transformation in the non-sterile river water did not fit first order kinetics because of the low correlation coefficients ($R^2 = 0.4789$ at 37°C and 0.7313 at 25°C). The effect of microorganisms in the river water on malathion transformation could be considerable and thus the kinetics deviated from the first-order pattern. In fact, malathion transformation in the non-sterile river water occurs by a combination of biological and non-biological reactions (Howard 1991). Malathion undergoes transformation readily in both bacterial and fungal cultures (Guerant et al.1970). Therefore, both hydrolysis and biodegradation could be important forcess for malathion transformation. Transformation of malathion in batch cultures of bacteria would follow second order kinetics (Wolfe et al. 1980). Without further supply of additional oxygen during the transformation, the rate of malathion transformation decreased greatly after that initial period but the rate in non-sterile river water was still higher than that in the sterile distilled water,

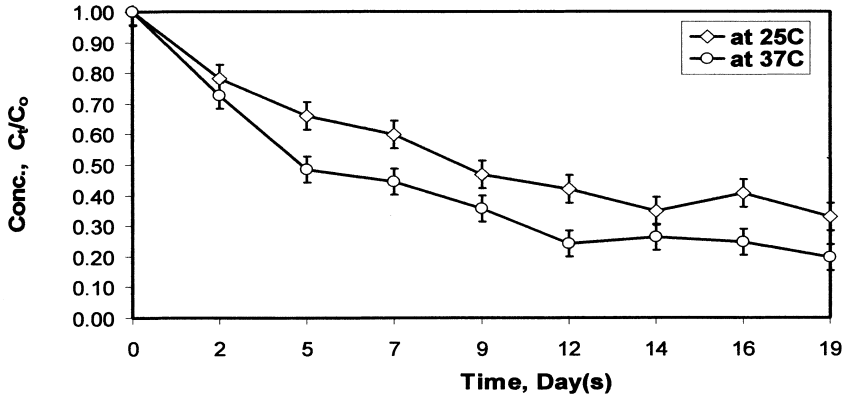

Figure 7. Effect of temperature on malathion transformation in the sterile river water.

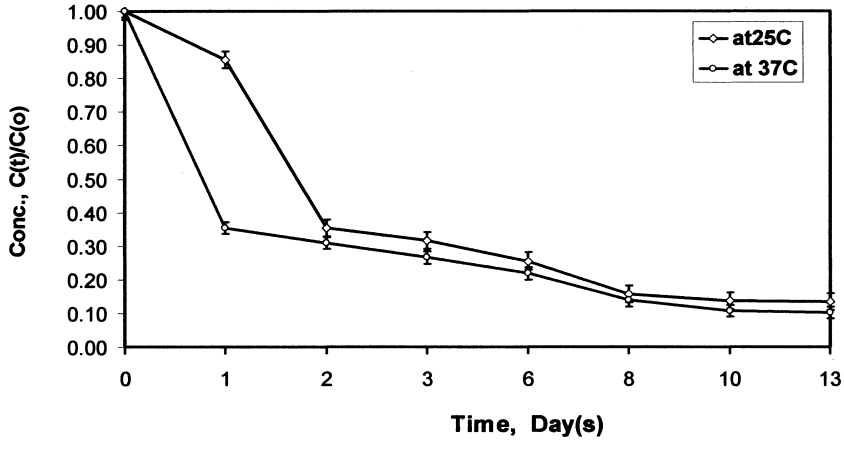

Figure 8. Effect of temperature on malathion transformation in the river water.

which suggests that significant microbial degradation of malathion in the river water could be a combination of aerobic and anaerobic degradations.

Both the matter dissolved in river water and microorganisms would greatly enhance the transformation of malathion in water. The matter dissolved in river water would include mainly inorganic and organic compounds, dead cells and some suspended solids. What the specific chemical in water is and how it affects the degradation of malathion is not clear. Further investigation is needed and it cannot be discussed in detail in this study. In comparison with malathion hydrolysis in the sterile distilled water, the matter dissolved in the river water and microorganisms reduced the time needed to transform 50% malathion at 25°C in water by 50 % and 35%, respectively.

Temperature is an important factor not only for the hydrolysis reaction but also for microbial degradation of malathion in water. Increasing temperature increased

the transformation rate of malathion in either the sterile or the non-sterile river water, as shown in Fig. 7 and Fig. 8. By increasing the temperature from 25°C to 37°C, the transformation rate of malathion increased by 6% in the sterile distilled water and by 51% in the sterile river water, respectively. For malathion transformation in the non-sterile river water, the time needed to transform 50% of malathion was reduced by up to 50% with the temperature increasing from 25°C to 37°C. The results obtained suggest that, in the natural environment, malathion in water would persist longer in cold weather than in warm weather regions.

Acknowledgments This research was supported by the U.S. Department of the Army Research and Development grant # W912H2-04-2-0002 to Jackson State University.

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