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EFFECT OF ATRAZINE ON THE TOXICITY OF SOME ORGANOPHOSPHORUS INSECTICIDES TO *CHIRONOMUS RIPARIUS* (DIPTERA: CHIRONOMIDAE)

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ABSTRACT: The acute toxicity of two organophosphorothioates (profenofos and diazinon) and one organophosphorodithioate (dimethoate) insecticides were evaluated individually and in binary combination with the herbicide atrazine against the fourth-instar larvae of the aquatic midge, *Chironomus riparius*. Atrazine alone, up to 1000µg/L, did not show significant toxicity to the midges in a 24-h bioassay. However, the presence of atrazine at much lower concentrations (40-200µg/L) increased the toxicity of profenofos, and diazinon. Atrazine did not increase the toxicity of dimethoate. Atrazine by itself did not reduce the level of AChE activity, but in combination with profenofos significantly decreased AChE activity as compared to profenofos alone at the lowest concentration. Also, similar trends were existed for dimethoate and diazinon without significant differences between them. These results suggest that the enhanced inhibition of AChE by the lowest levels of OPs plus atrazine may be due to affecting one or more indirect mechanisms by atrazine.

Key words: **Atrazine, Toxicity, Organophosphorus, *Chironomus riparius***

INTRODUCTION

Atrazine is a selective pre- and post emergence triazine herbicide applied to many major food crops including corn, sorghum, and sugarcane as well as grassland and forestry. In spite of its extensive use, atrazine is considered to be relatively safe herbicide with a short half-life and insiginificant bioaccumulation and

biomagnification (Solomon *et al.* 1996; Pratt *et al.* 1997; Gianessi 1998). Organophosphorothioates (profenofos and diazinon) and organophosphorodithioate(dimethoate) are members of the highly diverse class of organophosphate (OP) insecticides (Matsumura 1985). They are extensively used in the urban and agricultural areas. Although these two types of OPs are structurally different, each is acutely toxic and is intended to interfere with normal cholinergic nerve transmission by inhibiting the enzyme acetylcholinesterase (AChE). Pharmacologically, organophosphorodithioate such as dimethoate require in *vivo* oxidation activation into more toxic, AChE-inhibiting O-analogs by cytochrome P450 monooxygenases in living organisms whereas the organophosphorothioates profenofos and diazinon do not. These two organophosphorothioates are O-analogs in their parent form (Chambers 1992; Egaas *et al.* 1993; Miota *et al.* 1999).

Pesticides applied to crops, lawns, and animals are commonly found in soil or water, and water contamination is often caused by more than one pesticide at relatively low concentrations. Recent reports have revealed that high dose of atrazine induce abnormalities and deformities in non-target organisms (Hayes *et al.* 2002). In addition, atrazine has been shown to act synergistically with the organophosphorothioates chlorpyrifos, and methyl parathion thereby increasing the toxicities of these OPs to the aquatic midge larvae *Chironomus tentans* and the aquatic amphipod *Hyalella azteca* (Jin-Clark *et al.* 2002; Anderson and Lydy 2002). These increases in toxicity were determined to be associated with decreased AChE activity in both organisms. Thus, atrazine may induce cytochrome P450 momnooxygenase in order to confer the synergistic effects on the toxicity of organophosphorothioates.

This study examined the structural features of organophosphorothioate and organophosphorodithioate insecticides in relation to the toxicity changes in the presence and absence of atrazine. Herein, we report: (1) the toxicities of the individual insecticide against the fourth-instar *Chironomus riparius* larvae, (2) the effects of atrazine on the toxicity of profenofos, diazinon and dimethoate to *Chironomus riparius*, (3) the AChE activity of *Chironomus riparius* exposed to binary combinations of atrazine and each of the tested OPs.

MATERIALS AND METHODS

Organisms

The fourth-instar larvae of the aquatic midge *Chironomus riparius* were originally obtained from the Department of Biological Sciences, Royal Holloway, University of London, UK. Midges were cultured in the laboratory according to the standard procedures (ASTM 1999).The strain has been maintained at the institute of Graduate Studies and Research, University of Alexandria since August 2000. The fourth-instar larvae were used for the pesticide bioassays as well as the enzyme activity assays (Ibrahim *et al.* 1998).

Chemicals

Profenofos [O-4-bromo-2-chlorophenyl O-ethyl *S*-propyl phosphorothioate] (99% purity), diazinon [O,O-diethyl O-2-isopropyl-6-methyl-4-pyrimidinyl phosphorothioate] (99%), dimethoate [O,O- dimethyl *S*-methylcarbamoylmethyl phosphorodithioate] (99%), and atrazine [2-chloro-4-ethylamino-6-isopropylamino-*S*-triazine] (99%) were obtained from Zeneca Agrochemicals Co., UK. Acetylthiocholine iodide (ATC), Foline ciocalteau solution, bovine serum albumin, 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB), and Triton X-100 were purchased from Sigma (Fancy Road, Poole, Dorset. BH12 4 QH, England).

Bioassays of the Tested Pesticides

The test procedures generally followed those outlined by the ASTM, 1999.The acute toxicity bioassays were performed for 24 h using fourth-instar *Chironomus riparius* larvae exposed to five concentrations of each pesticide. The appropriate dilutions of each pesticide were prepared in acetone. The pesticide was delivered by adding 100 µl of pesticide solution to 1-L reconstituted water containing 10 midges in a glass beaker. The same procedure was used to treat midges with corresponding concentrations of acetone in water as controls. Each beaker was aerated and maintained at 21± 2 °C. The endpoint for each bioassay was measured as an effective concentration (EC). The midges that were unable to perform an active movement upon gentle probing were considered as affected. Log-probit analysis (SAS Institute 1996) was used to estimate the toxic endpoint concentrations for each OP.

In order to assess the combined effect of atrazine and each OP insecticide, fourth-instar *Chironomus riparius* larvae were exposed to each OP at the EC1, EC5, EC15, and EC50 levels individually and in combination with atrazine treatments of 10, 40, 100, and 200µg/L. The pesticide exposure methods were the same as described above in the acute toxicity tests. Statistical comparisons were performed using SAS, 1991. Multiple comparisons using the Tukey Honestly significant difference test were used to detect significantly different treatment pairs.

In Vivo Inhibition of Acetylcholinesterase Activity

Acetylcholinesterase (AChE) activity was determined according to the method of Ellman *et al.* 1961. The larvae of fourth-instar *Chironomus riparius* were exposed to each OP at the EC1 level individually and in combination with a fixed concentration of atrazine (200µg/L). Additionally solvent controls (acetone) and positive controls OP concentrations corresponding to EC50 values) were performed to monitor baseline AChE activity and demonstrate that increased exposure resulted in change activity. The pesticide exposure methods were the same as described above in the acute toxicity tests. All surviving midges were collected from each beaker as a sample. Each sample was homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.1% (v/v) Triton X-100. The homogenates were centrifuged at 10,000g for 15 min at 4°C, and the supernatants were then used as the enzyme sources.

The concentration of total protein in each AChE preparation was determined using the method of Lowry *et al.* 1951 using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Toxicity of Pesticides and Their Binary Mixtures

Atrazine up to 1000µg/L was not acutely toxic to the midges under our bioassay conditions. At this atrazine level, less than 3% effect was observed which was similar to controls. Individual OP toxicity results are shown in Table (1). Profenofos was the most toxic compound, followed by dimethoate and diazinon. Although, atrazine at levels as low as 40 µg/L significantly increased

the toxicity of profenofos and diazinon. Analysis of variance results indicated that significant differences were present for both treatment classes for atrazine + profenofos mixtures (P <0.0001), and atrazine + diazinon mixtures (P <0.0001) (Table 2). Tukey's test results indicate that for profenofos, the addition of larger concentrations of atrazine significantly increased the greater than additive response (P <0.05). Higher concentrations of atrazine also increased the average percent effect for diazinon treatment, but the increases were not statistically significant. Both compounds were significantly affected by atrazine at 40 µg/L (P<0.05). Additional Tukey's tests were performed for comparing atrazine treatments with each OP level. These tests illustrated that atrazine affected the toxicity of profenofos and diazinon at each level of OP tested (Table 2). The presence of atrazine did not influence dimethoate toxicity at the levels used in this study (Table 2).

These results indicate that environmentally relevant concentration of atrazine (40µg/L) increased the acute toxicity of profenofos and diazinon, but had no effect on dimethoate. Several other investigators have reported synergistic or greater than additive toxicity involving atrazine mixtures. For example, greater than additive toxicity has been noted for mixtures of atrazine and alachlor in amphibians (Howe *et al.* 1998), whereas atrazine and parathion mixtures have been shown to cause greater than additive toxicity in mosquito larvae (Lichenstein *et al.* 1973). However, other investigations have indicated that atrazine in binary combination with mevinophos and methoxychlor exhibited less than additive toxicity to *Chironomus tentans* (Pape-Lindstrom and Lydy 1997). The variety of joint actions produced by atrazine mixtures indicates that the effect of atrazine on an organism is dependent on the species, co contaminant, and the levels of atrazine used.

The differences in the joint action noted for the three OPs tested compounds in the present study may be explained by differences in chemical structures. Profenofos and diazinon are phosphorothioates and have an aromatic side chain. Dimethoate is a phosphorodithioate and has an aliphatic side chain. These results are in agreement with Belden and Lydy (2000).

AChE Activity

The results of AChE activity measurements in *Chironomus riparius* are shown in Table 3. Atrazine did not significantly affect AChE activity when applied alone in any of the experiments. However, when midges were exposed to atrazine in combination with profenofos, AChE activity was significantly reduced ($P < 0.05$) than that of the low concentration of profenofos treatment only. The average percent inhibition of AChE activity of the atrazine and profenofos treatment was similar in value with profenofos 24-h EC50 level. Atrazine did not significantly reduce AChE activity when applied in combination with diazinon or dimethoate. For all three OPs, the acute EC_{50} concentration significantly decreased midge AChE activity ($P < 0.05$), demonstrating that increased inhibition levels were measurable.

Since atrazine itself had no measurable effect on AChE activity, the increased AChE inhibition found in profenofos-treated chironomids exposed to atrazine demonstrates that atrazine might influence the toxicokinetics of profenofos (resulting in increased formation of the O-analog). These results are in agreement with those previously reported by Belden and Lydy (2000); Kao *et al.* (1995). The atrazine concentration used in this study (200µg/L) was chosen to ensure that magnitude of the increased toxicity was sufficient to result in measurable differences in AChE activities. Therefore, the enhanced inhibition of AChE by the OPs may be the result of atrazine's effect on an indirect mechanism.

This study demonstates that atrazine can cause sublethal effects at environmentally relevant concentrations; therefore, addition study is also necessary to evaluate the effects of chronic exposure to atrazine.

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Table 1: Effective concentration (EC, µg/L) determined for each OP tested compound corresponding to different levels of *Chironomus riparius* larval mortality.

EC levels	Profenofos	Dimethoate	Diazinon
EC_1	0.14(0.09-0.19)*	0.24(0.12-0.36)	4.2 (1.8-6.6)
EC_5	0.18(0.13-0.23)	0.43(0.28-0.58)	7.4(4.2-10.6)
EC_{15}	0.24(0.19-0.29)	0.69(0.49-0.89)	11 (7.4-14.6)
EC_{50}	0.40(0.34-0.46)	1.6 (1.3-1.9)	28 (22-34)

* Figures between parenthesis represent 95% confidence limits .

Table 2: Percentages of *Chironomus riparius* larval mortality treated with some OP compounds singly or in binary mixtures with atrazine.

	Atrazine (µg/L)					
OP (µg/L)	0	10	40	80	200	Total
Profenofos						
0.14	0 (0)^{a}	2.0 (2.0)^{a}	6.6 (4.4)^{ab}	25.2 (7.6)^{b}	60.0(3.2)^{c}	18.8 (4.8)^{A}
0.18	2.0 (2.0)^{a}	2.1 (2.1)^{a}	31.0 (5.0)^{b}	62.5 (5.4)^{c}	85.6(4.1)^{d}	36.6 (7.0)^{B}
0.24	8.2 (3.6)^{a}	13.6 (5.1)^{a}	40.4 (6.0)^{b}	78.6 (4.8)^{c}	98.2(2.0)^{d}	47.8 (7.2)^{C}
0.40	39.4 (3.8)^{a}	45.5 (7.6)^{a}	88.2 (5.2)^{b}	95.8 (2.4)^{b}	99.5(0.0)^{b}	73.7 (5.5)^{D}
Total	12.4 (3.7)^{1}	15.8 (4.6)^{1}	41.5 (7.0)^{2}	65.5 (6.3)^{3}	85.8(3.9)^{4}	
Dimethoate						
0.24	4.1 (2.2)^{a}	0 (0)^{a}	0 (0)^{a}	8.2 (3.5)^{a}	6.4(3.6)^{a}	3.7 (2.2)^{A}
0.43	6.0 (2.3)^{a}	4.2 (2.2)^{a}	7.5 (4.5)^{a}	12.8 (3.5)^{a}	13.6(5.6)^{a}	8.8 (2.4)^{A}
0.69	23.2 (7.6)^{a}	27.1 (5.0)^{a}	23.8 (6.8)^{a}	25.6 (4.1)^{a}	25.4(6.4)^{a}	25.0 (3.2)^{B}
1.6	44.8 (5.6)^{a}	47.0 (4.9)^{a}	60.0 (6.1)^{a}	46.2 (9.8)^{a}	47.6(6.5)^{a}	49.1 (3.3)^{C}
Total	19.5 (4.6)^{1}	19.6 (4.7)^{1}	22.8 (5.6)^{1}	23.2 (4.7)^{1}	23.3(4.7)^{1}	
Diazinon						
4.2	4.0(2.2)^{ab}	0 (0)^{a}	10.6 (4.5)^{ab}	17.5(6.5)^{b}	19.1(7.0)^{b}	10.2 (2.2)^{A}
7.4	4.2(2.5)^{a}	4.0(2.4)^{a}	15.6 (5.5)^{ab}	20.6(7.2)^{ab}	26.8(6.2)^{b}	14.0 (2.4)^{A}
11	10.4(3.6)^{a}	13.5(5.2)^{ab}	32.5 (4.8)^{ab}	35.0(6.0)^{ab}	38.2(7.0)^{b}	25.9 (3.0)^{B}
28	42.7(4.4)^{a}	44.0(5.4)^{a}	62.6 (5.2)^{ab}	69.2(4.8)^{b}	75.2(4.5)^{b}	58.7 (3.2)^{C}
Total	15.3(4.0)^{1}	15.4(4.2)^{1}	30.3 (5.3)^{2}	35.6(5.2)^{2}	39.8(5.6)^{2}	

Each cell represents the average and standard error of the percent effects found for that mixture. Totals represent the average and standard error for all samples evaluated within a treatment class .Cell atrazine, or chlorpyrifos values that are not significantly different, as indicated by Tukey,s test, are marked with the same lowercase letter, number, or uppercase letter, respectively. Each row (OP treatments) was considered independently. In all cases, a signicant difference occurred if $p < 0.05$.

Table 3: Specific activity of acetylcholinesterase (AChE) (nmol/min/mg) in the fourth-instar *Chironomus riparius* larvae of the indicated treatment

Treatments	Concentrations (µg/L)	AChE Activity
Profenofos	0.14	3.2 ± 0.4^{B}
	0.40	1.2 ± 0.3^{C}
Atrazine	200	5.3 ± 0.9^{A}
Prof. + Atraz.	0.14 + 200	1.8 ± 0.2^{C}
Dimethoate	0.24	4.3 ± 0.4^{AB}
	1.6	1.4 ± 0.2^{C}
Atrazine	200	5.4 ± 0.9^{A}
Dime. + Atraz.	0.24 + 200	2.8 ± 0.3^{BC}
Diazinon	4.2	3.7 ± 0.2^{B}
	28	1.8 ± 0.3^{C}
Atrazine	200	5.2 ± 0.8^{A}
Diaz. + Atraz.	4.2 + 200	2.7 ± 0.2^{BC}
Control	0.0	5.2 ± 0.2^{A}

-The lower concentrations of the tested organophosphate insecticides correspond to the EC1 while the higher ones correspond to the EC_{50} as described in the text. All values are means±SE ($n = 5$).

-Enzyme activity values marked with the same letter are not significantly different ($P < 0.05$).

تأثير مبيد الحشائش الأترازين على سمية بعض المبيدات الحشرية الفوسفورية ضد حشرة الكيرونوموس ريبارس

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تم تقدير سمية مبيد الحشائش الأترازين و المبيدات الحشرية الفوسفورية (البروفينوفوس، الدايميثوت، و الدايزينون) على حدة و أيضا تم تقدير سمية هذه المبيدات الفوسفورية عند خلطها بمبيد الأترازين بتركيزات تتراوح ما بين ٤٠-٢٠٠ ميكروجرام/ لتر على العمر الرابع من يرقات حشرة الكيرونوموس ريبارس لمدة ٢٤ ساعة.

أوضحت النتائج أن الأترازين بمفردة ليس له سمية ضد هذه الحشرات حتى بتركيز ١٠٠٠ ميكروجرام/ لتر بينما كانت السمية عالية للمبيدات الحشرية المختبرة. كما وجد زيادة في سمية مخلوط الأترازين مع البروفينوفوس، الأترازين مع الدايزينون و لم يحدث زيادة فى سمية مخلوط الأترازين مع الدايميثوت.

و لتقييم فعالية هذه المركبات على نشاط أنزيم الأسيتيل كولين أستيريز فى هذه الحشرات، تم تعريض الحشرات لتركيز ٢٠٠ ميكروجرام/ لتر من الأترازين وبتركيز مساو لأقل جرعة مؤثرة(EC1) من المبيدات الحشرية و لمخلوط كل من الأترازين و المبيدات الثلاثة على حدة لمدة ٢٤ ساعة. وقد أوضحت النتائج أن الأترازين لا يؤثر على نشاط الأنزيم، ولكن وجد تثبيطا عاليا لنشاط الأنزيم عند المعاملة بمخلوط هذه المركبات.

يتضح من هذه الدراسة أن وجود الأترازين مع المركبات الفوسفورية المختبرة يزيد من الفعل السام لهذه المركبات من خلال تأثيره على نشاط أنزيم الأسيتيل كولين أستيريز.