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SUSCEPTIBILITY AND RESISTANCE TO INSECTICIDES OF CHAGAS DISEASE VECTORS

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Abstract Chemical control of Chagas disease vectors appears to be the best practical way to reduce the incidence of the disease. DDT was initially tested in the 1950s for the campaigns of control of Chagas disease vectors. Its low level of effectiveness against triatomine caused the failure of these control actions. HCH was then introduced in the southern cone and Dieldrin in the north of Latinoamerica. Starting in the late 1960s anticholinesterasic organophosphorus and carbamate compounds were introduced in the control of Chagas vectors. The use of pyrethroid compounds began in 1980. This family of insecticides is now the most important tool in triatomines control because of its favorable toxicological properties. Other types of insecticides also studied for Chagas vector control were the insect growth regulators and the antifeeding compounds. Because of the mode of action of these insecticides they are now considered just a potential complement of neurotoxic insecticides for integrated programmes of Triatomines control. Innovative formulations such as fumigant canister and insecticidal paints have been successfully developed in Latinoamerica with the World Health Organization support. Resistance to insecticides of triatomines is not yet a great problem in Chagas vectors. However, some resistant strains to pyrethroids have been found in *Rhodnius prolixus* from Venezuela and in *Triatoma infestans* from Brazil. Some cases of *T. infestans* incipient resistance to deltamethrin have been detected in Argentina. According to the control tools now available it is possible to expect the interruption of vector transmission of Chagas disease in the near future.

Resumen ***Susceptibilidad y resistencia a insecticidas de los vectores de la enfermedad de Chagas.*** Una de las pocas alternativas prácticas de controlar la transmisión de la enfermedad de Chagas es a través del control de los triatominos vectores. Inicialmente las campañas antivectoriales en la década del '50 utilizaron DDT como activo, cuya falta de efectividad triatomicida hizo fracasar estas acciones. HCH fue entonces utilizado en el Cono Sur y Dieldrin en el norte de Latinoamérica. A partir de fines de los '60 comienzan a usarse los anticolinesterasicos fosforados y carbamatos. En 1980 se introducen los piretroides como herramientas de control de vectores de Chagas, que hoy día constituyen el grupo de insecticidas más usados por sus favorables propiedades toxicológicas. Otros insecticidas también estudiados para combatir los triatominos han sido los inhibidores de crecimiento de los insectos (IGR) y los antialimentarios. Debido a su modo de acción este tipo de compuestos pueden considerarse en la actualidad sólo útiles como complemento de insecticidas neurotóxicos en control poblacional. Formulaciones innovadoras como el pote fumígeno y la pintura insecticida han sido exitosamente desarrolladas en Latinoamérica con el apoyo de la Organización Mundial de la Salud. La resistencia a insecticidas no representa aún un problema importante para el control de vectores de la enfermedad. No obstante, se han encontrado focos de resistencia a piretroides en *Rhodnius prolixus* en Venezuela y a *Triatoma infestans* en Brasil. En Argentina ya se han detectado casos de resistencia incipiente a deltametrina en *T. infestans*. Considerando las herramientas de control disponibles hoy día, la interrupción de la transmisión vectorial de la enfermedad de Chagas parece posible en un futuro cercano.

Key words: insecticides, Chagas disease, triatomines, vector, *Triatoma infestans,* resistance, *Rhodnius prolixus*

Chagas disease or American trypanosomiasis is found on the American continent in the area between 42° N latitude and 45° S latitude where it infects about 16-18 million people. Some 100 million people, a quarter of all the inhabitants of Latin America, are at risk of contracting the disease[1].

Chagas disease is a chronic and incurable parasitic infection that causes disability and death. It is caused by a flagellate protozoan, *Tripanosoma cruzi*, which is transmitted to humans in the feces of blood sucking triatomine reduviid bugs. There are 3 genera of triatomines particularly incriminated in the transmission of Chagas disease: *Triatoma*, especially *T. infestans*, *T. dimidiata* and *T. sordida*; *Rhodnius*, especially *R. prolixus* and *R. pallescens* and *Pastrongylus*, especially *P. megistus*[2].

The number of disability-adjusted life years lost because of Chagas disease amounts to 2 740 000 and represents the third largest tropical disease burden after malaria and schistosomiasis.

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Because no treatment is available for the chronic forms of the disease and there is no immunological protection, chemical control of the vectors appears to be the best way to reduce the incidence of the disease[2, 3].

Chemical control has been based principally on spraying the dwellings and the peridomiciliary areas with formulations applied by professional sprayers. The active principles used since the 1960's were chlorinated hydrocarbons, organophosphorus and pyrethroid insecticides. Chemical vector control programmes at the national level have been implemented in Argentina, Brazil and Venezuela.

Activities for the control of Chagas disease vectors involve three stages[4].

1. *Preparatory phase. It i*ncludes the mapping of the area to be treated, the programme of control activities and estimation of resources.
2. *Attack phase.* In this phase a massive insecticide spraying of infested houses takes place followed by a second spraying of reinfested houses not more than 6 months later.
3. *Surveillance phase.* When the objective of the attack phase has been reached vigilance activities are performed to detect and control residual foci of triatomines.

The control activities based in the above 3 stages is not a strict strategy. In some cases, the second spraying of the attack phase is not carried out, or a mixture of attack and surveillance phase is carried out with non-professional formulations.

Historical background

After the war the most important use of DDT was the control of malaria - carrying mosquitoes[5]. A campaign to eliminate malaria from the whole of Italy was started in 1945. In 1957 it was stated that not a single death from malaria had occurred in Italy since 1948[5]. The wartime success of DDT in controlling malaria stimulated Latinamerican entomologists to test this insecticide in the 1950s for the control of Chagas disease. As an unexpected result, DDT had to be discarded for this purpose because of its low level of effectiveness against triatomine. The low triatomicidal power of DDT has been justified taking into account two particular degradative pathways established in *Triatoma infestans*[6, 7]. These pathways are mediated by a DDT– dehydrochlorinase and by a DDT hydroxylase which produce the degradation of DDT to DDE and kelthane[6, 7]. A delayed penetration of DDT in starved nymphs of *T. infestans* was informed as a complementary cause of the tolerance to this insecticide[8]. After the unexpected failure of DDT, the first option between the chlorinated hydrocarbons was the HCH which was successfully introduced for the control of the Chagas disease vectors in 1947[9].

Active principles

Chlorinated hydrocarbons

As stated above HCH was the first synthetic insecticide introduced for Triatomines control. This product is a mixture of isomers of hexachlorocyclohexane. Only five isomers have been found: $\alpha,\beta,\gamma,\delta$ and ε[10]. The g isomer, commonly called lindane is recognized as the active component of HCH. This compound was the only one isomer with insecticide activity against *Triatoma infestans* (B. D'Agostino and E. Zerba, unpublished results). The dosage of HCH expresed as γ isomer for the control of Chagas disease vectors was 500 mg/m^2. The treaments based in the use of HCH were expensive and time consuming because two successive spray cycles were necessary for a successful control. The initial application would eliminate the nymphs and adults while the second between 1 to 6 months later, would eliminate the nymphs born from eggs hatched before the end of the residual activity of the insecticide.

Venezuela introduced dieldrin in 1947 for the control of the principal vector in the region: *Rhodnius prolixus*[9]. The use of dieldrin was a consequence of the DDT failure in the initial control actions developed in Venezuela to reduce the incidence of Chagas' disease.

In the early 1960's the enormous impact of Rachel Carson's "Silent Spring" called the attention to the potential of chlorinated hydrocarbons for causing adverse effects on human health and the environment.

The high chemical stability and the potential toxicological and ecotoxicological risk of chlorinated insecticides caused their progresive substitution by compounds with more favorable properties. Organophosphorus and carbamate insecticides were an alternative of less persistent and non bioaccumulative insecticides for the control of Chagas' disease vectors.

Anticholinesterasic compounds

Organophosphorus and carbamate insecticides kill animals, both vertebrate and invertebrate by inhibiting cholinesterase with consequent disruption of nervous activity caused by accumulation of acetylcholine at nerve ending[10]. Propoxur was the first anticholinesterasic insecticide used for the control of triatomine vectors of Chagas disease. The triatomicidal effect of this carbamate was established in 1968 and the initial field trials were performed in Chile during the period 1969-1971[9]. The phosphothionates malathion and fenitrothion were introduced in 1975 into Chagas vector control programmes.

Table 1 – Triatomicidal Effect of Anticholinesterasic Insecticides on Fifth-Stage Nymphs of *T. infestans*.

Insecticide	LD_{50} (μg/g)	Confidence Limits
Malathion	49.2	37.6 – 64.4
Fenitrothion	5.6	2.5 – 12.5
Pirimiphos Methyl	12.8	7.5 – 21.9
Dichlorvos	39.5	37.0 – 42.0
Propoxur	18.5	15.1 – 24.6
Bendiocarb	20.0	15.3 – 26.2

Topical application (References 13 and 14)

These anticholinesterasic compounds with ovicide action[11], lesser vapour pressure and a higher initial impact of control than HCH allowed a spacing between applications of 1 year. Phosphorothionates have the disadvantage of a strong and impleasant smell which results in villagers resistance to the house treatments. These compounds are a good alternative for treatments in outhouses and other peridomestic structures[12]. The organophosphate DDVP had a reduced use as a "dry fog" or as slow release formulations. Other anticholinesterasic compounds, such as pyrimiphos methyl or bendiocarb were evaluated at the laboratory level but not used in regular campaigns against the vectors of Chagas' disease. In Table 1, the LD_{50} values of anticholinesterasic compounds obtained in nymphs V of *T. infestans* are shown[13, 14].

Pyrethroids

The obtention of new compounds by modification of the chemical structure of natural pirethrins resulted in a new family of insecticides: pyrethroids. Synthetic pyrethroids are neurotoxics acting on the axons in the peripheral and central nervous systems by interacting with sodium channels in mammals and/or insects. Because their selectivity based in a great insecticide activity, the low application rates and their rapid degradation in the environment, these compounds have been successfully used for the control of house hold pests and insects of public health importance[15]. Allethrin was the first commercially successful pyrethroid, introduced in 1949. Allethrin and other photolabile compounds principally obtained by esterification of chrysantemic acid constitute the first generation of pyrethroid compounds.

This type of pyrethroid compounds was not successfully used in the field for the control of Chagas vectors in spite that some of them showed excellent triatomicidal activity in laboratory bioassays as shown in Table 2[16]. The failure of first generation pyrethroids for Chagas vector control was principally caused by their lack of residual activity.

Table 2 – Triatomicidal Effect of Some Pyrethroid Insecticides Derived from Chrysanthemic Acid.

Insecticide	LD_{50} (μg/g)	Confidence Limits
Bioresmethrin	0.6	0.3 – 1.4
N – Phemothrin	2.8	2.2 – 3.7
Cyphenothrin	2.9	2.8 – 3.0
Bioallethrin	10.7	7.1 – 16.2
Allethin	90.7	77.4 – 106.4

Triatoma infestans, *Nymphs V, Topical application (References 15 and 16).*

Deltamethrin first described in 1974 was the first photostable pyrethroid tested in Triatomines at the laboratory level. It showed a very high toxic effect on *Triatoma infestans* [17]. Deltamethrin and cypermethrin have been successfully applied in the field for Chagas' vector control since 1980[9]. Permethrin had a limited use in Brazil because the dosage necessary to obtain adequate control resulted in expensive treatments, but its cis isomer was recently described as one of the most toxic pyrethroid to *T. infestans*[18, 19]. The effect difference in *T. infestans* between cis-trans permethrin and the isolated cis isomer showed in Table 3 could be explained by a clear antagonism between both isomers[19]. In other words, the less active trans isomer produces an antagonistic effect which masks the high toxicity of the cis isomer when they are simultaneously applied on nymphs *of T. infestans*. These results are opening interesting possibilities for the cis isomer of permethrin as a new tool for control of the vector of Chagas disease.

In the 1990's the pyrethroids compounds used in Chagas vector control were restricted to cypermethrin and a select group of third generation cyanopyrethroids with a demonstrated high triatomicidal activity (Table 4).

Second generation pyrethroids is the name given to the whole isomeric mixtures of photostable compounds such as cypermethrin and permethrin. Pyrethroids belonging to third generation are constituted by the more active

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

Cis : trans	LD_{50} (ng/insect)	
	Third Stage Nymphs	Fifth Stage Nymphs
100 : 0	5.8	16.8
82 : 18	11.2	47.5
24 : 76	139.6	325.3
0 :100	144.0	899.8

Adapted from reference 19

Table 4 – Triatomicidal Effect of the Principal Pyrethroid Insecticides used at the Present time in Chagas Vectors

Insecticide	LD_{50} (mg/g)	Confidence Limits
Deltamethrin	1.54	0,85 – 2,48
Beta-cypermethrin	1.56	0,93 – 4,34
Beta-cyfluthrin	0.32	0,18 – 0,46
Lambda-cyhalothrin	0.11	0,06 – 0,21
Cypermethrin	2.86	0,95 – 6,67

Triatoma infestans*, Nymphs V, Topical application according to WHO protocol, 1994 (Reference 39)*

isomers obtained by isomeric enrichment. Deltamethrin, one of 8 possible isomers, is the best example of a third generation pyrethroid. Besides deltamethrin, the group of third generation pyrethroids used at the present time in Chagas vector control is also constituted by lambda-cyhalothrin and beta-cyfluthrin. The successful results of recent laboratory bioassays, field trials and national campaigns of Chagas vector control in Argentina allowed the incorporation of beta-cypermethrin to this pyrethroid group[20].

Other insecticides

Many compounds from a wide variety of classes have now been shown to possess juvenile hormone (JH) activity for different insect orders. It is known that the activity of these JH compounds is specific to each order or even family of insects[21]. In the case of *T. infestans* the Vth instar nymphs are the most susceptible to JH analogues[22]. In spite of the high selectivity and effectivity against triatomines established in JH experimental compounds[23, 24] and commercial products such as fenoxycarb[22] this particular type of insect growth regulators (IGR) are not used in the field at the present time for the control of Chagas disease vectors. The principal criticism to the use of JH insecticides in Chagas vectors control is their particular mode to action. The interference with normal metamorphosis performed by JH compounds could produce abnormalities after moult and delayed ecdysys[22]. These effects result first in delayed population control than in fast mortality of individual insects. In summary, JH compounds would tend to prolong the disease transmitting nymphal stage and, hence, show little promise for the control of Chagas disease vectors. But it seems reasonable to explore its complementary use with neurotoxic insecticides with established toxicity to triatomines.

Since the Vth instar nymph is the stage least susceptible to neurotoxic insecticides and that abnormal moulted individuals caused by JH compounds could present a low vector potential, mixed formulations of both type of insecticides may well result in a satisfactory integration of the tools.

Antifeeding compounds are substances which are not necessarily food repellents but cancel out the signal to the appropriate organ in the insect to iniciate feeding on the host. After the contact with an antifeeding compound the insect may starve to death.

It was demonstrated that N-ethylmaleimide (NEM) and other sulfhydryl reagents inhibited the feeding response of *Triatoma infestans*[25]. *Triatoma* feeding was also deterred when a gauze cloth impregnated with NEM was located between the food source and the nymphs. The antifeeding effect is attributed to a receptor blockage produced by sulfhydryl reagents[25]. A series of cis isomers of methyl esters of N-substituted maleamic acid were synthesized and their antifeeding activity by topical application on Vth instar nymphs of *T. infestans* was demonstrated[26]. Likewise, the continuous exposure of *T. infestans* experimental populations to antifeeding maleamates produced a significant reduction in the insect and egg numbers[27].

The population management caused by antifeeding compounds could be considered an alternative method of Chagas disease vectors control. As in the case of JH insecticides, antifeeding compounds, specially natural products, deserve more research as a complementary tool for integrated programmes of Triatomines control.

Innovative formulations

In recent years with the support of the Tropical Disease Research Programme (TDR) of WHO, two new vector control tools have been developed for use against Chagas disease: a fumigant canister and paints that incorporate insecticides in a slow release formulation.

Fumigant canister was developed in Argentina in the Research Center of Pest and Insecticides (CIPEIN)[1, 4, 12, 15, 28]. The latest version of the fumigant canister (CIPEIN – PF-6) was introduced into the market in 1994. It consists of a disposable canister containing a solid fumigant mixture and a plastic vial. The fumigant mixture includes beta-cypermethrin incorporated into the mixture using adequate protective measures to avoid thermal or chemical descomposition during the combustion. The heat produced during combustion, causes fusion of the vial and the subsequent delivery of dichlorrvos and permethrin contained in the vial in form of vapor and fume[29].

Allthough fumigant canisters were initially designed to be used during the surveillance phase their success in centralized or community based attack phase has been demonstrated in different field trials[29]. However, the principal rol of the fumigant canister could be to make the surveillance phase of Chagas vector programs more sustainable in areas where domiciliary vectors are the major

targets so improving the overall efficiency of the programs[30].

On the other hand the use of fumigant canisters is expected to reduce the financial costs of vector programs and to create opportunities for wider participation of the community at risk in Chagas disease control activities[29, 30].

At present, the fumigant canister for Chagas disease vector control is used in governmental campaigns only in Argentina. In 1991 the Ministry of Health of Argentina adopted the CIPEIN – PF-5 version of the fumigant canister in its national Chagas disease strategy. In the period of 1991-1995 the government of Argentina had purchased a total of 495.000 canisters[30].

According to the analysis of Fujisaki and Reich[30], the extensive use of the fumigant canister by the Argentinean Ministry of Health since 1991 probably contributed to the reduction of the prevalence of Chagas disease in recent years in Argentina.

With reference to insecticidal paints, many slow release formulation, mostly based on organic polymers has been developed and tested in the control of Chagas Vectors in Brazil with the support of TDR[1, 12, 3]1.

Field trials of a malathion/polyvinil acetate slow – release emulsion paint have shown it to be an insecticide tool of long indoor effectiveness. The stability and adherence to the substrates of the paints could be an advantage over conventional formulations, preserving insecticide effect where other chemical control alternatives would probably be washed[31]. This property could be to make the paints an effective alternative for the control of Chagas vectors located in peridomestic areas.

Resistance

Despite prolonged and intensive control campaigns against Chagas vectors few studies have been made on the possible development of insecticide resistance in Triatomines[32].

In Argentina was selected a strain of *T. infestans* resistant to malathion and determined phosphorotriesterase as the causal mechanism[33]. Resistance to dieldrin in *R. prolixus* from Venezuela is the first well documented evidence of field resistance. Dieldrin resistance was first detected in the state of Trujillo and later in the states of Yaracuy, Tachira, Cojedes y Portuguesa[34, 35].

Other authors have reported the survival of *T. infestans, R. prolixus* and *P. megistus* colonies established from the field and exposed to papers impregnated with discriminating concentration of insecticides (for a review see reference 32).

High resistance ratios to gamma HCH (>1200) and dieldrin (550) were established in a strain of *Rhodnius prolixus* (Santo Domingo) by topical application of the insecticides. This strain was susceptible to pyrethroid compounds[36].

A Venezuelan *R. prolixus* strain collected in Carabobo has shown higher resistance to all five pyrethroids evaluated than for dieldrin (RR 3): pyrethroid RR values ranged between 11.4 for deltamethrin and 12.4 for cypermethrin to 4.5 for lambacyhalothrin[37]. This was surprising, taking into account that *R. prolixus* control was mostly done with organochlorines in Venezuela[34]. In the State of Carabobo house spraying employed dieldrin, HCH and fenitrothion. Exposure of *R. prolixus* to pyrethroids might be due to their intensive for mosquito control in Carabobo State (Molina de Fernández D. personal communication). Deltamethrin resistance in this *R. prolixus* strain was strongly reduced by PBO synergism suggesting involvement of mixed function oxidases in pyrethroids resistance.

A *T. infestans* strain arising from Porto Alegre, Brazil, showed higher resistance to deltamethrin (7 x) than to cypermethrin (3.3 x) or betacyfluthrin (3.6 x) but was normally susceptible to betacypermethrin and lambdacyhalothrin[37]. Pyrethroid resistance in this strain could be associated with intensive use of deltamethrin and cypermethrin for control of Chagas disease in Brazil since 1982[38]. PBO synergism of deltamethrin resistance in this strain, suggests oxidative metabolism as a cause of resistance[37].

A program of detection of resistance to deltamethrin in Argentina is now in progress. After a screening performed by topical application of deltamethrin according to the WHO protocol[39]. Four resistant strains were found in San Luis, Mendoza, La Rioja and Catamarca (Picollo M.I. and Zerba E., unpublished results). The resistance monitoring in Argentina is performed in the frame of a more extensive latinoamerican program sponsored by the TDR of WHO.

Future of the control

An important progress to the control of Chagas' disease in South America started in 1991 when the Southern Cone Initiative was lanched by Ministries of Health of Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay. This Initiative is coordinated by the Pan American Health Organization, Regional Office for the Americas of the WHO[1, 4, 40]. Later in 1997 the governments outside the Southern Cone launched the Andean and Central American Countries Initiatives. These initiatives represent a big effort of latinoamerican countries to interrupt transmission of Chagas' disease by eliminating its vector[40, 41].

The impact of these measures are starting to be in evidence with a reduction in morbidity and mortality, specially in the most advanced countries like Argentina, Brazil, Chile and Uruguay[40, 41]. The control tools for interrupting the domestic cycle of Chagas disease are available.

Sustained implementation of vector control measures is expected to achieve the interruption of vector transmission of Chagas' disease in the near future.

Field and laboratory research, especially performed in latinoamerican countries, should be a support of this objective.

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