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Independent and Joint Action of *cis*- and *trans*-Permethrin in *Triatoma infestans* (Hemiptera: Reduviidae)

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The toxicity of pure *cis*- and *trans*-permethrin or mixtures of the two isomers topically applied to first, third, and fifth instar nymphs of *Triatoma infestans* (Klug) at 26°C was determined. The *cis*-isomer was more active than the *trans*-isomer in the three stages evaluated. When the two isomers were simultaneously applied to first instar nymphs, an additive effect was observed. Similar treatments of third and fifth instar nymphs resulted in an antagonistic effect. In third instar nymphs, the *cis*-isomer was more active than *trans*-isomer at all the three temperatures assayed (16°, 26°, and 36°C). The toxicity of the *cis*-isomer was lower at 36°C than at either 16° or 26°C. Temperature had no significant effect on the toxicity of the *trans*-isomer within the temperature range assayed. The toxicity of either isomer to third instar nymphs was not affected by pretreatment of nymphs with PBO (an inhibitor of mixed-function oxidases activity) or TPP (an inhibitor of esterase activity), suggesting that these detoxification pathways are not relevant in the metabolism of *cis*- or *trans*-isomers. Arch. Insect Biochem. Physiol. 37:225–230, 1998.

Key words: *Triatoma infestans*; pyrethroids; joint action; antagonism; temperature coefficient

INTRODUCTION

All the pyrethroid insecticides exist in different isomeric forms, and their insecticidal action is extremely stereospecific; only certain stereoisomers out of a number of possible ones are active (Naumann, 1990). Assuming that most commercially available pyrethroids are mixtures of optical and geometric isomers, it is important to study the toxicity of their component isomers individually and in combination, and then decide whether it is more beneficial/effective to apply a mixture or only the more active pure molecules in pest control programs.

Generally, the pyrethroids show a negative temperature coefficient (Naumann, 1990). This means that the toxicity increases as the post-treatment temperature decreases. However, the effect of the temperature on the pyrethroid toxicity depends on several factors and either positive or neutral (= 1) temperature coefficients can

Abbreviations used: CI, combination index; ED_{50}, effective dose 50%; PBO, piperonyl butoxide; TTP, triphenyl phosphate

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be observed in some conditions (Sparks et al., 1983; Toth and Sparks, 1988; Johnson, 1990).

Esterases and mixed-function microsomal oxidases are enzymes usually involved in pyrethroid biotransformation (Ruigt, 1985). In bioassays in which the toxicity of pyrethroids is evaluated, the use of specific enzyme inhibitors suggests the metabolic pathway associated with insecticide metabolism. Such enzyme inhibitors are called synergists. Piperonyl butoxide (PBO) is an example of mixed-function microsomal oxidase activity inhibitor (Fukuto, 1976); and triphenyl phosphate (TPP) is an example of esterase activity inhibitor (Oppenoorth, 1985).

Chagas disease is endemic in Argentina (WHO, 1991). Its causative agent, the protozoan *Trypanosoma cruzi*, is vectored by the blood-sucking bug *Triatoma infestans* (Klug). Permethrin is a noncyanopyrethroid not usually used in Chagas disease vector control because it is not cost-effective. However, the high insecticidal activity of its *cis*-isomer against *T. infestans* (Klug) recently observed in our laboratory (Alzogaray and Zerba, 1996, 1997), suggests its potential use in the control of Chagas disease vectors. The specific aim of this work was to evaluate the toxicity of *cis*- and *trans*-permethrin individually and in combination on different instars of *T. infestans*, to investigate how particular changes in post-treatment temperature affect the toxicity of the two isomers when they are individually applied, and to determine whether the treatment of the insects with biotransformant enzyme inhibitors has some synergistic effect on the toxicity of these pyrethroids.

MATERIALS AND METHODS

Biological Material

First, third, and fifth instar nymphs of *T. infestans* were obtained from a colony maintained in our laboratory at 26°C and a photoperiod 12:12 (L:D) h. The experimental work was done on 7- to 18-day-old nymphs starved from the moult into the specific instar.

Chemicals

Cis- and *trans*-permethrin [3-phenoxybenzyl (1RS)-*cis,trans*-3-2,2-dichlorovinyl-2,2-dimethylcyclopropanecarboxylate] were obtained from the Environmental Protection Agency (Washington, DC) reference standards (>99% purity). The 82:18 *cis–trans* mixture was a gift from Chemotechnica Sintyal (Buenos Aires, Argentina); the 24:76 mixture was prepared by mixing the pure isomers. PBO was a gift from Chemotechnica Sintyal and TPP was from Aldrich (Milwaukee, WI).

Bioassays

Pyrethroids were topically applied to the ventral abdominal surface in 0.2 μl of acetone. Control groups were treated with acetone only. A minimum of four concentrations for each compound and 5–10 nymphs for each concentration were used to estimate the dose required to affect 50% of the nymphs (ED_{50}). After the application of the insecticide, the nymphs were maintained at 16°, 26°, or 36°C in a controlled temperature chamber (RH varied between 60% and 90%). The effects of the compounds on the nymphs were evaluated 24 h after treatment. By examing locomotor activity, nymphs were considered to have shown a toxic effect if they remained in the same place after being touched with stainless steel forceps, even if tremors and convulsions occurred in their legs. Each experiment was replicated three times.

Similar assays were performed after treating the nymphs with either the enzyme inhibitors PBO or TPP, according to an experimental procedure previously reported (Alzogaray and Zerba, 1997). The enzyme inhibitors were applied to Whatman #1 filter paper (0.7 mg/cm^2). A glass ring (diameter 5 cm, height 3 cm) was placed on the filter paper, and the nymphs were introduced into it. The device was covered with a transparent film and held for 24 h at 26°C.

ED_{50} values and slopes ±SE were calculated according to the Litchfield and Wilcoxon (1949) method. Significant differences among values were determined by the test of potency described by Litchfield and Wilcoxon.

Combination indices (CI) were calculated for each replicate according Chou and Talalay (1984). CI has been defined as

$$(D)_1/(D_x)_1 + (D)_2/(D_x)_2 + \alpha\,(D)_1\,(D)_2/(D_x)_1\,(D_x)_2$$

where $(D)_1$, $(D)_2$ is the dose of drug 1 and 2 in the mixture; $(D_x)_1$, $(D_x)_2$ is the dose of pure drug 1 and 2 required to produce a particular effect in the ×% of treated individuals (e.g., ED_{50}); $\alpha = 1$, if drugs are mutually exclusive; $\alpha = 0$, if drugs are nonexclusive. When CI = 1, it indicates an additive effect (lack of interaction); when CI < 1, it indicates a synergistic effect; when CI > 1, it

indicates an antagonistic effect. The mean CI ± SD was calculated. Values significantly different from 1 were tested with a one-sample t-test.

There is a graphic method to determine whether the drugs are mutually exclusive or nonexclusive. Because we could not clearly determine the mutual or nonmutual exclusivity of our data, and following the suggests by Chou and Chou (1987), we only considered the CI values corresponding to a mutually exclusive assumption, which always predicts less antagonism than the mutually nonexclusive assumption. Therefore, the more conservative CI values are presented here.

The temperature coefficient has been defined as the quotient between two ED_{50} values estimated at two different temperatures (Toth and Sparks, 1988). This quotient is calculated by dividing the larger ED_{50} value by the smaller ED_{50} value. The temperature coefficient represents how a particular change in temperature affects the toxicity of a compound at the ED_{50} level. Temperature coefficients are designated as negative if the ED_{50} value at the higher temperature is larger than the ED_{50} value at the lower temperature, and positive if the opposite occur. Using the ED_{50} values estimated at 16°, 26°, and 36°C, we calculated temperature coefficients for the ranges 16°–26°, 26°–36°, and 16°–36°C.

Synergism ratios were calculated by dividing the ED_{50} values for the insecticide alone by the ED_{50} values for the insecticide plus synergist (Casabé et al., 1988).

Both temperature coefficients and synergism ratios were considered significantly different from 1 when the ED_{50} values from which they were calculated were significantly different.

RESULTS AND DISCUSSION

Isomer Interaction

The ED_{50} values in first instar nymphs held at 26°C are shown in Table 1. The *cis*-isomer was about threefold more effective than *trans*-isomer, the difference between the respective ED_{50} values are statistically significant ($P < 0.05$). The CI for the two mixtures assayed were not significantly different from 1 ($P > 0.05$). This result indicates a lack of toxicological interaction between the two permethrin isomers. The effect of permethrin mixtures on first instars could be considered additive effects of each geometric isomer.

TABLE 1. Toxicity of *cis*- and *trans*-Permethrin Topically Applied Either Individually or in Different Combinations to First Instar Nymphs of *T. infestans*

cis : trans	Slope ±SE	ED_{50} (95% CL), ng/insect	CI[a] ±SD
100 : 0	9.6 ± 1.7	0.8 (0.1–1.0)	—
82 : 18	12.2 ± 1.7	0.8 (0.6–1.0)	0.9 ± 0.1*
24 : 76	4.7 ± 0.7	2.6 (1.7–7.1)	2.0 ± 1.3*
0 : 100	6.8 ± 1.2	2.3 (2.0–2.7)	—

[a]CI = combination index = $(D)_1/(D_x)_1 + (D)_2/(D_x)_2 + \alpha\,(D)_1\,(D)_2/(D_x)_1\,(D_x)_2$, where $(D)_1$, $(D)_2$: dose of drug 1 and 2 in the mixture; $(D_x)_1$, $(D_x)_2$: dose of pure drug 1 and 2 required to produce a particular effect in the x% of treated individuals (e.g., ED_{50}); $\alpha = 1$, if drugs are mutually exclusive; $\alpha = 0$, if drugs are nonexclusive. CI = 1 indicates an additive effect (lack of interaction).
*Not significantly different from 1 ($P > 0.05$). Each value was calculated from the data obtained in at least three replicates.

The ED_{50} values in third instar nymphs are shown in Table 2. The *cis*-isomer was about 25 times more effective than the *trans*-isomer, the difference between the respective ED_{50} values being significant ($P < 0.05$). The CI values for the two isomer mixtures assayed were significantly different from 1 ($P < 0.05$), showing an antagonistic effect.

Table 3 shows the ED_{50} values for fifth instar nymphs. The *cis*-isomer was about 54 times more effective than the *trans*-isomer; the difference between the respective ED_{50} values being significant ($P < 0.05$). The CI values were significantly different from 1 in the two isomer mixtures assayed ($P < 0.05$), showing an antagonistic effect.

The comparative toxicity of geometric isomers of permethrin has been investigated by

TABLE 2. Toxicity of *cis*- and *trans*-Permethrin Topically Applied Either Individually or in Different Combinations to Third Instar Nymphs of *T. infestans*

cis : trans	Slope ±SE	ED_{50} (95% CL), ng/insect	CI[a] ±SD
100 : 0	4.4 ± 0.8	5.8 (4.6–7.0)	—
82 : 18	3.7 ± 0.9	11.2 (8.3–13.3)	1.7 ± 0.1*
24 : 76	3.6 ± 0.4	139.6 (111.5–169.4)	8.8 ± 1.0*
0 : 100	2.5 ± 0.7	144.0 (113.0–242.3)	—

[a]CI = combination index = $(D)_1/(D_x)_1 + (D)_2/(D_x)_2 + \alpha\,(D)_1\,(D)_2/(D_x)_1\,(D_x)_2$, where $(D)_1$, $(D)_2$: dose of drug 1 and 2 in the mixture; $(D_x)_1$, $(D_x)_2$: dose of pure drug 1 and 2 required to produce a particular effect in the x% of treated individuals (e.g., ED_{50}); $\alpha = 1$, if drugs are mutually exclusive; $\alpha = 0$, if drugs are nonexclusive. CI > 1 indicates an antagonistic effect.
*Significantly different from 1 ($P < 0.05$). Each value was calculated from the data obtained in at least three replicates.

TABLE 3. Toxicity of *cis*- and *trans*-Permethrin Topically Applied Either Individually or in Different Combinations to Fifth Instar Nymphs of *T. infestans*

cis : trans	Slope ±SE	ED_{50} (95% CL), ng/insect	CI[a] ±SD
100 : 0	3.1 ± 0.5	16.8 (11.6–24.3)	—
82 : 18	3.0 ± 0.5	47.5 (33.0–74.1)	2.6 ± 0.1*
24 : 76	4.5 ± 0.8	325.3 (218.6–435.3)	4.1 ± 1.4*
0 : 100	4.1 ± 0.6	899.8 (668.7–1233.7)	—

[a]CI = combination index = $(D)_1/(D_x)_1 + (D)_2/(D_x)_2 + \alpha\ (D)_1\ (D)_2/(D_x)_1\ (D_x)_2$, where $(D)_1$, $(D)_2$: dose of drug 1 and 2 in the mixture; $(D_x)_1$, $(D_x)_2$: dose of pure drug 1 and 2 required to produce a particular effect in the x% of treated individuals (e.g., ED_{50}); $\alpha = 1$, if drugs are mutually exclusive; $\alpha = 0$, if drugs are nonexclusive. CI > 1 indicates an antagonistic effect.
*Significantly different from 1 ($P < 0.05$). Each value was calculated from the data obtained in at least three replicates.

other authors in a few insects. The *cis*-isomer having a higher insecticidal activity than *trans*-isomer. The difference ranged from 1.7 in third instar of the soybean looper *Pseudoplusia includens* (Dowd and Sparks, 1988) to 7.1 in third instar of the cabbagge looper *Trichoplusia ni* (Toth and Sparks, 1988). Intermediate values were reported for the house fly *Musca domestica*, the German cockroach *Blatella germanica* and the mosquito *Culex quinquefasciatus quinquefasciatus* (Ruigt, 1985; Naumann, 1990), and the tobacco budworm *Heliothis virescens* and adults of *P. includens* (Dowd and Sparks, 1988).

The effects resulting from the mixture of different molecules are commonly called interactions (Wilkinson, 1976). If the result of the interaction is to enhance the biological activity with respect to the pure molecules, the effect is called synergism. If the activity decreases, the effect is called antagonism. The additive effect is not considered as an interaction. Our results indicate that an antagonistic effect and the poor biological activity of *trans*-permethrin mask the important activity of *cis*-permethrin in both the third and fifth instars of *T. infestans*.

Effect of Temperature

Table 4 shows the values of ED_{50} for *cis*- and *trans*-permethrin at 16°, 26°, and 36°C, and the temperature coefficients in the ranges 16°–26°, 26°–36°, and 16°–36°C for third instar nymphs of *T. infestans*. Only *cis*-permethrin showed a significant negative temperature coefficient in the ranges of 26°–36° and 16°–36°C ($P < 0.05$). The ED_{50} of *trans*-permethrin did not vary significantly in the ranges of temperature assayed ($P > 0.05$); and then, the respective temperature coefficients were not significantly different from 1.

The *cis* isomer was significantly more toxic than *trans* not only at 26 but also 16°C ($P < 0.05$). At 36°C, the ED_{50} values for *cis*- and *trans*-permethrin were not significantly different ($P > 0.05$).

The negative temperature coefficient observed for *cis*-permethrin means that toxicity is higher when the temperature decreases. This phenomenon is usually associated with pyrethroid insecticides (Ruigt, 1985). Previously, a negative temperature coefficient for cyanopyrethroids was reported by our laboratory in third instar nymphs of *T. infestans* (Alzogaray and Zerba, 1993). The sign and magnitude of the coefficient for the same molecule can change among different temperature ranges (Sparks et al., 1983; Johnson, 1990), methods of insecticide application, and species (Toth and Sparks,

TABLE 4. ED_{50} of *cis*- and *trans*-Permethrin at 16°, 26°, and 36°C in Third Instar Nymphs of *T. infestans*

Insecticide	Temp, (°C)	Slope ±SE	ED_{50} (95% CL), ng/insect	Temperature coefficients[a] 16°–26°C	26°–36°C	16°–36°C
cis-permethrin	16	2.6 ± 0.4	4.4 (3.3–5.6)			
				–1.3		
	26	4.4 ± 0.8	5.8 (4.6–7.0)			–13.1*
					–10.0*	
	36	2.5 ± 0.4	57.8 (46.4–71.1)			
trans-permethrin	16	2.5 ± 0.5	146.3 (111.3–213.1)			
				1.0		
	26	2.5 ± 0.7	144.0 (113.0–242.3)			1.4
					1.3	
	36	1.3 ± 0.3	107.8 (60.9–161.9)			

[a]Larger ED_{50}/smaller ED_{50}. Negative values mean ED_{50} at the larger temperature > ED_{50} at the smaller temperature.
*Significant difference ($P < 0.05$) between the ED_{50} for each compound at two different temperatures. Each value was calculated from the data obtained in at least three replicates.

TABLE 5. ED_{50} of *cis*- and *trans*-Permethrin in Third Instar Nymphs of *T. infestans* Pretreated With Synergists

Insecticide	Pretreatment[a]	Slope ±SE	ED_{50} (95% CL), ng/insect	Synergism ratio[b,c]
cis-permethrin	PBO	5.2 ± 0.7	4.2 (3.5–4.8)	1.4*
	TPP	3.1 ± 0.6	5.6 (3.8–7.1)	1.0*
trans-permethrin	PBO	7.9 ± 2.4	161.1 (142.2–189.2)	0.9*
	TPP	9.7 ± 1.5	206.2 (117.6–282.6)	0.7*

[a]Applied as films on filter papers (0.7 mg/cm^2) for 24 h before the insecticide application.
[b]ED_{50} for insecticide alone/ED_{50} for insecticide plus synergist.
[c]Calculated with values of ED_{50} for insecticide alone from Table 2.
*Not significant difference ($P > 0.05$) between the ED_{50} for each compound with and without synergist. Each value was calculated from the data obtained in at least three replicates.
PBO, pyperonil butoxide; TPP, triphenyl phosphate.

1988). The *cis* isomer of permethrin was more toxic in *T. ni* than the *trans*-isomer at three temperatures (15.6°, 26.7°, and 37.8°C) and was independent of the treatment technique (topically applied or incorporated into the diet) (Toth and Sparks, 1988).

The possible mechanisms involved in a negative temperature coefficient are penetration differences (Blum and Kearns, 1956), reduced rate of metabolism (Ruigt, 1985), and a drastic increment in intrinsic neurotoxicity (Gammon, 1978; Miller and Adams, 1982).

Effect of Synergists

Table 5 shows the values of ED_{50} and the synergism factors for *cis*- and *trans*-permethrin in third instar nymphs of *T. infestans* previously exposed to filter paper treated with 0.7 mg/cm^2 of either PBO, an inhibitor of the mixed-function oxidase activity, or TPP, an inhibitor of the esterase activity. There were no significant differences between the ED_{50} with or without previous treatment with synergists ($P > 0.05$); consequently, the respective Synergism Factors were not significantly different from 1. These results mean that neither PBO nor TPP produced a significant synergism on the activity of the isomers.

The esterases and the mixed-function oxidases are relevant in pyrethroid degradation (Ruigt, 1985). According to other authors, of the two isomers *trans*-permethrin is preferentially hydrolyzed in *M. domestica* and *T. ni* (Shono and Casida, 1978), and in *P. includens* and *H. virescens* (Dowd and Sparks, 1988). This differential rate of hydrolysis can explain in some cases the differences in the toxicity between the two isomers. On the other hand, it could be rationalized that any modification of this metabolic pathway may affect the potential toxicity of the pyrethroids. In fact, a number of inhibitors of insect detoxifying enzymes have been reported as synergists of *trans*-permethrin (Dowd and Sparks, 1986).

The lack of synergism by either TPP or PBO suggests than neither esterases nor mixed-function oxidases are relevant in the *cis*- or *trans*-permethrin metabolism in third instars of *T. infestans*.

Differences in the synergism and antagonism effects observed in first, third, and fifth instar nymphs could be interpreted taking into account that nymphal stage profoundly influences the toxicokinetic of permethrin isomers. Previous results of our laboratory demonstrated in *T. infestans* that penetration through integument (Fontán and Zerba, 1987) and enzymatic activity involved in insecticidal effect (Zerba et al., 1987) are dependent of the developmental stage.

In conclusion, a very different pattern of insecticidal effect of permethrin isomers against nymphs of *T. infestans* was observed. A clear antagonism between both isomers was demonstrated in third and fifth instars nymphs. This particular case of toxicological interaction deserves further study, and opens interesting possibilities for the *cis* isomer of permethrin as a new tool for control of the vector for Chagas disease.

LITERATURE CITED

Alzogaray RA, Zerba EN (1993): Temperature effect on the insecticidal activity of pyrethroids on *Triatoma infestans*. Comp Biochem Physiol 104(C):485–488.

Alzogaray RA, Zerba EN (1996): Comparative toxicity of deltamethrin and *cis*-permethrin on first instars of *Triatoma infestans* (Hemiptera: Reduviidae). J Med Entomol 33:58–62.

Alzogaray RA, Zerba EN (1997): Incoordination, paralysis and recovery after pyrethroid treatment on nymphs III of *Triatoma infestans* (Hemiptera: Reduviidae). Mem Inst Oswaldo Cruz 92:431–435.

Blum MS, Kearns CW (1956): Temperature and the action of pyrethrum in the American cockroach. J Econ Entomol 49:862–865.

Casabé N, Melgar F, Wood EJ, Zerba EN (1988): Insecticidal activity of pyrethroids against *Triatoma infestans*. Insect Sci Appl 9:233–236.

Chou J, Chou T-C (1987): Dose–Effect Analysis With Microcomputers. Cambridge: Elsevier.

Chou TC, Talalay P (1984): Quantitation analysis of dose–effect relationship: The combined effects of multiple drugs or enzyme inhibitors. Adv Enzymol Regul 22:27–55.

Dowd PF, Sparks TC (1986): Inhibition of *trans*-permethrin hydrolysis in *Pseudoplusia includens* (Walkers) and use of inhibitors as pyrethroid synergists. Pestic Biochem Physiol 27:237–245.

Dowd PF, Sparks TC (1988): Relative toxicity and ester hydrolysis of pyrethroids in the soybean looper and tobacco budworm (Lepidoptera: Noctuidae). J Econ Entomol 81:1014–1018.

Fontán A, Zerba EN (1987): Mode of entry of insecticides in *Triatoma infestans*. Arch Insect Biochem Physiol 4:313–323.

Fukuto TR (1976): Physicochemical aspects of insecticidal action. In Wilkinson CF (ed): Insecticide Biochemistry and Physiology. New York: Plenum, pp 397–428.

Gammon DW (1978): Neural effects of allethrin on the free-walking cockroach *Periplaneta americana*: An investigation using defined doses at 15 and 32°C. Pestic Sci 9:79–91.

Johnson DL (1990): Influence of temperature on toxicity of two pyrethroids to grasshoppers (Orthoptera: Acrididae). J Econ Entomol 83:366–373.

Litchfield JT, Wilcoxon F (1949): A simplified method of evaluating dose–effect experiments. J Exp Ther 96:99–113.

Miller TA, Adams ME (1982): Mode of action of pyrethroids. In Coats JR (ed): Insecticide Mode of Action. San Diego: Academic Press, pp 3–27.

Naumann K, (1990): Chemistry of Plant Protection. Vol 4: Pyrethroid Insecticides: Structures and Properties. Berlin: Springer-Verlag.

Oppenoorth FJ (1985): Biochemistry and genetics of insecticide resistance. In Kerkut GA, Gilbert LI (eds): Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol 12. Oxford: Pergamon, pp 731–773.

Ruigt GSF (1985): Pyrethroids. In Kerkut GA, Gilbert LI (eds): Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol.12, Oxford: Pergamon, pp 183–262.

Shono T, Casida JE (1978): Species-specificity in enzymatic oxidation of pyrethroid insecticides: 3-phenoxybenzyl and α-cyano-3-phenoxybenzyl 3-(2,2-dihalovinyl)-2,2-dimethylcyclopropanecarboxylates. J Pestic Sci 3:165–168.

Sparks TC, Pavloff AM, Rose OL, Clower DF (1983): Temperature–toxicity relationships of pyrethroids on *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) and *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae). J Econ Entomol 76:243–246.

Toth SJ, Sparks TC (1988): Influence of treatment technique on the temperature–toxicity relationships of *cis*- and *trans*-permethrin in the cabbage looper (Lepidoptera: Noctuidae). J Econ Entomol 81:115–118.

Wilkinson CF (1976): Insecticide interactions. In Wilkinson CF (ed): Insecticide Biochemistry and Physiology, New York: Plenum, pp 605–647.

World Health Organization (1991): Control of Chagas Disease. Geneva: WHO.

Zerba EN, de Licastro SA, Wood EJ, Picollo de Villar MI (1987): Insecticides: Mechanism of action. In Brenner RR, Stoka AM (eds): Chagas' Disease Vectors, Boca Raton, CRC Press, pp 101–123.