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# Enantiomeric Resolution and Biotoxicity of Methamidophos

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Despite the fact that the biological processes of chiral pesticides are enantioselective, the biotoxicity of methamidophos with respect to enantioselectivity has so far received limited research. In this study, the enantiomeric separation and biotoxicity of the two enantiomers of methamidophos were investigated. Successful enantioseparation of methamidophos using high-performance liquid chromatography on a Chiralcel OD column was achieved. As indicated by the optical rotation and circular dichroism detection, (+)-methamidophos was eluted prior to (−)-methamidophos. The *t* test at the 95% level of confidence indicated significant differences between the enantiomers in their in vitro inhibition toward acetylcholinesterases of bovine erythrocytes and *Electrophorus electricus* and in vivo acute aquatic toxicity to *Daphnia magna*. The in vitro assays showed that (−)-methamidophos was about 8.0−12.4 times more potent to the enzymes than its (+)-form. In contrast, the (+)-enantiomer was 7.0 times more toxic to *D. magna* in 48 h tests. The toxicity of racemic methamidophos was intermediate in both in vitro and in vivo bioassays. These results suggest that the biotoxicity of chiral OPs to nontargeted organisms is enantioselective and therefore should be reevaluated with their pure enantiomers.

**KEYWORDS: Chiral pesticide; methamidophos; enantiomeric separation; enantioselective toxicity**

## INTRODUCTION

Chirality is an important concept in synthetic agrochemicals because enantioselectivity occurs not only in their insecticidal activities to targeted organisms but also in their toxicity to nontargeted organisms. About 25% of pesticides in current use are chiral, and this percentage is still increasing as more pesticides with complex structures are introduced into application (*1*). Organisms constitute a chiral environment, and most enzymatic processes are stereospecific. It is thus common to find enantiomeric selectivity for chiral pesticides in many biological activities such as toxicity (*2*, *3*), endocrine disruption (*4*), and environmental fate (*5*−*8*).

Organophosphorus pesticides (OPs), first introduced in the 1950s for use on fruits, vegetables, and other crops, are often chiral. For example, of the 70 OP standards available from a popular commercial source of standard pesticides, 30 are chiral. Both the phosphorus and carbon atoms may be the chiral centers (*8*). Today, OPs are still marketed in their racemic forms, that is, an equimolar mixture of enantiomers. It is thus important to assess the environmental safety of individual enantiomers of OPs, which has been made possible with the development of chiral separation technology. The enantiomers of some chiral OPs have so far been successfully resolved by high-performance liquid chromatography (HPLC) involving the use of different chiral stationary phases (CSPs) (*9*, *10*). Liu et al. reported that the (−)-forms of fonofos and profenofos were about 10 times more toxic to *Ceriodaphnia dubia* (*C. dubia*) and *Daphnia magna* (*D. magna*) than their corresponding (+)-forms (*3*). The acute aquatic toxicity of fenamiphos and leptophos to *D. magna* was also found to be enantioselective, with the (+)-forms of the two OPs being more toxic than their corresponding (−)-forms (*11*, *12*). Presently, the separation, analysis, and toxicity of testing of pesticide enantiomers remain a challenging task.

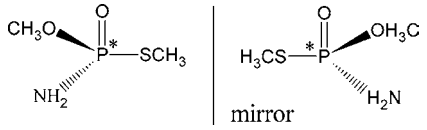


**Figure 1.** Enantiomers of methamidophos (* indicates chiral center).

Methamidophos [(*RS*)-*O*,*S*-dimethyl phosphoramidothioate] with an asymmetric center at the phosphorus atom and hence containing one pair of enantiomers (**Figure 1**) is one of the high-toxicity and broad-spectrum OPs. It is a potent acetylcholinesterase (AChE) inhibitor used to control chewing and sucking insects and spider mites on a variety of crops such as brassica, cotton, tobacco, sugar beet, lettuce, potatoes, and tree fruits (*13*). Methamidophos is highly toxic to aquatic organisms with the $LC_{50}$ (half-lethal concentration) of 25−51 mg $L^{-1}$ at 96 h for rainbow trout. Another 96-h toxicity test showed that a methamidophos concentration as low as 0.22 mg $L^{-1}$ was lethal to larval crustaceans (*14*). With a high water solubility (>2000 g $L^{-1}$ at 25 °C), methamidophos residue in the environment was thought to be a potential groundwater con-

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taminant (*15*). As a chiral compound, the (+)-enantiomer of methamidophos was about 6.3 times more potent to houseflies than its (−)-form (*16*). The lack of information on the enantioselectivity and environmental behavior of methamidophos, however, warrants further studies.

In this study, the enantiomeric separation and biotoxicity of methamidophos were investigated. Enantiomer resolution was conducted using HPLC with a Chiralcel OD column. The biotoxicity of the resolved enantiomers and the racemate was assayed by in vitro inhibition on the AChE of bovine erythrocytes (BE-AChE) and *Electrophorus electricus* (EE-AChE) and by in vivo acute aquatic toxicity to *D. magna* under static conditions. As current information on methamidophos is largely derived from its racemic form, the data from this study may significantly contribute to a more comprehensive understanding of the ecotoxicological risk posed by its individual enantiomers.

## MATERIALS AND METHODS

**Chemicals.** Analytical standard of racemic methamidophos (purity > 99.0%) was obtained from Kefa New Technology Development Co. (Shenyang, China). Acetylthiocholine iodide (ATCh-I), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), and acetylcholinesterases of bovine erythrocytes (BE-AChE, type XII-S) and *E. electricus* (EE-AChE, type V-S) were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals and solvents were of analytical or HPLC grade. Working solutions of the enzymes were made in 0.1 M potassium phosphate buffer (pH 8.0), in which the hydrolysis rates of ATCh-I were approximately 0.05−0.10 absorbance unit/min (*17*). Methamidophos was dissolved in mobile phase at 1000 mg $L^{-1}$.

**Chromatographic Conditions and Resolution of Enantiomers.** Enantiomer separation was performed on a Jasco LC-2000 series HPLC system (Jasco, Tokyo, Japan) equipped with a PU-2089 quaternary gradient pump, a mobile phase vacuum degasser, an AS-1559 autosampler with a 100-$\mu$L loop, a CO-2060 column temperature control compartment, a variable-wavelength CD-2095 circular dichroism (CD) detector, an OR-2090 optical rotation (OR) detector, and an LC-Net II/ADC data collector. Chromatographic data were acquired and processed with the computer-based ChromPass software (version 1.7.403.1, Jasco). Separation was achieved at 25 °C on a Chiralcel OD column (250 mm × 4.6 mm) with the enantioselective phase [cellulose tris(3,5-dimethylphenyl carbamate)] coated onto a 5-$\mu$m silica gel substrate. The injection volume was 20 $\mu$L. The flow rate of the mobile phase was 0.5 mL $min^{-1}$. The detection wavelength of CD was set at 230 nm. The specific rotation of enantiomers was determined by the on-line OR-2090 chiral detector. The light source for the chiral detector was a 150-W Hg−Xe lamp, and the tapered cell path was 25 mm with a volume of 44 $\mu$L. The rotation sign ("+" or "−") was indicated by a positive or negative peak on the chromatogram.

The resolved enantiomers in the mobile phase were manually collected at the HPLC outlet of the CD detector during the observation of the UV absorbance. Individual enantiomer solutions were evaporated to dryness, redissolved in a known volume of acetone, and used as the stock solutions for biotoxicity assays (the final amount of acetone in assay solution was <0.04%). The concentrations of enantiomers were determined by assuming the same response factor for enantiomers as for the racemate and also by analyzing an aliquot on a gas chromatograph coupled with a nitrogen−phosphorus detector (GC-NPD).

**Assay for AChE Inhibition.** *Theoretical Consideration.* It is recognized that the acute toxicities of OPs are due to their inhibition toward AChE. The inhibition mechanism can be chemically described as (*18*)

$$\text{Enz–OH} + \text{OP–X} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} [\text{Enz–OH·OP–X}] \xrightarrow{k_p} \text{Enz–OP} + \text{X}^- \qquad (1)$$

$$\text{Enz–OH} + \text{OP–X} \xrightarrow{k_i} \text{Enz–OP} + \text{X}^-$$

where X represents the leaving group in OP and $k_+$, $k_-$, $k_p$, and $k_i$ are the rate constants of association, dissociation, phosphorylation, and bimolecular inhibition, respectively. The dissociation constant of the intermediate complex [Enz−OH·OP−X], $K_d$, is defined as

$$K_d = \frac{k_-}{k_+} \qquad (2)$$

The constant of bimolecular inhibition, $k_i$, quantifies the inhibitory power of an OP toward AChE. The relationship between the rate constants is (*19*)

$$k_i = \frac{k_p k_+}{k_-} = \frac{k_p}{K_d} \qquad (3)$$

If the lowest concentration of OPs remains much higher than that of the enzyme, reaction *1* follows first-order kinetics over a wide range of inhibitor concentrations. For reaction *1*, the following relationship exists (*18*):

$$\ln\frac{[E_r]}{[E_0]} = -\frac{k_p t}{1 + K_d/[I]} \qquad (4)$$

In eq 4 $[E_0]$ is the initial activity of AChE, $[E_r]$ is the remaining activity at the time of measurement, $t$ is the reaction time, and $[I]$ is the concentration of inhibitor, that is, OPs. According to Main (*19*), an apparent phosphorylation rate constant, $k_{app}$, may be calculated for different concentrations of inhibitor by using the following equation:

$$k_{app} = -\ln\frac{[E_r]}{[E_0]}/t \qquad (5)$$

$$k_{app} = \frac{k_p}{1 + K_d/[I]} \qquad (6)$$

Thus Equation 5 indicates that $k_{app}$ can be obtained by the linear regression of $\ln[E_r]/[E_0]$ versus the incubation time for each $[I]$. Equation 6 can be converted to the following linear form:

$$\frac{1}{k_{app}} = \frac{1}{k_p} + \frac{1}{k_i} \times \frac{1}{[I]} \qquad (7)$$

By plotting the reciprocal of $k_{app}$ versus the reciprocal of $[I]$, $k_p$ and $k_i$ can be obtained from the slope and intercept of the linear line.

As an indicator of enzyme sensitivity to inhibitor, $IC_{50}$ (the concentration of inhibitor producing half-inhibition of enzyme activity) in 30 min can be calculated using the following equation (*20*):

$$IC_{50} = \frac{\ln 2}{k_i \times 30} \qquad (8)$$

*Enzyme Assay.* The in vitro inhibition of AChE activities was evaluated using two enzymes, BE-AChE and EE-AChE. The AChE inhibition rate constants were obtained on the basis of the equations proposed by Kitz and Wilson (*18*) and Main (*19*) and the method described by Filho et al. (*21*). Briefly, test solutions (20 $\mu$L) at various concentrations of each enantiomer that could inhibit enzyme activity by 10−90% were added into 500-L centrifuge tubes, followed by the addition of 180 $\mu$L of AChE solution. The mixture was incubated at 37 °C. Meanwhile, control samples were also prepared by use of 20 $\mu$L of phosphate buffer (pH 8.0) in place of test solution, and $[E_0]$ was measured. At 2, 4, 6, 8, and 10 min, 20 $\mu$L of the AChE-inhibitor solution was taken to measure $[E_r]$. The AChE activity was spectrophotometrically determined at 37 °C with a Bio-Rad model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA) according to a published procedure (*22*). Briefly, 210 $\mu$L of DTNB solution and 20 $\mu$L of ATCh-I solution were added to the wells on a 96-well microtiter plate. Enzyme standard or enzyme−inhibitor (20 $\mu$L) was subsequently added to make the final concentrations of DTNB and ATCh-I at 0.28 and 0.5 mM, respectively. The enzymatic activities of these mixtures were determined at 405 nm for 5 min at intervals of 1 min from the

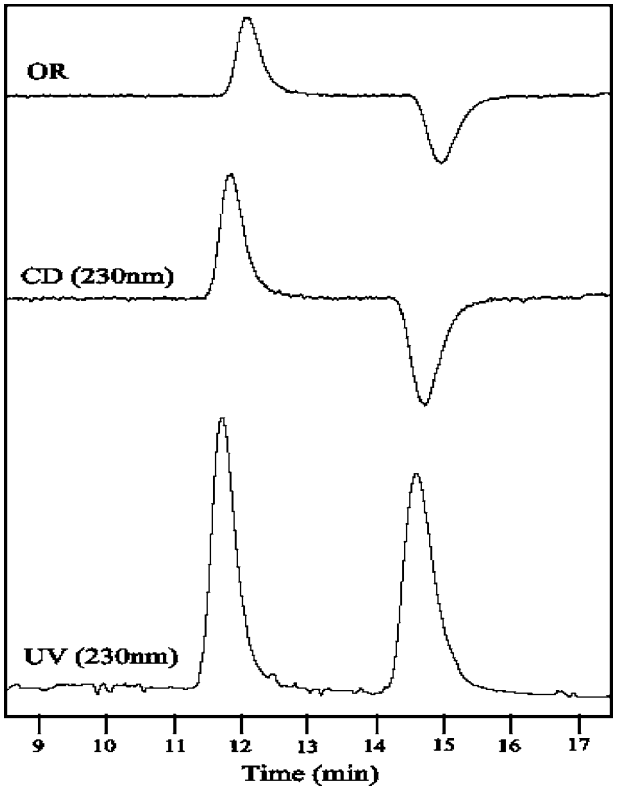


**Figure 2.** HPLC chromatograms for enantiomeric separation of methamidophos on the Chiralcel OD column.

addition of enzyme. All of the above tests and measurements were performed in four replicates.

**Acute Aquatic Toxicity.** Enantioselectivity of aquatic toxicity was evaluated through in vivo assay using *D. magna*. The test organisms were obtained from continuous culture maintained at 22 ± 1 °C in M4 culture medium (*23*) with a photoperiod of 12 h/day and a density of <50 animals per liter. Stock organisms were originally obtained from the Chinese Academy of Protection and Medical Science. The medium was renewed three times a week, and daphnids were fed daily with the alga *Scenedesmas obliquus*, which were cultured in the laboratory using a nutrient medium. Offspring were separated at regular intervals. The test animals used in this experiment were juveniles aged between 6 and 24 h. Prior to testing, a sensitive test for daphnids to potassium dichromate ($K_2Cr_2O_7$) was performed as a positive control, and the $LC_{50}$ (24 h) value was in the range of 0.6−1.7 mg $L^{-1}$. The overall acute toxicity test was conducted according to the standard protocol (*24*). Briefly, five neonates were transferred into glass beakers filled with 20 mL of blank or test solutions of various enantiomer concentrations. Four replicates for each treatment were prepared. The test animals were not further fed and were incubated at 22 ± 1 °C for 48 h. Mortality of daphnids was observed after incubation for 24 and 48 h.

## RESULTS AND DISCUSSION

**Enantiomer Resolution.** Baseline separation of methamidophos enantiomers was obtained on a Chiralcel OD column with a mobile phase of *n*-hexane/isopropanol (80:20, v/v) at the flow rate of 0.5 mL $min^{-1}$. A typical chromatogram is shown in **Figure 2**. The separated enantiomers can be easily distinguished with positive or negative peaks in the OR and CD chromatograms. (+)-Methamidophos (peak I) was eluted prior to its (−)-form (peak II). According to Miyazaki et al. (*16*), it was confirmed that peak I was $(R)_P$-(+)-methamidophos and peak II $(S)_P$-(−)-methamidophos. The CD chromatogram offers further information that $(R)_P$-(+)-methamidophos had a positive signal and the *S*-enantiomer had a negative signal at 230 nm. The methamidophos enantiomers were also previously resolved on other CSPs (*9*, *10*). However, the chiral recognition remains unclear. Because of the complexity in chiral recognition mechanisms, we did not attempt to elucidate any structural features of methamidophos or CSP that correlated with this result. The developed method was used to obtain the pure enantiomers of methamidophos for subsequent biotoxicity assays. The excellent enantioseparation warranted the sufficient purity of the enantiomers collected at the outlet of HPLC for biotoxicity assays.

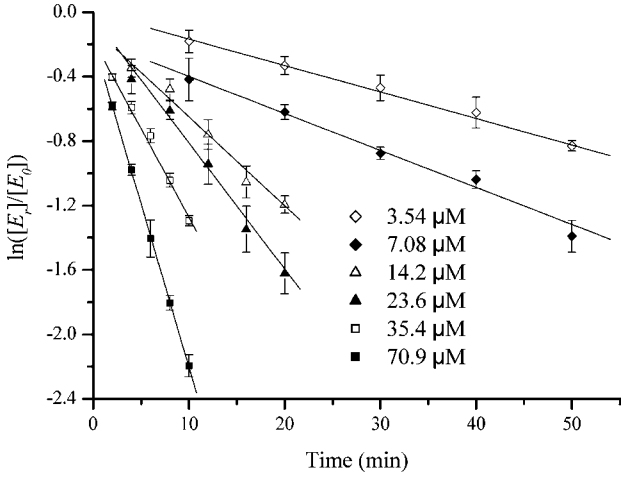


**Figure 3.** Typical plots for the determination of $k_{app}$ in BE-AChE with various concentrations of (±)-methamidophos.

**Enantioselectivity in AChE Inhibition.** According to eq 5, plotting $\ln[E_r]/[E_0]$ versus incubation time yields linear lines, as illustrated in **Figure 3** for (±)-methamidophos inhibition of BE-AChE. The slopes of these lines were readily determined to obtain the $k_{app}$ values. Similarly, the plots and $k_{app}$ values were also obtained for (±)-methamidophos inhibition on EE-AChE and for individual enantiomer inhibition of both enzymes (plots not shown). Subsequently, linear lines of $1/k_{app}$ values for various concentrations versus $1/[I]$ were plotted, as shown for BE-AChE and EE-AChE (**Figure 4**). According to eq 7, the rate constants were calculated as $k_i$ = 1/slope, $k_p$ = 1/*Y*-intercept, and $K_d = k_p/k_i$. The $IC_{50}$ values were calculated from $k_i$ values using eq 8. The rate constants and $IC_{50}$ values are presented in **Table 1**.

The *t* test at the 95% level of confidence was used to compare individual $IC_{50}$ data. Significant differences were observed in $IC_{50}$ between the (+)- and (−)-enantiomers for both enzymes (**Table 1**). As a lower $IC_{50}$ indicates a more potent inhibition, the $IC_{50}$ values manifest that (−)-methamidophos was a stronger inhibitor than (+)-methamidophos and the racemate. The $IC_{50}$ values suggest that to both enzymes (−)-methamidophos was 8.0−12.4 times stronger than its (+)-form.

Examination of the rate constants in **Table 1** offers an insight into the inhibitory process. The observed enantioselectivity appears to occur in the association process between the inhibitor and the enzyme. Taking the EE-AChE inhibition as an example, the $K_d$ value for (+)-methamidophos was 13.6 times that for the (−)-enantiomer. The (−)-methamidophos was thus preferentially bound to EE-AChE as compared to its (+)-enantiomer. Although the cause for this is not clear, this result is consistent with the association of other phosphates with AChE (*25*, *26*). By comparison, the $k_p$ values remained relatively constant for the racemate and enantiomers, indicating that the phosphorylation in this case was only slightly or not enantioselective. As $k_i$ reflects a comprehensive influence of both association and phosphorylation, it is not surprising to observe the enan-

**Table 1.** Inhibition Parameters for Enantiomers and Racemate of Methamidophos against BE-AChE and EE-AChE at 37 °C

| | BE-AChE | | | EE-AChE | | |
|---|---|---|---|---|---|---|
| | (+) | (±) | (−) | (+) | (±) | (−) |
| $IC_{50}$,[a,b] mg $L^{-1}$ | 2.26 ± 0.22 | 0.73 ± 0.06 | 0.28 ± 0.03 | 1.49 ± 0.12 | 0.29 ± 0.02 | 0.12 ± 0.00 |
| $k_i$,[b] $M^{-1}$ $min^{-1}$ | 1.46 ± 0.14 | 4.50 ± 0.38 | 11.82 ± 1.14 | 2.20 ± 0.18 | 11.32 ± 0.62 | 27.72 ± 0.33 |
| $K_d$,[b] $10^{-5}$ M | 21.20 ± 6.78 | 6.75 ± 0.18 | 4.47 ± 0.64 | 19.20 ± 5.24 | 3.98 ± 0.34 | 1.41 ± 0.30 |
| $k_p$,[b] $min^{-1}$ | 0.30 ± 0.09 | 0.30 ± 0.06 | 0.50 ± 0.02 | 0.42 ± 0.09 | 0.45 ± 0.02 | 0.39 ± 0.08 |
| $r$[c] | 0.99 | 0.98 | 0.97 | 0.99 | 0.99 | 0.99 |

[a] $IC_{50}$ in 30 min. [b] All values are mean ± standard deviation of the mean ($n = 4$). [c] Correlation coefficient of determination of the replot for $k_i$ calculation.

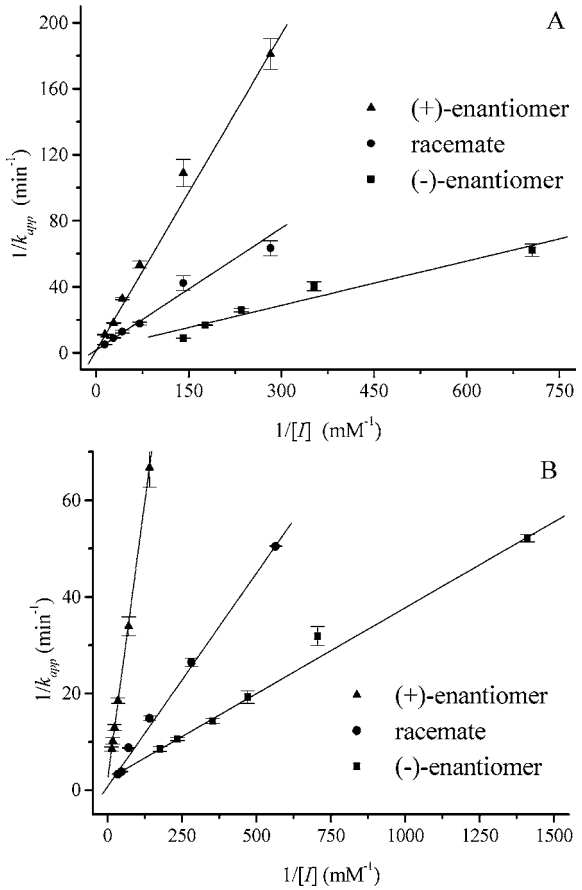


**Figure 4.** Double-reciprocal plot of $k_{app}$ versus [*I*] in the inhibition of (**A**) BE-AChE and (**B**) EE-AChE for enantiomer and racemic methamidophos.

**Table 2.** $LC_{50}$ for Racemate and Enantiomers of Methamidophos to *D. magna* (Micrograms per Liter)

| test time | (±)-methamidophos | (+)-methamidophos | (−)-methamidophos |
|---|---|---|---|
| 24 h | 108.7 ± 10.3 | 70.7 ± 0.0 | 277.1 ± 11.4 |
| 48 h | 34.0 ± 5.2 | 33.8 ± 3.6 | 237.9 ± 14.5 |

tioselectivity in $k_i$ (**Table 1**). Similar results of the inhibitory mechanism can be derived for BE-AChE. It is thus concluded that the enantioselective inhibition of methamidophos toward BE-AChE and EE-AChE arose primarily from the preferential binding of the (−)-enantiomer relative to the (+)-enantiomer.

**Enantioselectivity in Aquatic Toxicity.** The acute aquatic toxicity of methamidophos racemate and individual enantiomers to *D. magna* was determined through 24- and 48-h static tests. The $LC_{50}$ values are listed in **Table 2**. The *t* test indicated significant differences in $LC_{50}$ between the two enantiomers, with the (+)-enantiomer being 7.0 times more toxic than its (−)-form in 48-h tests. This result is consistent with that from an in vivo assay by Miyazaki et al. that (+)-methamidophos was more potent to houseflies (*16*). However, it is inconsistent with the enzyme assays reported earlier where the (−)-enantiomer was a stronger inhibitor to two test enzymes. The causes for the opposite toxicity observed using the two different biological targets are not clear, but may be attributed to one or both of the following two factors. First, the enzymes of different species may have opposite sensitivities to the two enantiomers. In addition, the in vivo toxicity of enantiomers is the result of a complex biological process that may be influenced by many other life activities such as transportation, transformation, assimilation, accumulation, and metabolism in the organism. Stereospecificity in one or more of these processes may result in different amounts of each enantiomer reaching the active sites of target enzymes (*27*).

The enantioselective biotoxicity also varies among chiral OPs. Similar to our findings, the in vivo toxicities of fenamiphos (*11*) and leptophos (*12*) to *D. magna* and of acephate (*16*) to houseflies all arose primarily from their (+)-enantiomers. In contrast, the toxicity to *C. dubia* and *D. magna* of other two chiral OPs, fonofos and profenofos, was contributed primarily from their (−)-enantiomers (*3*). It is generally true that the biotoxicity of most chiral OPs is enantioselective and that only one of the enantiomers contributes significantly to the toxicity of their racemates.

**Conclusions.** Racemic methamidophos can be enantiomerically resolved on a chiral HPLC column, and pure enantiomers can be successfully obtained. This work reveals that the biotoxicity of methamidophos to BE-AChE (in vitro), EE-AChE (in vitro), and *D. magna* (in vivo) is enantioselective. The significant enantioselectivity of methamidophos observed in AChE inhibition and aquatic toxicity suggests that enantiomers need to be individually assessed for their environmental safety to evaluate the overall toxicity of chiral OPs. The inconsistent toxicity of enantiomers between in vitro and in vivo bioassays suggests that the biological activities of chiral OPs to nontargeted organisms should be evaluated by both in vitro and in vivo tests. Because most chiral pesticides are still marketed as racemates, more comprehensive studies are needed to evaluate their enantioselective environmental behaviors.

## LITERATURE CITED

(1) Williams, A. Opportunities for chiral chemicals. *Pestic. Sci.* **1996**, *46*, 3−9.

(2) Liu, W. P.; Gan, J. Y.; Qin, S. J. Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. *Chirality* **2005**, *17* (S1), 127−133.

(3) Liu, W. P.; Gan, J. Y.; Schlenk, D.; Jury, W. A. Enantioselectivity in environmental safety of current chiral insecticides. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (3), 701−706.

(4) Hoekstra, P. F.; Burnison, B. K.; Neheli, T.; Muir, D. C. G. Enantiomer-specific activity of *o,p′*-DDT with the human estrogen receptor. *Toxicol. Lett.* **2001**, *125*, 75−81.

(5) Kohler, H. E.; Angst, W.; Giger, W.; Kanz, C.; Müller, S.; Suter, M. J. F. Environmental fate of chiral pollutants—the necessity of considering stereochemistry. *Chimia* **1997**, *51*, 947−951.
(6) Lewis, D. L.; Garrison, A. W.; Wommack, K. E.; Whittemore, A.; Steudler, P.; Melillo, J. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* **1999**, *401*, 898−901.
(7) Buser, H. R.; Müller, M. D.; Poiger, T.; Blamer, M. E. Environmental behavior of the chiral acetamide pesticide metalaxyl: enantioselective degradation and chiral stability in soil. *Environ. Sci. Technol.* **2002**, *36*, 221−226.
(8) Garrison, A. W. Probing the enantioselectivity of chiral pesticides. *Environ. Sci. Technol.* **2006**, *40*, 16−23.
(9) Wang, P.; Jiang, S. R.; Liu, D. H.; Zhang, H. J.; Zhou, Z. Q. Enantiomeric resolution of chiral pesticides by high-performance liquid chromatography. *J. Agric. Food Chem.* **2006**, *54*, 1577−1583.
(10) Ellington, J. J.; Evans, J. J.; Prickett, K. B.; Champion, W. L., Jr. High-performance liquid chromatographic separation of the enantiomers of organophosphorus pesticides on polysaccharide chiral stationary phases. *J. Chromatogr. A* **2001**, *928*, 145−154.
(11) Wang, Y. S.; Tai, K. T.; Yen, J. H. Separation, bioactivity, and dissipation of enantiomers of the organophosphorus insecticide fenamiphos. *Ecotoxicol. Environ. Saf.* **2004**, 57346−353.
(12) Yen, J. H.; Tsai, C. C.; Wang, Y. S. Separation and toxicity of enantiomers of organophosphorus insecticide leptophos. *Ecotoxicol. Environ. Saf.* **2003**, *55*, 236−242.
(13) WHO. *Methamidophos Health and Safety Guide 79*; World Health Organization: Geneva, Switzerland, 1993.
(14) Tomlin, C. *The Pesticide Manual, a World Compendium*, 10th ed.; British Crop Protection Council: Croydon, U.K., 1994.
(15) Yen, J. H.; Lin, K. H.; Wang, Y. S. Potential of the insecticides acephate and methamidophos to contaminate groundwater. *Ecotoxicol. Environ. Saf.* **2000**, *45*, 79−86.
(16) Miyazaki, A.; Nakamura, T.; Kawaradani, M.; Marumo, S. Resolution of biological activity of both enantiomers of methamidophos and acephate. *J. Agric. Food Chem.* **1988**, *36*, 835−837.
(17) Rodriguez, O. P.; Muth, G. W.; Berkman, C. E.; Kim, K.; Thompson, C. M. Inhibition of various cholinesterases with the enantiomers of malaoxon. *Bull. Environ. Contam. Toxicol.* **1997**, *58*, 171−176.
(18) Kitz, R.; Wilson, I. Ester of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. *J. Biol. Chem.* **1962**, *237*, 3245−3249.
(19) Main, A. Affinity and phosphorylation constant for the inhibition of esterases by organophosphates. *Science* **1964**, *144*, 992−993.
(20) Johnson, J. A.; Wallace, K. B. Species-related differences in the inhibition of brain acetylcholinesterase by paraoxon and malaoxon. *Toxicol. Appl. Pharmacol.* **1987**, *88*, 234−241.
(21) Filho, M. V. S.; Oliveira, M. M.; Salles, J. B.; Bastos, V. L. F. C.; Cassano, V. P. F.; Bastos, J. C. Methyl-paraoxon comparative inhibition kinetics for acetylcholinesterases from brain of neotropical fishes. *Toxicol. Lett.* **2004**, *153*, 247−254.
(22) Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88−95.
(23) Organization for Economic Cooperation and Development (OECD). *Report of the Final Ring Test of the Daphnia magna Reproduction Study*; 1995.
(24) ISO6341, 1996(E): Water quality—determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacca)—acute toxicity test; 1996.
(25) Kemp, J. R.; Wallace, K. B. Molecular determinants of the species-selective inhibition of brain acetylcholinesterase. *Toxicol. Appl. Pharmacol.* **1990**, *104*, 246−258.
(26) Fukuto, T. R.; Metcalf, R. L. The effect of structure on the reactivity of alkylphosphonate esters. *J. Am. Chem. Soc.* **1959**, *81*, 372−377.
(27) Konwick, B. J.; Garrison, A. W.; Avants, J. K.; Fisk, A. T. Bioaccumulation, biotransformation, and metabolite formation of fipronil and chiral legacy pesticides in rainbow trout. *Environ. Sci. Technol.* **2006**, *40*, 2930−2396.

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