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**Field testing and evaluation of insecticides for
indoor residual spraying against domestic
vectors of Chagas disease**

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Communicable Disease Control, Prevention and Eradication
WHO Pesticide Evaluation Scheme (WHOPES)**

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PREFACE

This document outlines the general procedures for field trials of residual insecticides against domestic Triatominae, where the aim is to eliminate the domestic bug populations and inhibit reinfestations. The procedure is suitable for village-scale field trials of a specified formulation applied at a single target dose rate, whose toxicological safety has already been approved. The procedure also allows for multiple village trials in order to compare the effects of different products, or different formulations of the same product, or different doses of the same formulation.

The basic procedure involves a pretrial entomological evaluation, followed by spraying and then a series of post-spray evaluations over a period of 12 months. Pre- and post-spray evaluations primarily involve manual capture of bugs infesting houses and peridomestic habitats, but post-spray evaluation also includes residue analysis and bioassays of residual activity on treated surfaces. Results are then expressed in terms of the proportion of houses apparently free of bugs over different periods of time post-spray, together with an estimation of the power of the residual product to inhibit any subsequent infestations.

CONTENTS

	Page
Acknowledgements	i
Preface	ii
1. Introduction	1
1.1 Chagas Disease in Latin America	1
1.2 Experience in Chagas Disease Vector Control	2
2. Rationale and Objectives of the Trial	4
2.1 Domestic Infestations	4
2.2 Peridomestic Infestations	5
3. Implementation of the Trial	6
3.1 Preparatory Phase	6
3.1.1 Statement of Objectives	6
3.1.2 Selection of Product	8
3.1.3 Selection of Target Dose	8
3.1.4 Selection of Trial Site	9
3.1.5 Personnel and Materials	10
3.2 Prespray Evaluation	11
3.2.1 Informed Consent of Local Authorities	11
3.2.2 Household Visits	12
3.2.3 Operational Sketch Map	13

3.2.4	Geographical and Climatic Data	14
3.2.5	Baseline Infestation Levels	14
3.2.5.1	Manual Searches	14
3.2.5.2	Passive Monitoring Tools	15
3.2.5.3	Householder Collections	15
3.2.5.4	Back Correction of the Infestation Data	15
3.2.5.5	Storage of Captured Bugs	16
3.2.6	Epidemiological Data	16
3.3	Spray Intervention	17
3.3.1	Preparation of House for Spraying	18
3.3.2	Procedure for Monitoring Applied Dose Rate	18
3.3.2.1	Tank Charges	18
3.3.2.2	Sentinel Filter Papers	19
3.3.3	Spray Application	20
3.3.4	Disposal of Waste	20
3.4	Postspray Evaluations	21
3.4.1	Postspray Infestation Levels	21
3.4.2	Wall Bioassays	21
4.	Interpretation of Results	22
4.1	Spray Deposit Rates	23
4.2	Wall Bioassays	24
4.3	House Infestation Rates	25

5.	Interpretation of Reinfestations	28
6.	References Cited	31
Annex 1	Summary Chronogram of Trial Activities	37
Annex 2	Basic Material and Equipment Requirements	38
Annex 3	Supervisors Checklist	40
Annex 4	Sample Data Entry Forms	44
	4.1 Example of Visit Card to be Affixed Inside the Door of Each House	44
	4.2 Example of Data Entry Sheet For Each Visit to Each House	45
	4.3 Example of Supervisors Data Control Form	47
Annex 5	Suggested Procedure for Morphometric Analysis of Reinfestations	48
Annex 6	Analysis of Costs	52



1. Introduction

1.1 Chagas Disease in Latin America

Chagas disease is ranked by the World Bank as the most serious parasitic infection of the Americas, outranking the combined burden of malaria, schistosomiasis, leishmaniasis and others in terms of economic impact estimated as disability-adjusted life years (DALYs) (World Bank 1993). In 1990, the World Health Organization estimated there were 16-18 million people infected, with a further 100 million considered at risk (WHO, 1991b), although following from extensive vector control campaigns the prevalence estimates have since been revised downwards to about 11-12 million people (Schmunis, 1999). No treatment is currently suitable for large-scale use against the disease, and vaccines are unavailable, so that control relies almost entirely on interrupting transmission – mainly by eliminating the domestic insect vectors.

Chagas disease takes its name from Brazilian clinician Carlos Chagas who first described it in 1909. It is caused by infection with the protozoan parasite *Trypanosoma cruzi*, mainly transmitted to man in the faecal droppings of infected triatomine bugs. About 90% of transmission is due to domestic species of Triatominae. Other routes of transmission include blood transfusion and organ transplant from infected donors, and transplacental transmission from infected mothers to the foetus.

Well over 100 species of Triatominae are now recognised, but many have little epidemiological significance because of their silvatic habits and consequent rarity of contact with humans. Some species however, have become highly adapted to domestic environments and represent highly significant vectors. Amongst

the most important vector species are *T. infestans* in the southern cone countries (Argentina, Bolivia, Brazil, Chile, Paraguay, Peru and Uruguay), *T. brasiliensis* in northeastern Brazil, and *Rhodnius prolixus* and *T. dimidiata* in regions north of the Amazon basin. These species are well adapted to human habitations, generally living in cracks and crevices of poorer quality rural houses, and emerging at night to suck the blood of the sleeping people and domestic animals. The bugs take large blood meals and seem to contribute to chronic iron-deficiency anaemia, as well as transmitting *T. cruzi*.

At present, there is no treatment suitable for large-scale use against Chagas disease, and vaccines are unavailable. In contrast, the biological characteristics of the domestic insect vectors make them particularly vulnerable to control. They are slowly-reproducing (e.g. *T. infestans* generally has only 1 or 2 generations per year) with a consequently low rate of genetic rearrangement. In addition, they have low population variability, and other genetic and demographic characteristics rendering it difficult to select for attributes such as insecticide resistance (see Dujardin et al., 1996; Schofield, 1994; Schofield et al., 1999). Most importantly, vector species occur predominantly in domestic and peridomestic habitats, and have a relatively low capacity for active dispersal (see Lehane et al., 1992). Thus, although domestic Triatominae are well adapted to stable domestic habitats (exhibiting strong *K*-selection, in contrast to the *r*-selection of, say, mosquitoes [see Rabinovich, 1974]) they seem poorly adapted to respond to instabilities such as the changes implied by vector control interventions.

1.2 Experience in Chagas Disease Vector Control

Early trials in Brazil (Dias & Pellegrino, 1948) and Argentina (Romaña & Abalos, 1948) showed good control of domestic populations of *Panstrongylus megistus* and *T. infestans* using a wettable powder (WP) formulation of BHC (also known as

HCH, Lindane or Gammexane). These trials examined various application methods, including fumigant generation, concluding that the best results were obtained with a WP formulation containing not less than 30% gamma isomer, applied with backpack sprayers at target dose rates of 500-2000 mg active ingredient (a.i.) per square metre over all internal and external wall surfaces and the internal roof surface. For many years this remained the standard for Chagas vector control in many countries, especially Argentina and Brazil (e.g. SUCAM, 1980), although dieldrin was also widely used in Venezuela during the 1950s and 1960s in large scale campaigns against *R. prolixus*.

During the 1960s and 1970s, several other organochlorine insecticides were trialed against domestic Triatominae, as well as a number of organophosphates and carbamates. However, BHC remained the most widely used compound until the 1980s when synthetic pyrethroids were shown to give superior results, often at lower operational costs (see Pinchin et al., 1980, 1982). Subsequently, many countries of Latin America have used synthetic pyrethroids of various types, generally giving very satisfactory results (see PAHO 1999; Schofield & Dias, 1998).

In line with this experience, methods of trial implementation and evaluation have become broadly standardised throughout Latin America. This document draws upon that experience in order to provide a standardised trial protocol designed to assist efficient and effective evaluation of new products and formulations. In summary, the trial proceeds through five main phases:

1. Preparatory - product and site selection, materials, transport , personnel and training.
2. Prespray Evaluation - approval of householders and local authorities, site mapping, prespray infestation levels.
3. Spraying - application of trial product; monitoring of applied dose; passive evaluation tools.

4. Postspray evaluation - monitoring of infestation levels at 1, 3, 6, 9, and 12 months postspray; wall bioassays for residual product activity; household interviews.
5. Interpretation of Results.

Note that to reinforce the participation of the community in the conduct of the trial, it is recommended that any houses found to be infested during the final evaluation at the end of the trial should be resprayed. However, this final respray does not affect the interpretation of final results.

A chronogram of activities is given in Table 1, and a supervisors checklist for each phase is given in Annex 1.

2. Rationale and Objectives of the Trial

2.1 Domestic Infestations

The objective of applying insecticide(s) against domestic Triatominae is to eliminate the triatomine population, so that the underlying rationale of the trial is that application of the specified product will kill all bugs in the treated houses. Thus the 'null hypothesis' of the trial is that application of the product will make no change to the house infestation rate (ie. proportion of houses infested), and it is against this null hypothesis that the trial results are compared. The underlying assumption therefore, is that the house infestation rate will not change during the course of the trial, except as a result of the control intervention. This assumption has been supported by field observation and experimentation with *Triatoma infestans* and *Panstrongylus megistus* (e.g. Dias, 1955; Gorla & Schofield, 1989; Schofield, 1980) showing that in general, house infestations tend to be stable over time. However, this assumption will not hold if there is significant immigration of bugs into the trial site, which is why the trial should be carried out in a relatively discrete area

where immigration can be considered unlikely (see Section 3.1.4) and any live bugs that appear in the treated houses after the intervention must be checked to see if they could represent an immigrant population (see Section 5).

The above rationale allows the trial design to be internally evaluable, so that no untreated control site is required, although a control site treated with a known standard may be used for comparison (see Section 3.1.1). The idea of an untreated control site is also considered unethical, because the marginal cost of spraying a house (compared to the cost of visiting it to check for infestation) is estimated to be no more than 10-15% (Oliveira Filho, 1989). In other words, if the trial budget were to be sufficient to include evaluation of another village, then it would be unethical not to spray that village as well.

A pre-spray evaluation is carried out to determine the existing house infestation rate, and a series of post-spray evaluations are carried out to see how the house infestation rate has changed after the intervention. This means that the primary indicator of the trial results is the presence or apparent absence of bugs in the treated houses. However, since sampling methods are imprecise, we can never be absolutely certain that bugs are absent, even when none can be found during a given evaluation. For this reason, several sampling techniques are used, and a series of checks is made, including back-correction of the results (see Section 3.2.5) all designed to improve the reliability of knowing whether bugs are present or absent from a house.

2.2 Peridomestic Infestations

The above rationale applies to trials whose aim is to demonstrate the effectiveness of intervention against domestic infestations of *Triatominae*. In many situations however, the peridomestic habitats may also be infested, either with the same or a different species. (Here, the peridomestic habitats are defined as artificial

structures associated with the house, such as chicken coops, stables, goat corrals, etc.) In such situations, the peridomestic habitats will generally be sprayed and evaluated in the same way as the houses, but the results will be presented separately for houses and for peridomestic habitats (see Section 4). It may be however, that the trial is being designed primarily to evaluate the effects of intervention on peridomestic populations, where the aim is to diminish these populations rather than necessarily eliminating them entirely. This does not affect the underlying rationale of the trial (see Section 2.1), but may imply more detailed analysis of changes in the age-structure and distribution of the peridomestic bug populations, in addition to their presence or apparent absence.

3. Implementation of the Trial

3.1 Preparatory Phase

3.1.1 Statement of Objectives

Planning and implementation of the trial is greatly assisted by a clear statement of the primary objective, together with any secondary objectives that may be considered (see below). For a single village trial, the primary objective is to demonstrate how effective is a specified product formulation, applied at a specified target dose, in eliminating domestic and/or peridomestic populations of the target vector species. For a multi-village trial, the objective may be to compare the effectiveness of two or more products, formulations, dose rates, or application methods. It is important to note however, that since the objective is to eliminate the target vector populations, neither clinical nor parasitological studies will have particular relevance (although additional information can be obtained if epidemiological data is available - see Section 3.6). Instead,

emphasis is given to entomological parameters throughout the trial.

Examples of Primary Trial Objectives:

1. *To demonstrate the effectiveness of product-X in eliminating domestic infestations of species-A*
2. *To compare the effectiveness of product-X and product-Y in eliminating domestic infestations of species-A.*

Note however, that there may be secondary objectives, such as comparing the ease of use of different formulations, comparing their side-effects (e.g. degree of irritation to spraymen) or acceptability to householders (e.g. marks on house walls, smell), or demonstrating their effects on other household pests such as cockroaches, scorpions, houseflies and so on. Moreover, operational effectiveness may not be the only motive for the trial, since cost factors may also be relevant (see Annex 6). The definition of primary and secondary objectives will affect the design of the data entry sheets (see Annex 4).

In defining the trial objectives, it is important to recall that the control of Triatominae implies elimination of bug populations from individual houses. Thus the unit of study is the individual house, and the key factor to be measured is the presence or apparent absence of bugs in each house under consideration. It should also be noted that the trials are designed to be internally evaluable, so that untreated control communities are not required. A characteristic of domestic infestations with triatomine bugs is that they tend to be stable in the absence of control interventions (see: Schofield, 1980, 1994). For this reason, the effectiveness of the product will be judged in terms of its ability to reduce existing infestations, so that the baseline

(prespray) infestation levels represent the internal controls for the study.

3.1.2 Selection of Product

Product formulations to be tested in village scale trials must be of known physical and chemical nature, and must be provided with full toxicity data and guidelines for safe use and waste disposal. The toxicity data should include oral, dermal, and inhalation studies on vertebrates, with acute and long-term exposure, in accordance with established hazard guidelines and product registration procedures (see FAO, 1982; WHO, 1991a). In no case should village scale trials be carried out using product formulations of unknown composition, or products lacking toxicological data or product registration documents. Products for use should be supplied in original and unopened containers.

Full details of safety precautions required for householders, domestic animals, and sprayers must be provided (including monitoring of enzyme levels or other functions if necessary) together with written guidance in case of intoxication.

3.1.3 Selection of Target Dose

In general, the target dose will be selected as the minimum rate compatible with effective use, based either on previous field experience and/or laboratory studies. In no case should a dose rate be selected that could present acute or chronic hazard to householders and their families, domestic animals or sprayers.

For dose-finding trials, it is usual to compare the new product with a known standard, applying dose rates derived from laboratory studies. In general, the laboratory studies are designed to estimate the LD₅₀ of the new product from exposure of fifth-instar nymphs of the target species to sprayed substrates

– usually sprayed filter papers, and mud blocks or other substrate prepared to mimic one or more of the main materials used in house construction (eg. Rojas de Arias et al., 2001). Field trials can then be carried out using dose rates equivalent to the LD₅₀, twice the LD₅₀, and half the LD₅₀ of the new product, together with a similar trial using a well-known standard (e.g. deltamethrin at 25mg a.i./m²). An appropriate operational dose for the new product can then be estimated as the dose expected to give a result similar to the standard, for example from a plot of the proportion of infested houses where infestation appears to have been eliminated, against dose rate.

3.1.4 Selection of Trial Site

For a single village trial, the community should be selected initially in accordance with four criteria: 1) proportion of houses (or domestic units - UDs - including peridomestic habitats) with known or suspected presence of the target vector; 2) acceptability of the trial by local householders and community authorities; 3) accessibility throughout the 12 month trial and evaluation period; 4) physical isolation from neighbouring communities.

Ideally the village should have at least 40-100 UDs of which at least 50% are expected to be infested with the target vector species (if less than 50% then the village should have a minimum of 20 houses infested). The village should be accessible at all times of the year, and should be physically discrete (ie. without great distances between neighbouring UDs, that would render access difficult for the spraymen). Householders and local authorities should be aware of the domestic bug infestations and prepared to accept the use of their village for the trial. The outermost UDs of the village should be at least 3km from the nearest neighbouring villages (this is to reduce the likelihood of reinvasion of treated houses by bugs

flying in from untreated communities). If possible, a scale map of the village is also greatly advantageous.

For multi-village trials, each village should be of a similar size and similar characteristics to those outlined above. Each village should be broadly similar in housing materials and styles, and should be within similar climatic and geophysical regions.

3.1.5 Personnel and Materials

The timing and implementation of the trial is crucially dependent on the availability of adequate personnel and materials. For a single village trial of 100 houses, at least 20 man-days will be required for each of the evaluations (pre and post-spray) and for the spraying itself. This assumes that one two-man team will be able to evaluate or spray a maximum of 10 houses per day. However, fewer houses may be covered by each team if the UDs are more dispersed.

In general, a village trial involving 100 UDs will require 4 two-man teams together with supervisor/driver, so that each evaluation or spray round can be comfortably completed within a 5 day period. Each person should be trained in basic biology of the target vector, including transmission risks and sampling procedures. Manual sampling (section 3.2.5.1) is carried out in each UD for a set 30 minute period using a torch to see into crevices, together with long blunt-ended forceps to withdraw any bugs and put them in a sealable plastic flask. It is important that pointed forceps not be used since these can rupture the bug, risking contamination of the operator with the bug's intestinal contents. In any case of suspected contamination, the skin should be swabbed with 70% alcohol (supervisors should carry eyewash with disinfectant eyewash for any case of contamination in the eye). Basic equipment requirements for each person are given in Annex 2.

In planning the trial, it is important to ensure that trained personnel, materials, equipment and transport will be available for each phase and each evaluation period throughout the trial (see Table 1 for trial chronogram). Where possible, it is preferable to carry out the prespray evaluation about 2-4 weeks before spraying. This allows time for passive monitoring of infestations just prior to intervention (see Section 3.2.5.2) providing an additional check on baseline infestation levels.

3.2 Prespray Evaluation

The aim of the prespray evaluation is to obtain the following:

- informed consent of local authorities
- informed consent and cooperation of householders
- operational sketch map of village, with all houses numbered and readily identifiable
- geographical and climatic data about the village
- baseline levels of domestic and peridomestic infestations with triatomine bugs
- epidemiological data if available

3.2.1 Informed Consent of Local Authorities

It is important that the nature, purpose, and timescale of the trial, and its expected results, be fully explained to the local authorities – usually by the team supervisor. Often a brief written agreement ('convenio') is signed between the appropriate representative of the trial team and the competent authorities. It is advantageous if the authorities can offer some form of temporary accommodation for the spray team, where they can sleep and store equipment. The local authorities can also help to communicate about the trial to the local householders.

3.2.2 Household Visits

Once local authorities have agreed, each house is visited by one of the two-man teams. They explain the nature of the trial and its expected results, and enquire to what extent the householders may be aware or bothered by triatomine bugs or other household pests (e.g. fleas, flies, cockroaches, scorpions etc). It is likely that the trialed product may have a beneficial effect against other domestic insects, although this will probably be evaluated only in terms of householders comments before and after the trial. It is helpful if each team member has a preprepared specimen of the target vector species (or life-size colour photographs) that he can show to the householders in order to see if they recognise it.

The teams should explain to each householder what they propose to do in terms of prespray evaluation, spraying and postspray evaluations, noting that householders are asked to participate in the following ways:

- accept the use of passive monitoring tools in their houses (see section 3.2.5.2)
- agree to manual searches of their houses to find triatomine bugs (see section 3.2.5.1)
- collect any bugs they find into the plastic bag which will be provided (see section 3.2.5.3)
- prepare their houses for the spraying (see section 3.3.1)

At this time, the teams should plot the position of each house on the sketch map, affix a number to the house (preferably painted on the doorframe), and note the number of the house and the name of the householder, together with any additional information about householder reports of domestic bugs or other insects. The number and approximate ages of all household residents should be noted, together with presence and approximate number of domestic animals. The approximate size

of the house and peridomestic structures should also be measured (e.g. by pacing along the length and width). This will be useful in estimating the amount of product to be sprayed (see section 3.3.2). A manual search for bugs can also be carried out at this time, or the teams may arrange to return later in order to do this (see section 3.2.5.1).

Each house should have a visit card affixed to the inside of the door frame. This card should bear the house number and name of the head of the household, with columns to note the date of each visit, name of the sprayman or inspector who visited the house, and a note of activities carried out at that time (eg. spray with product X at xx mg a.i./m²; manual search positive or negative) (see Annex 4.1)

3.2.3 Operational Sketch Map

The operational sketch map should be as accurate as possible, noting the position of all houses (appropriately numbered) together with geographic features such as hills, rivers, streams, roads, tracks etc. Distances between houses can be estimated by pacing. Together with the sketch map, the supervisor should collate the team reports showing number of each house, approximate dimensions, name of householder, number and ages of residents, nature of peridomestic structures, number of animals, householders reports of triatomine bugs and other domestic insects.

Where possible, it is recommended that a GPS (Global Positioning Sensor) be used to provide precise geographic coordinates and altitude of each village in the trial, and each house within the village. Use of a commercial GPS will greatly assist in accurate mapping of the trial area, although commercial systems can be expected to have random errors of up to ± 100 metres in position sensing.

3.2.4 Geographical and Climatic Data

The supervisor should prepare a description of the village, indicating location, altitude, vegetation type (eg. forest, cerrado, chaco, arid etc), soil type (e.g. laterite, sandy etc), and try to get a general description of house styles and predominant construction materials. A brief description of main industries (eg. cotton, cattle, etc) is also helpful. It is also important to know approximate annual temperature fluctuations and rainfall patterns, and if possible, the supervisor should arrange for one householder to use a maximum and minimum thermometer on a weekly or monthly basis.

3.2.5 Baseline Infestation Levels

During the prespray evaluation, each house (including public buildings such as churches, clinics, schools etc.) should be checked for the presence of bugs. Three procedures are employed: manual searches (with and without dislodgant agents), passive monitoring tools and householder collections.

3.2.5.1 Manual Searches

A manual search involves two men searching the house and peridomestic structures for a period of 30 minutes each, using a torch to look into cracks and crevices throughout the fabric of the buildings, and behind pictures on the walls, and especially under bedding material. All live bugs and dead bugs (adults and nymphs), exuviae (cast nymphal skins), eggs and eggshells, should be collected and placed in the plastic flask numbered with the same number as the house together with the date. In addition, note should be taken of any evidence of faecal streaks of bugs on walls, pictures etc. After 20 minutes, if no live bugs have been found, a dislodgant agent (eg. 0.2% tetramethrin in water) is sprayed into cracks and roof space, and the search continued for a further 10 minutes.

3.2.5.2 Passive Monitoring Tools

Once the 30 minute manual search is completed, regardless of the result, the teams pin one sheet of white paper (A4 size or similar) on one wall of each room in the house (at approximately head height). The paper should bear the date and number of the house, and will serve to monitor possible faecal streaks left by bugs as they walk over it. This has been found to be a sensitive and cheap method of passive monitoring for the presence of live triatomine bugs, although other more expensive devices are also available (e.g. Gómez-Núñez box; TDR Biosensor; Cohen trap etc). An identification key is available to check the origin of any streaks that may be subsequently found on the papers (Schofield et al., 1986).

3.2.5.3 Householder Collections

The third approach to monitor infestation rates involves collections of bugs by the householders themselves. At the end of the manual search, the teams will give a self-sealing plastic bag to the householders, requesting them to collect and keep any bugs that they may find.

3.2.5.4 Back Correction of the Infestation Data

The baseline (prespray) infestation level (proportion of houses infested) is an estimate based on the finding of live bugs or other evidence of infestation prior to spraying. However, none of the methods indicated above is ideal, and some houses reported as apparently negative may in fact have low level infestations that were not detected during the initial survey work. Such houses may be found to have been infested, by the appearance of bugs during the spraying itself – for example, by live bugs being seen as the house is sprayed, or by live or freshly dead bugs being collected by the householders during the month after spraying (section 3.2.5.3), or by bugs, exuviae, or faecal streaks detected

during the month after spraying by a passive sampling device (section 3.2.5.2). In such cases, the baseline infestation level should be 'back-corrected' to include those houses that initially appeared to be uninfested but were subsequently shown to be infested during the month after spraying.

Example: *During a trial of Cyfluthrin 10WP against T. dimidiata in Nicaragua, prespray evaluation by timed manual collection in 49 houses in one locality revealed bugs in six of them (12.2%). Immediately after spraying, however, householder collections in the plastic bags showed that one house reported as negative during the prespray evaluation did in fact have a low level of infestation with T. dimidiata. By back correction therefore, 7 houses (rather than 6) could be considered as positive at prespray (14.3%). (data from: Acevedo et al., 2000).*

3.2.5.5 Storage of Captured Bugs

From the capture data, three measures are derived: 1. the number of houses with live triatomine bugs; 2. the number of houses with either live bugs or possible infestations (i.e. evidence of infestation such as cast exuviae or faecal streaks), 3. the number of houses in which only peridomestic infestations are found. Once these measures have been derived, all bugs should be killed (e.g. by exposing the flasks to direct sunlight or otherwise heating to above 40°C for 30 minutes) and stored dry for subsequent morphometric examination (see Section 5). Note that it is not necessary to dissect bugs to determine parasite rates, since this information has no direct bearing on the trial results (see section 3.2.6).

3.2.6 Epidemiological Data

For a trial aimed at demonstrating the effectiveness of a specified intervention to reduce house infestation rates, there is

no need to collect epidemiological information such as parasite rates or seropositivity of householders. Over the trial period of 12 months, serological indicators are unlikely to change significantly because those residents already infected with *T. cruzi* will remain so. Similarly, since the proportion of infected bugs simply reflects their likelihood of taking an infected blood meal, this is also unlikely to change significantly (except through density-dependent effects, see: Kirk & Schofield, 1987; Trumper & Gorla, 1991). Moreover, dissection of bugs to determine parasite rate is laborious, time-consuming, and represents an unnecessary hazard for the investigator. In addition, it destroys baseline material that can be useful in determining the source of any reinfestations discovered during the trial (see Section 5).

Nevertheless, it is often the case that serological or other epidemiological data is already available for a particular trial region, in which case it forms a useful background to be included in the description of the trial site.

3.3 Spray intervention

Once the prespray evaluation is completed, the spray intervention can be implemented. All UDs and other buildings should be sprayed, together with all peridomestic structures, regardless of infestation rates. However, one uninfested public building (such as a church or school) should be left unsprayed and designated as control for wall bioassays carried out during the postspray evaluations (see section 3.4.2.). In the event that a UD cannot be entered (e.g. the householder is absent or bedridden) then the house should be sprayed only over the external surfaces and peridomestic structures.

Spraying is normally carried out using manually operated constant pressure backpack sprayers of 8 or 10 litre capacity, fitted with a manometer able to measure up to 60psi, together with an application lance fitted with a Teejet 8002 nozzle or

similar, that will provide an application flow rate of about 750-800 ml/minute at a pressure of about 50psi (see WHO, 2000).

3.3.1 Preparation of House for Spraying

Just prior to spraying, all foodstuffs, kitchen utensils, and domestic animals should be removed from the building, and all furniture should be pulled away from the walls to allow access behind. Clothes and bedding materials should also be removed and hung out in the sun¹. Residents should stay clear of the house while spraying is being carried out, and should preferably not re-enter until at least 30 minutes afterwards.

3.3.2 Procedure for Monitoring Applied Dose Rate

It is vital to have a good estimate of the applied dose rate, in order to check that the average target application dose has been achieved, and to detect possible operational errors in product preparation, dilution, and application. The application rate is monitored in two ways, firstly in relation to the number of tank charges used per UD, and secondly by subsequent HPLC residue analysis from sentinel filter papers.

3.3.2.1 Tank Charges

Assuming that all internal and external wall surfaces are to be sprayed, together with the interior roof surface and exterior of furniture, the approximate surface area to be sprayed (A) can be estimated as: $A = 4h(w+l) + 1.5wl$ where h is the average height of the house walls, w is the width of the house, l is the length of the house (all measured in metres). This is not a precise measure, but will provide a guide to the number of tank charges required for spraying each house.

¹ Exposure to tropical sunlight for 10-15 minutes is usually lethal to any triatomine bugs that may be hiding amongst the clothes or bedding.

Depending on the product, it is usually feasible to calculate the required product concentration in each tank charge which, when sprayed at a rate of 40ml/m², will achieve the desired application dose per square metre. For example, one 10 litre tank charge will spray a total of 250 m² at 40 ml/m², so that the tank charge must be made up to contain (250 x target dose rate)mg active ingredient. Thus, if the target dose rate is a mg/m² and the product is delivered at a concentration of $b\%$, then the tank charge should contain $25a/b$ gms formulated product. Similarly, an 8 litre tank charge should contain $20a/b$ gms formulated product.

Example: *Product is supplied as a 5% wettable powder (5WP), to be sprayed at a target application dose of 50mg a.i./m², using an 8 litre spraypack. Therefore, the tank charge should contain $20 \times 50 / 5 = 200$ gms of the 5WP formulated product.*

3.3.2.2 Sentinel Filter Papers

Just prior to spraying, the supervisor should affix a series of clean filter papers (Whatman no.1) at various heights on several walls of each house. 4-5 papers per house is usually sufficient, and each paper should be labelled in pencil with the house number and wall height. About 1 hour after spraying (to allow the papers to dry) the papers are removed (using forceps) and placed individually into folders of aluminium foil, which can then be placed into self-sealing plastic bags to be shipped to the laboratory where a sample of the papers can be analysed by HPLC. The main purpose of this residue analysis is to check that the average target application dose has been achieved.

3.3.3 Spray Application

In accordance with the labelled safety instructions of the product to be applied, spraymen should wear protective overalls, boots, gloves and transparent face mask [note that the absolute minimum protection in all cases is a transparent face mask or vizor]². They should spray all internal and external walls of each UD (including peridomestic habitats) together with the internal roof surface and principal hard furnishings. Normally the spraying is made with the nozzle held about 45cm from the target surface, moved in swaths of about 40-50 m/sec to deliver an average of about 40ml/m² (see WHO, 2000). It is important that proper coverage be given even to 'hidden' areas such as under seats, under beds, around roof joists etc. Spray application can be reinforced over deep cracks in the walls.

Throughout the spray procedure, the supervisor should monitor the spraymen for any reports of skin or eye irritation, or other signs of discomfort. In the case of skin contact carefully remove contaminated clothing and wash affected area with soap and clean water. In case of persistent irritation, seek medical advice. In case of eye contact, rinse immediately with clean water for at least 15 minutes and obtain medical aid. In case of accidental ingestion, wash out mouth with water. Keep patient at rest and obtain medical advice immediately (WHO, 1997).

3.3.4 Disposal of Waste

After spraying, spraymen should wash themselves and their protective clothing with water. Used product containers should be counted (as a check on the quantity of product used – see Section 6.1.) and then burned and the ashes buried.

² If using alpha-cyano pyrethroids, it may be advisable for spraymen to apply vaseline or barrier cream to face and hands, in order to reduce any transient skin irritation that these products can sometimes cause.

3.4 Postspray Evaluations

Postspray evaluations are carried out at 1, 3, 6, 9, and 12 months after completion of the spray interventions. They are primarily designed to monitor bug infestation levels and residual activity of the applied product. However, householders should also be asked for their comments – positive and negative – about the results of the spray intervention, especially in terms of noticeable effects on triatomine bugs and other domestic pests, smell and appearance of the spray deposits, and any effects on themselves or their domestic animals.

3.4.1 Postspray Infestation Levels

Each UD should be visited by a two-man team who will check the papers for evidence of bug faeces (see Section 3.2.5.2) and the plastic bags for any bugs collected by the householders (see Section 3.2.5.3). The teams then carry out a manual search following the procedure outlined in section 3.2.5.1, ensuring that any bugs captured are carefully labelled and stored for subsequent analysis (see Section 5). [Note that evidence of infestation up to 1 month after spraying in houses previously considered as uninfested, can be used to back-correct the baseline infestation levels (see Section 3.2.5.4)]

3.4.2 Wall Bioassays

Wall bioassays can be used as a measure of operational spraying efficiency and/or of residual presence of the applied insecticide. Note however, that these bioassays are not designed in any way to provide information on possible insecticide resistance.

In a preselected sample of at least 4 houses (plus the untreated control building, see section 4) wall bioassays should be carried out on each of two wall surfaces of each house during each of the postspray evaluations (note that this will require a minimum

of 100 fifth-instar triatomine nymphs for each evaluation). The technique is to attach an open-sided box to the wall using nails and adhesive tape, so that the open side is against the wall surface. The nature of the wall surface should be noted in each case (e.g. unplastered mud, painted mud, wood etc.) together with its orientation (e.g. shaded, internal, external etc) and boxes should be positioned on an adjacent part of the same surface for each successive evaluation (i.e. never place the boxes in exactly the same position). The boxes can be made from used X-ray film. Each box should be approximately 12 x 14 cms, angled to be 4-5cm deep at the top but flush at the bottom. The upper edge is then prised open before sealing in order to introduce 10 laboratory-reared fifth instar nymphs for the assay. The nymphs are left in place for 24h and then transferred to clean containers for readings of knockdown and mortality at 24h, 7 days and 14 days, after which the bugs are destroyed. Knockdown is defined as a bug unable to walk normally (even though it may appear to be alive) while mortality can be defined as a bug unable to cling to a piece of inclined filter paper³ (see Section 4.2).

Note that the exposure time of 24h is rarely sufficient to achieve 100% mortality, even just after spraying. It is preferable therefore, to choose a longer exposure time (e.g. 48h) but this is not always possible for operational reasons. Whatever exposure time is chosen however, it should be the same for all bioassays during each evaluation.

4. Interpretation of Results

The following results can be expressed graphically and/or in tabular form (see Guillen et al., 1997):

³ An alternative is to define as dead those insects that are unable to walk over a filter paper, either spontaneously or when prodded (WHO, 1994)

4.1 Spray Deposit Rates

- correlation between target dose as estimated by number of used containers and applied tank charges

Example: *In a trial of lambda-cyhalothrin 10WP, the product was supplied in water-soluble sachets containing 60gm of formulated product, with each sachet contained in an individual plastic outer sachet. The target dose rate was 30mg a.i./m², using 8-litre backpack sprayers, so that one sachet was sufficient for one tank charge. 100 houses were to be sprayed, each of around 200 m², so that the supervisor could expect at least 100 sachets to be used. After spraying, the spraymen reported a total of 110 tank charges used, and so should have returned 110 empty outer sachets to the supervisor.*

- variation of target dose as estimated by HPLC residue analysis of filter paper deposits

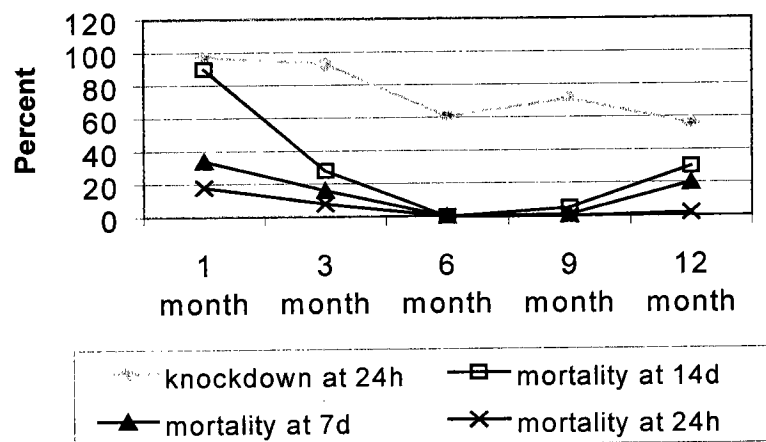
Example: *In a comparative trial of deltamethrin 2.5SC, bifenthrin 10WP, and cyfluthrin 10WP, sentinel filter papers were attached to internal house walls of 20-29 houses for each product, at heights of 0.5, 1.0, 1.5, and 2.0 metres above floor level. A random sample of these papers was analysed by HPLC. The results showed wide variation, but the average delivered dose of bifenthrin (50.9 ±40.9 mg a.i./m²) was satisfactorily close to the target dose of 50 mg a.i./m², and the average delivered dose of cyfluthrin (45.6 ±19.1mg a.i./m²) did not differ significantly from the target dose of 40 mg a.i./m². For deltamethrin however, the average delivered dose revealed by HPLC analysis (11.1 ±9.4 mg a.i./m²) was significantly below the target of 25 mg a.i./m², and suggested an operational error in the initial*

calculation of the dilutions (data from Ramsey et al., 2000).

4.2 Wall Bioassays

These results should be expressed as % knockdown after 24h exposure, at each of the postspray evaluations, together with % mortality at 24h, 7 days and 14 days. (Note that if a bug is classified as dead at 24h it must also be included as having been knocked down in that period.)

Example: *During a trial of deltamethrin 2.5SC in southern Bolivia, applied at 25mg a.i./m², 300 T. infestans fifth and fourth stage nymphs were subsequently exposed to treated walls at each of the evaluation periods in order to measure % knockdown and mortality. The results were expressed graphically as follows (from Guillen et al., 1997) (reproduced with kind permission of Memorias do Instituto Oswaldo Cruz):*

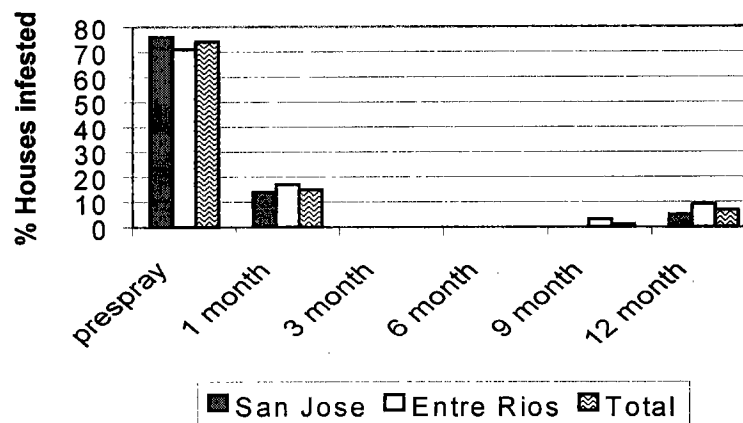


Note that, as expected, the % knockdown declined steadily with the age of the deposits, but that the % mortality showed an increase towards the end of the trial. This was interpreted to reflect negative temperature-dependence, which is well-known for the activity of several pyrethroids (see: Leahy, 1985), because the increasing activity corresponded to the onset of the cooler winter months.

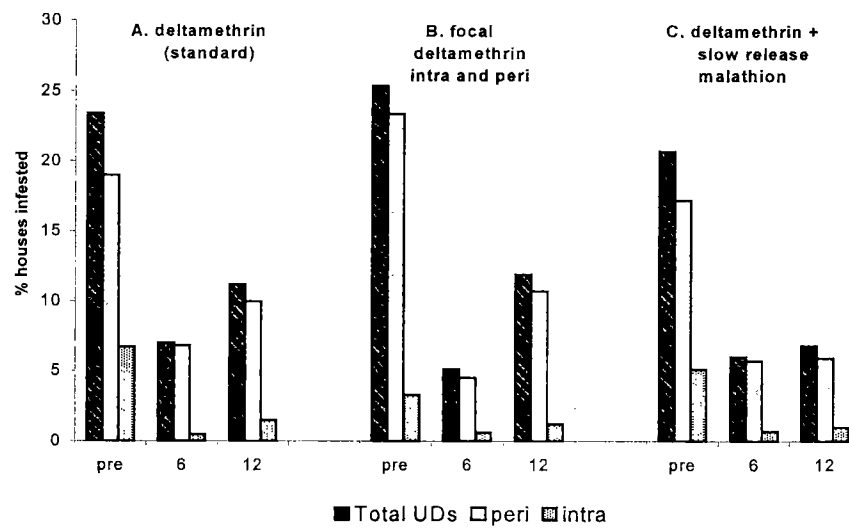
4.3 House Infestation Rates

- apparent infestation rates (% of total UD's) against time (months) after spraying (prespray evaluation is denoted as time 0). This should be expressed as (a) observed infestation rates according to live bugs captured (b) corrected infestation rates, whereby the prespray rate includes UD's with live bugs, together with UD's showing evidence of infestation such as exuviae or faecal streaks, and the postspray rates include houses where the paper sheets indicated new faecal marks from bugs (see examples 1 and 2).

*Example 1. In a trial of deltamethrin 2.5SC against *T. infestans* in southern Bolivia, two villages (San Jose and Entre Rios) were sprayed at a target dose of 25mg a.i./m², and subsequently evaluated by timed manual collection at 1,3,6,9, and 12 months post spray. House infestation rates in the two villages were expressed as % houses in which live bugs were captured, as follows (from Guillen et al., 1997): These results were interpreted as showing that the treatment completely eliminated all apparent bug infestations 1-3 months after spraying. However, some bugs were again found at the 9 and 12 month evaluations, which in this particular study were attributed partly to bugs surviving in peridomestic habitats, and partly to accidental passive carriage of bugs by people visiting from another region of Bolivia (reproduced with kind permission of Memorias do Instituto Oswaldo Cruz).*



Example 2. In a trial against *T.brasiliensis* in NE Brasil, several treatments were evaluated for their effectiveness in controlling peridomestic infestations. These included: A – deltamethrin 5SC at 25mg a.i./m² (used as standard), B – deltamethrin applied only to suspected foci where the bugs might have been hidden (such as chicken roosts), C – deltamethrin applied inside the houses, and a slow-release formulation of malathion at 2g a.i./m² applied to the peridomestic areas. Each of the sprayed communities was evaluated by timed manual collection at 6 and 12 months post-spray, and house infestation rates expressed as follows (data from Oliviera Filho et al., 2000): Note that in this case, the peridomestic habitats were highly infested, and although all treatments produced an initial reduction in the infestation rates, none was able to eliminate the infestations altogether (reproduced with kind permission of Cadernos de Saude Publica).



In the above examples, trial results have been concisely expressed as the proportion of houses or UDs infested before and after the intervention, expressed either as an aggregate for houses and peridomestic habitats together (example 1) or showing separately the peridomestic and domestic infestations (example 2). The proportion of houses infested is also known as the 'house infestation rate' or 'house infestation index' and is one of a series of indices proposed by a WHO working group (WHO, 1991b) as follows⁴:

$$\text{a. Infestation index} = \frac{\text{number of houses infested}}{\text{number of houses examined}} \times 100$$

$$\text{b. Density index} = \frac{\text{number of bugs captured}}{\text{number of houses examined}}$$

⁴ The original WHO document inadvertently gave errors in the formulae for density and crowding indices.

$$\text{c. Crowding index} = \frac{\text{number of bugs captured}}{\text{number of houses infested}}$$

$$\text{d. Dispersion index} = \frac{\text{number of localities infested}}{\text{number of localities examined}} \times 100$$

$$\text{e. Colonisation index} = \frac{\text{number of houses with nymphs}}{\text{number of houses infested}} \times 100$$

$$\text{f. Natural Infection index} = \frac{\text{number of bugs infected with } T. \text{ cruzi}}{\text{number of bugs examined}} \times 100$$

Except for the infestation index, and to a lesser extent the colonisation index, these measures are rarely used. The natural infection index is irrelevant for trial work (see Section 3.2.6) but is sometimes used in long-term monitoring of large-scale control interventions.

5. Interpretation of Reinfestations

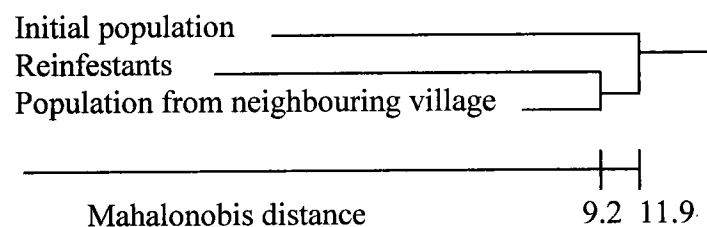
The expected result of a thorough control intervention is the complete elimination of all domestic and peridomestic bug infestations within about 1 - 3 months. If bugs are discovered in treated houses after this time then they may be due to bugs surviving the initial treatment (indicating poor control coverage) or they may be due to bugs that have migrated into the trial site

after the initial intervention. It is important to distinguish between the two effects.

Interpretation of apparent reinfestations is difficult, because it may involve either survivors or true immigrants that have come into the trial community either by passive carriage or active flight. As a first step, householders in UDs where reinfestations are found should be asked about any visitors they have received who may have come from other infested areas, or visits they may have made to infested areas. The position of reinfested UDs should be also noted on the sketch map. It may be that reinfestations are found only on the periphery of the community, which is suggestive of active flight from untreated habitats, or it may be that reinfestants are found only in houses where visitors have come from untreated regions, which is suggestive of passive carriage. If the apparent reinfestations involve nymphal stages, these should be identified to species and stage, and then reared to adults. These can then be compared by morphometric analysis to determine if they conform to the same morphometric profile of the original bug populations from the prespray captures (Dujardin et al., 1997a,b, 2000).

Example: *In a trial of deltamethrin 2.5SC against T. infestans in the village of Abapo, Bolivia, an apparent reinfestation of bugs was found in some houses 6 and 8 months after spraying. These 'reinfesting' bugs (R) were compared with a previously collected sample of the initial prespray population (I) and with bugs collected from the neighbouring village of Rio Seco that had not been sprayed (V). The test hypotheses were as follows: If $I = R$ and $R \neq V$, then the reinfestants are a residual population that survived treatment; If $I \neq R$ and $R = V$, then the reinfestants have been accidentally carried in from the neighbouring village; If $I \neq R$ and $R \neq V$, then the reinfestants have probably been accidentally carried in from an unknown locality; If $I = R$ and*

R = V, then no conclusions are possible. *The comparison was done by size-in and size-free morphometry of head characters, and confirmed by isoenzyme comparison (Dujardin et al., 1999). All three comparisons gave similar results, showing that the reinfestants were most similar to bugs from Rio Seco, rather than to the original population, suggesting that reinfestation had been due to bugs accidentally transported by people visiting from Rio Seco, rather than bugs surviving the insecticide treatment. The graph below shows the UPGMA comparison of Mahalanobis distances used to measure global metric differences between populations (from Dujardin et al., 2000) (reproduced with kind permission of Academie Royale des Sciences d'Outre Mer):*



Several markers have been developed for the analysis of reinfestations, including isoenzymes (but only if live bugs are available), RAPD, and microsatellite DNA sequence comparisons, but the simplest and probably most informative approach is to use morphometric comparisons based on measurements of the head capsule (see: Casini et al., 1996; Dujardin & Casini, 1996; Dujardin et al., 1996, 1997a,b, 1998, 2000). One of the simplest analyses is to use the method of Darroch & Mosimann (1985) (see Annex 5).

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Annex 1. Summary Chronogram of Trial Activities

(The time of spray intervention is designated as time 0)

Month -2	Preparatory phase (selection of trial site; preparation of materials, training of field personnel)
Month -1	Prespray evaluation (householder visits, prepare operational sketch map, collect climatic and geographical data, baseline house infestation levels, application of passive monitoring tools)
Month 0	Spray intervention (check passive monitoring tools; affix sentinel filter papers; spray all UDs (except for building designated control for wall bioassays))
Month 1	Postspray evaluation (householder interviews, check passive monitoring tools, manual search for bugs; wall bioassays)
Month 3	Postspray evaluation (as month 1)
Month 6	Postspray evaluation (as month 1)
Month 9	Postspray evaluation (as month 1)
Month 12	Postspray evaluation (as month 1)
Month 13	Evaluation of results (interpretation of reinfestations [if any]; preparation of final report) (Recommended respray of any houses found to be infested during final postspray evaluation).

Annex 2. Basic Material and Equipment Requirements

1. Prespray and Postspray Evaluations

Each person should be equipped with the following:

- watch
- pencil and record book
- torch (minimaglight or better)
- spare batteries and spare bulb (minimum of one set for each evaluation)
- blunt-ended forceps (at least 10cm reach)
- wide-mouthed clear plastic flasks with lid and label (one per house)
- prepared specimen of target vector species, or life-size photograph (to show as reference to the householders)
- plastic bottle containing approx. 100cc alcohol (70%)
- cotton wool or tissue paper swaps
- pistol-type plastic spraygun suitable for application of dislodging agent (0.2% aqueous tetramethrin or equivalent)
- pack of self-sealing plastic bags

The alcohol and swabs are for disinfecting the skin in case of accidental contamination with bug intestinal contents. In addition, the supervisor should carry an eyebath and eyewash in case of accidental contamination in the eye. Note that materials for the house numbering system (e.g. cards or paint) will also be required during the prespray evaluation.

2. Spray Interventions

Each two-man team should be equipped with the following:

- protective clothing (including face masks, boots, and gloves)
- backpack sprayer (e.g. 8 or 10 litre Hudson X-pert)
- sachets or bottles of product to be trialed

In addition, the supervisor should have appropriate tools and spares for the sprayers, together with requisite filter papers and self-sealing plastic bags for the application dose monitoring.

3. Wall Bioassays

Each bioassay will require one bioassay box, nails (hammer) and adhesive tape, together with 10 laboratory reared fifth-instar nymphs of the target vector species.

Annex 3. Supervisors Checklist

1. Preparatory Phase: Product and site selection, materials, transport, personnel and training.

- ⇒ product selected
- ⇒ dose rate(s) determined
- ⇒ spraymen trained
- ⇒ site(s) selected
- ⇒ transport available
- ⇒ quantity of product required for spraying
- ⇒ evaluation and spray equipment (including toolkit and spares)
- ⇒ visit cards, report cards, sketch map materials, maximum-minimum thermometer

2. Prespray Evaluation: Approval of householders and local authorities, site mapping, prespray infestation levels.

- ⇒ discussion with local authorities; consent given; convenio prepared and signed
- ⇒ one building declared for bioassay control to be left unsprayed
- ⇒ one householder assigned to note maximum and minimum temperatures (and days rainfall if possible)
- ⇒ teams assigned for house visits
- ⇒ teams equipped with torch, forceps, specimens or photographs of target insects (see Table 2)
- ⇒ teams have visit cards (plus nails or pins to affix these to inner doorframes)
- ⇒ teams have paint to number each house
- ⇒ teams have report cards to note householder consent and comments, and sketch map pad

- ⇒ teams have torch, forceps, dislodgant agent, paper sheets, plastic containers for captured bugs, self-sealing plastic bags to leave with householders.
- ⇒ teams dispatched for house visits
- ⇒ general site description prepared.
- ⇒ at return of teams, supervisor records evaluation results (manual searches intradomiciliary and peridomiciliary, householder reports, paper sheets to be checked for bug faecal streaks, householder collections in plastic bags).
- ⇒ live bugs should be killed and mounted on labelled pins in secure box

3. Spraying: Application of trial product; monitoring of applied dose; passive evaluation tools.

- ⇒ one public building to remain unsprayed for bioassay controls
- ⇒ teams assigned for house-spraying
- ⇒ copies of sketch map prepared
- ⇒ sentinel filter papers in place in approx. 4 houses
- ⇒ required quantity of product to be sprayed
- ⇒ teams dispatched for house-spraying
- ⇒ at time of spraying, spraymen collect householder plastic bags with bugs, and leave new bags.
- ⇒ at post-spray, supervisor records number of used product containers, spraymen report quantity of product used and number of tank charges per house.
- ⇒ supervisor collects sentinel filter papers
- ⇒ spraymen comment on ease of spraying, irritation or other negative aspects, accidents
- ⇒ disposal of used product containers
- ⇒ send sentinel filter papers for HPLC analysis

4. Postspray Evaluation: Monitoring of infestation levels at 1, 3, 6, 9 and 12 months postspray; wall bioassays for residual product activity; household interviews.

- ⇒ teams assigned for postspray evaluation
- ⇒ teams equipped with sketch map, torch, forceps, dislodgant agent, paper sheets, plastic containers for captured bugs, self-sealing plastic bags to leave with householders.
- ⇒ teams dispatched for house evaluation
- ⇒ houses selected for wall bioassays
- ⇒ supervisor has bioassay boxes, nails and tape, groups of 10 laboratory reared nymphs
- ⇒ at return of teams, supervisor records evaluation results (manual searches intradomiciliary and peridomiciliary, householder reports, paper sheets to be checked for bug faecal streaks, householder collections in plastic bags).
- ⇒ collected bugs should be killed and mounted on labelled pins in secure box

5. Interpretation of Results

- ⇒ objectives of trial
- ⇒ product description and methodology
- ⇒ general site description
- ⇒ prespray infestation rates
- ⇒ presence of other household pests
- ⇒ local authority and householder comments (recognition of target and non-target insects)
- ⇒ sprayman comments (ease of use, smell, irritation, accidents)
- ⇒ householder comments (acceptability of spray, smell, irritation, effect on target and non-target insects)
- ⇒ HPLC results (average achieved dose)

- ⇒ domestic and peridomestic infestations at prespray (month 0) and postspray (months 1, 3, 6, 9, 12) (timed manual collections intradomiciliary and peridomiciliary; householder collections; faecal streaks on paper sheets)
- ⇒ bioassay results: knockdown and mortality at 1, 7, 14 days post exposure
- ⇒ temperature and rainfall (possible temperature dependence of product activity)
- ⇒ analysis of reinfestations (species, stage, morphometric comparisons)
- ⇒ discussion of acceptability and effectiveness

Annex 4. Sample Data Entry Forms

Annex 4.1 Example of Visit Card to be Affixed Inside the Door of each House

Name of Trial: _____		
Country: _____ ; Department/State/Province: _____ ;		
Municipality: _____ ; Village: _____ ;		
Locality: _____		
House number: _____ ; Name of Householder(s): _____		

Date of Visit	Activity	Inspector

Annex 4.2 Example of Data Entry Sheet for Each Visit to Each House

The conduct of the trial will be aided by clear data entry sheets prepared in accordance with the primary and secondary trial objectives (see Section 3.1.1). The unit of study is the individual house, so that one data sheet is required for each house in the trial. IT IS VITAL that each house be clearly and unequivocally identified (see Section 3.2). The following offers a basic data entry sheet suitable for each visit to each house, where items in black refer to the primary objectives, and items in italic refer to possible secondary objectives.

Name of Trial _____
Country: _____; **Department/State/Province:** _____;
Municipality: _____; **Village:** _____;
Locality: _____

House number _____; **Name of Householder(s)** _____

Usual number of occupants: _____;

Description of House: situation /main construction of walls (eg. wooden boards, palm logs, palm leaves, grass thatch, fired brick, adobe, tapiale, plastered or unplastered) /main construction of roof (eg. palm leaves, grass thatch, wooden boards, ceramic tiles, corrugated fibre-board, corrugated metal), /form of floor (eg. beaten earth, concrete, ceramic tiles), /number of rooms, /general state of repair and hygiene

Peridomestic habitats: chicken house(s) /goat corrals, /stable, /pigsty, /other

Date of visit: _____; Time of arrival: _____;
Time of departure: _____

Activity: manual sampling for mm minutes, / manual sampling
using dislodgant, / spraying with product X using xx doses or
tank charges

Inspector(s): _____

Bugs encountered (L-alive; D-dead)

Intradomestic:

Males Females N5 N4 N3 N2 N1 Eggs Exuvia
Dejecta

Other: Cockroaches /Scorpions /houseflies /fleas

Peridomestic:

Males Females N5 N4 N3 N2 N1 Eggs Exuvia
Dejecta

Other: Cockroaches /Scorpions /houseflies /fleas

Observations: _____

Inspector(s): _____

Annex 5.
Suggested Procedure for Morphometric Analysis of Reinfestations

Morphometric differences between bug populations of the same species may be shown as differences in size, or shape, or both. However, since differences in size may result from different growth rates (allometric differences) it is important to remove the effect of such differences from the analysis and so consider only differences in shape or form. The approach of Darroch & Mossimann (1985) has been adapted for use with Triatominae, and provides a way of removing the influence of size variation. The method is most easily applied to measurements of the head capsule of a sample of bugs (ideally at least 14 individuals of the same stage, preferably adults), which are then transformed using either a spreadsheet (such as EXCEL[®]) and analysed using a multivariate analysis package such as JMP[®], or the more specialised package NTSYS[®]. The sequence to be followed is summarised as follows:

Step 1. Take measurements of the head capsule of individual bugs using a binocular microscope (x25) equipped with a micrometer eyepiece (see Figure)(alternative methods include camera lucida or digital photography/image analysis).

Step 2. Enter the measurements (as mm) in the spreadsheet, organised as follows: columns are characters or variables (e.g. total length of head, postocular distance, etc), and rows are for individual specimens (ideally at least 14 individuals for each group).

Step 3. (spreadsheet) Log-transform the data. Without this transformation the analysis is not valid.

Step 4. (spreadsheet) Create an additional column containing the mean of each row. This is the “isometric estimator of size”.

Step 5. (spreadsheet) Create additional columns containing “shape variables”, which are the signed difference between the log-transformed data and the isometric estimator of size.

Step 6. Using the multivariate analysis package (e.g. JMP) perform a conventional Principal Components Analysis (PCA) on the shape variables to obtain “shape components”. Note that if the previous steps were correctly done, the last principal component should have null contribution to total heterogeneity.

Step 7. (JMP) Use the “shape components” (all the principal components except the last one) as input for a Canonical Variate Analysis (CVA). The resulting factor map will then show the “shape” differences between groups.

Step 8. (JMP) Perform a linear regression of relevant discriminant canonical factors (obtained by step 7) on ‘size’ (obtained by step 4) to verify the presence of any allometric residues (the determination coefficient indicates the amount of allometric content still present in the “shape components”).

Step 9. (JMP) Perform the calculation of Mahalanobis distances derived from the CVA performed on shape (step 7).

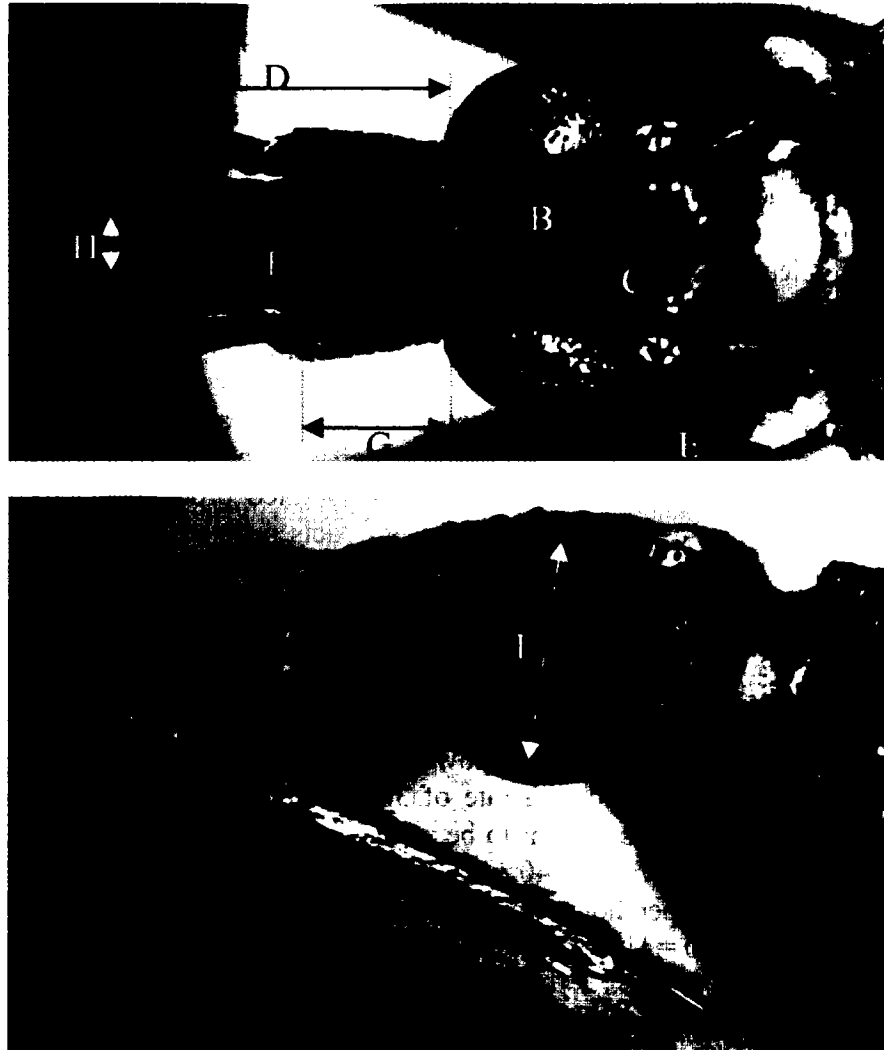
Step 10. (JMP) Construct an UPGMA tree using these distances.

References and Further Reading:

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- Darroch JN & Mossimann JE (1985) Canonical and Principal components of shape. *Biometrika* 72, 241-252
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- Klingenberg CP (1996) Multivariate allometry. In: *Advances in Morphometrics*. Proceedings of the 1993 NATO-ASI on Morphometrics (Marcus LF, Corti M, Loy A, Naylor GJP, Slice D. editors) *NATO-ASI ser.A, Life Sciences*, 23-49. Plenum Publications, New York.

Figure 1. Dorsal and lateral views of the head of an adult *Triatominae*, indicating morphometric measures that can be taken for group comparisons (reproduced with kind permission of Mr J.Patterson)



The proposed measures are as follows: A – maximum width across the eyes; B – width between the eyes; C – width between the outer edges of the ocelli; D – post-ocular distance; E – pre-ocular distance; F – head length; G – outer length of antenniferous tubercle; H – width of anteclypeus; L – maximum depth of eye (lateral view); R1, R2, and R3 – lengths of rostral segments.

Annex 6. Analysis of Costs

Detailed analysis of the full economic costs of house spraying is beyond the scope of these guidelines, although examples of such studies are given by Oliveira Filho (1989; 1994) and Basombrio et al. (1998). However, for the purposes of the field trials described here it may be desirable to compare the cost differences of two or more product applications. Such an approach provides for several simplifications, since it can be assumed that transport and labour costs are similar for each application, and that differences will occur only in the cost of the product and the rate at which it can be applied. These two cost factors can be estimated as follows:

1. Cost of Product: The cost of product applied is best estimated as an average unit cost per sprayed house, which will depend on the actual price of the formulated product and the average applied dose rate. Since products may be supplied in liquid or powder formulations, at various concentrations, it is recommended to make the calculations in terms of active ingredient. For example, suppose product A is supplied as a 5% suspension concentrate at a cost of US\$40 per litre, and is to be applied at a rate of 25 mg a.i./m². In contrast, product B is supplied as a 10% wettable powder at a cost of US\$60 per kilo, and is to be applied at a rate of 30 mg a.i./m². Assuming an average house has 200 m² to be sprayed, then the cost per house for product A will be $(40 \times 25)/(5 \times 10000) \times 200 = \text{US\$}4.00$, and the cost per house for product B will be $(60 \times 30)/(10 \times 10000) \times 200 = \text{US\$} 3.60$.

2. Rate of Application: The rate at which a product can be applied, in terms of the number of houses that can be treated per man per day, can have a marked effect on costs. This is because most of the cost of spraying a house is due to the costs of labour. For example, suppose product A is delivered in plastic 1 litre

bottles, whereas product B is delivered in 75gm sachets. Assuming the spray teams are using 10 litre compression sprayers, then for product B each sachet contains the precise amount for one tank charge, whereas for product A the spraymen must carefully measure out 80 ml of formulated product for each tank charge (and after 12 tank charges, there will be 40 ml remaining from each bottle, which may be wasted). This may mean that product B can be handled more quickly, without waste, and so lead to more houses being sprayed per man per day. To compare the cost implications of the two products in terms of their rate of application, the supervisor must have an estimate of the unit labour cost (daily wage of each sprayman) to be divided by the average number of houses sprayed per man per day, for each product.

References and Further Reading:

- Akhavan D (1998) *Análise de Custo-Efetividade do Programa de Controle da Doença de Chagas no Brasil*. Organização Pan-Americana da Saúde, Brasília. 271pp.
- Basombrio MA, Schofield CJ, Rojas CL, Del Rey EC (1998) A cost-benefit analysis of Chagas disease control in northwest Argentina. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 92, 137-143.
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