

Evaluation of commercial aerosol insecticides for control of *Aedes aegypti* susceptible or resistant to pyrethroids

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Abstract

Objective. To evaluate indoor use of commercial aerosols for dengue vector mosquito control, and estimate the number of treatable houses per can. **Materials and methods.** Four aerosol products containing combinations of pyrethroids (two containing propoxur and one containing synergists too), were evaluated with mosquitoes in a room of a Tapachula-style house. Eight cages containing 20 insecticide susceptible or resistant females were hung from tripods, another set was placed in sheltered areas of the room. From the entrance of the room, one of 4-9 concentrations was sprayed for each aerosol, leaving the mosquitoes for 30 min after sprayed. Mortality was recorded after 24 h and lethal concentrations were calculated. **Results.** Aerosol A had the highest LC_{50} with 0.308 g for mosquitoes hanging from tripods and 0.453 g for sheltered mosquitoes; followed by aerosols C, D and B, with statistical differences between types of exposure. **Conclusions.** Aerosols B-D could spray 20-25 3-room houses (56 m³-room), killing all resistant mosquitoes. Aerosols may become a good tool for indoor mosquito control, if the optimal concentration and correct spray method are used.

Keywords: *Aedes aegypti*; aerosols; insecticide resistance; control; indoor space spray

Resumen

Objetivo. Evaluar el uso en interiores de aerosoles comerciales para el control de mosquitos vectores de dengue, estimando el número de casas tratables por lata. **Material y métodos.** Se evaluaron cuatro aerosoles que contenían combinaciones de piretroides (dos propoxur y uno sinergistas también), en una habitación de casa estilo Tapachula con mosquitos dentro de jaulas. Ocho jaulas, cada una con 20 hembras susceptibles o resistentes a insecticidas, se colgaron en trípodos, junto con ocho jaulas escondidas en la habitación. Una de 4-9 concentraciones se roció desde la entrada de la habitación, dejando los mosquitos durante 30 min después del rociado; se estimaron las concentraciones letales con mortalidades después de 24 h. **Resultados.** El aerosol A tuvo los CL_{50} mayores, con 0.308 g para mosquitos de los trípodos y 0.453 g para mosquitos escondidos; seguido de aerosoles C, D y B, con diferencias estadísticas entre el tipo de exposición. **Conclusiones.** Los aerosoles B-D podrían rociar 20-25 casas de tres cuartos (56 m³ por cuarto), y matar a los mosquitos resistentes. Los aerosoles pueden convertirse en una herramienta para el control de mosquitos en interiores de casas, si se usa la concentración óptima y el método de rociado correcto.

Palabras clave: *Aedes aegypti*; aerosoles; resistencia a insecticidas; control; rociado espacial de interiores

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Controlling the primary vector is currently the most effective approach to reduce transmission for some vector borne diseases. Commercial aerosol insecticides are designed for usage in homes and recommended to control several types of pest arthropods,¹ but for vector-borne diseases could represent an additional tool in an integrated approach, particularly with community participation. Arboviruses transmitted by *Aedes aegypti* might be most controllable with commercial aerosols, since the vector is primarily confined to urban settings. For example, one study found that when aerosol cans were used in households, there were significantly reduced numbers of dengue cases when compared to those using traditional outdoor ultra-low volume (ULV) applications.² Others found aerosol cans to be among the most frequently used protective items purchased by households in Merida, Yucatan,³ demonstrating that it is a more accepted control measure compared to ULV applications.⁴

Adulticides are mainly applied as ULV by vehicle-mounted equipment, but backpack ULV sprayers or thermal foggers for indoor applications have been very useful for *Ae. aegypti* control.^{5,6} Indoor residual spraying (IRS) also has shown to be effective in controlling this species.⁷ Pyrethroids are commonly used insecticides, therefore pyrethroid-resistance in mosquitoes is high and broadly distributed worldwide.⁸⁻¹¹ Mexico is no exception to this, and different types of resistance with several mutations conferring knock down resistance (*kdr*) have been reported.¹²⁻¹⁶ Insecticide resistance will play a key factor on the efficacy of any aerosol applied indoors. Active ingredient (AI) composition and formulation could both contribute to the overall efficacy of the aerosol product. Permethrin resistance in Asian mosquito populations was most likely the reason for poor efficacy of a permethrin-based aerosol can as compared to a cypermethrin-based one.⁴ Also, all the products evaluated by Kuri-Morales and colleagues (2018)¹⁷ were pyrethroid-based formulations, and only 23% achieved 100% mortality.

In the context of insecticide resistance management, the impact that the use of commercial aerosols may have on both susceptible and resistant *Ae. aegypti* populations becomes relevant. Even if the same active ingredients are included in different commercial aerosols, the effectiveness may vary depending on its formulation. Therefore, it is recommended that assessments also be made on the different commercial aerosols containing the AI as well as synergists.¹⁸ Herein we evaluate the effectiveness of four commercial aerosols based on their calculated lethal concentrations (LC) against two strains of *Ae. aegypti*, one susceptible and one resistant to pyrethroids, under semi-field conditions in a Tapachula

style house. The feasibility and effectiveness of applying in the field the recommendations that we make according to our results would have to be evaluated together with the local control program, and the support of the community, where advice on the optimal concentration of the preferred aerosol insecticide and how to spray it, depending on the environmental and physical conditions of the houses, and whether the mosquitoes are resistant or susceptible to insecticides would be crucial for the expected success.

Materials and methods

Mosquito strains

The susceptible reference strain New Orleans, and the resistant field strain collected in Acapulco, México in 2015 with a calculated bifenthrin Resistant Ratio (RR) of 33 (WHO method) were used, and reared under insectary conditions at 27±2°C, 70±10 RH and 12:12 (L:D) h photoperiod. For each experiment, 1-3 day-old non blood-fed female mosquitoes were used, provided with 10% sucrose solution.

Test room and type of exposition for mosquitoes

The study was conducted in Tapachula from July to November 2016 (protocol evaluated by the ethics committee of the *Instituto Nacional de Salud Pública*, INSP). A typical room, 4.7 m long by 4 m wide and 3 m high, was used for the experiments (figure 1), with a couch set, bookshelf, TV, TV shelf, desk, center table, fan, and window curtains. All walls were plastered and painted at least three years before the tests and the roof consisted of zinc metal sheets without insulation. The room had 24 ventilation holes of 20 cm² located at 2.5 m height, distributed in three walls (figure 2A). Within the room and aligned from the front door to the next room's door, four PVC tripods 1.5 m high, were placed one meter apart. At both arms of each tripod, two cylinder-mesh-cages (1 000 cm³) containing 20 female mosquitoes each, were arranged side by side and hung from the tripod, with a total of eight cages, four with resistant and four with susceptible mosquitoes, placed interspersed. Another set of eight cages were placed in four "potentially" sheltered places, which were under the bookshelf, under the armchair, under the desk, and under the center table (figure 1). This set up was intended to simulate exposed and sheltered mosquitoes, respectively. All the spray concentration tests of the four commercial aerosol cans (three replicates each) were conducted in this room. Eight control cages, four with susceptible and

four with resistant mosquitoes, were located outside of the house, free of insecticide exposure, but under the same environmental conditions, every time a test was run (figure 1).

Commercial aerosol insecticides

A survey on the different products available in the local Tapachula market was conducted to identify the most representative formulations. Four commercial aerosol cans were purchased and tested on the resistant and susceptible mosquito strains (table I). None of the products mentioned the spraying time needed for effective control.

Droplet size emitted by each aerosol can was measured with a DC-III equipment (portable droplet measurement system KLD Laboratories, Huntington Station, NY) by applying the insecticide cloud at a right angle (horizontal) one meter away from the product outlet nozzle with respect to the location of the stem

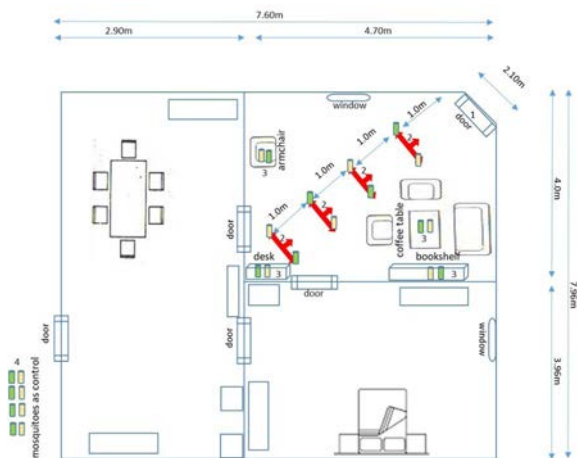


FIGURE 1. DIAGRAM OF THE TEST ROOM FROM A TAPACHULA, CHIAPAS-STYLE HOUSE. THE MESH CAGES ARE REPRESENTED BY THE COLORED CYLINDERS. THE GREEN CYLINDERS ARE CAGES WITH THE RESISTANT MOSQUITO STRAIN AND THE GOLD CYLINDERS ARE CAGES WITH THE SUSCEPTIBLE STRAIN. THE AEROSOL INSECTICIDES WERE EVALUATED FROM JULY TO NOVEMBER 2016. THE NUMBERS ON THE DIAGRAM REPRESENT THE FOLLOWING: 1) HOUSE ENTRANCE AND AEROSOL CAN SPRAY POINT; 2) TRIPODS ARE SHOWN IN RED, EACH WITH TWO ARMS FROM WHICH THE CAGES ARE HUNG, REPRESENTING THE EXPOSED MOSQUITO GROUPS; 3) LOCATIONS OF CAGES PLACED UNDER FURNITURE REPRESENTING THE SHELTERED MOSQUITO GROUPS; 4) LOCATION OF CONTROL CAGES



FIGURE 2. A) PICTURE OF TRIPOD SET UP WITH CYLINDER MESH-CAGES HANGING FROM TRIPOD ARMS REPRESENTING THE EXPOSED MOSQUITO GROUPS; AND B) PICTURE OF EXPERIMENTER SPRAYING ONE OF THE AEROSOL CANS IN THE MAIN ENTRANCE OF THE ROOM OF A TAPACHULA, CHIAPAS STYLE HOUSE. THE STUDY WAS CONDUCTED FROM JULY TO NOVEMBER 2016

Table I
COMPARISON OF THE COMMERCIAL AEROSOL CANS PURCHASED AT A LOCAL SUPERMARKET FROM TAPACHULA, CHIAPAS, MEXICO, TESTED ON THE RESISTANT AND SUSCEPTIBLE MOSQUITOES FROM JULY TO NOVEMBER 2016

Aerosol	Target	Active ingredients	Inert content	Formulation	Specifications for use
A Baygon, house and garden	Mosquitoes and flies	Tetramethrin (0.35%), permethrin (0.10%) and allethrin (0.10%)	Emulsifiers, solvents, propellants and perfume	Water-based	None
B H24 Citro Nox	Plants and insects	Tetramethrin (0.299%) and cyphenothrin (0.10%)	Water softener, emulsifying solvent, perfume, antioxidant, antifoam, propellant, piperonyl butoxide, N-octyl bicycloheptene dicarboximide	Water-based	Spray at a distance of 30 to 50 cm
C H24 long-acting, ultra-efficient fulminant power	Cockroaches and crawling insects, with scorpion killing power	Propoxur (0.46%), tetramethrin (0.103%) and fenvalerate (0.455%)	Deodorized naphtha solvent, perfume and hydrocarbon propellant	Oil-based	None
D H24	Cockroaches, crawling insects, ant, scorpion, spiders and bedbugs	Propoxur (0.151%), prallethrin (0.0093%) and deltamethrin (0.0315%)	Deodorized naphtha solvent, perfume and hydrocarbon propellant	Oil-based	Spray until surfaces or insects get wet

(drop collector), which was moved slowly side to side in a fanning motion, for 30 s as the default time set. Three repetitions of droplet size measurement were undertaken for each of the commercial aerosol products before the experiments. Environmental conditions during the measurements and experiments were on average 28.8 °C and 67 % RH. The mass (volume) median diameter varied between 7.5 and 12.2 microns for the water based aerosol products, while the oil-based products varied between 8.8 and 18.2 microns.

Lethal concentration determinations

The amount of each application (i.e. concentration) was calculated based on discharge rate, which was determined for each of the aerosol cans by measuring the total time in seconds to empty the entire contents of the can in grams, g/s. Roughly, the rate of release (net content ÷ total discharge, table II) was the same for the four products (0.6632-0.6640 g/s).

To generate baselines for lethal concentration determination for each aerosol can, seven to nine different concentrations (g/s) were applied to the test room to produce mosquito mortalities ranging between 10 and 90% when tested against resistant mosquito strain. Ranges for aerosol A were from 1 to 36 s, aerosol B from 0.5 to 12 s, aerosol C from 0.31 to 9 s, and aerosol D from 0.33 to 10 s. While the susceptible strain only needed the first four concentrations for each aerosol can to obtain 100% mortality. Another person operating an electronic stopwatch would tell the researcher when to start and to stop spraying.

Application of the commercial aerosol insecticides

In the test room, the researcher stood in the entrance doorway and applied the insecticide towards the living room. As seen in figure 2B, he held the aerosol can upwards at a 45 degree angle and pressed the actuator of the can, directing the spray from the left side to the right side of the room and always keeping the can at the height of the head. The researcher would spray the can for the predetermined period of seconds calculated for each aerosol product. Once they finished spraying, the entrance door was shut and mosquitoes were left exposed in the room for 30 min. After which, mosquitoes were removed from the cages, transferred to clean disposable cups covered with a mesh net and a cottonball soaked in 10% sucrose solution, and placed in the insectary. Mortalities were recorded after 24 h to determine LC₅₀ and LC₉₀. After an application, windows and doors were opened for 2-3 h. Four cages of susceptible mosquitoes were then set in sheltered and aired places of the test room and monitored for mortality after 30 min. If one or more mosquitoes died, the test room was aired overnight and then checked again for mortality the next day. Two more replicates of the same concentration were undertaken in the same conditions, giving a total of three replicates per concentration for each of the four aerosol products. One to three concentrations of an aerosol product were able to be tested per day, depending on the concentration sprayed and the aerosol product.

Table II
MORTALITY PERCENTAGES OF EXPOSED AND SHELTERED *Aedes aegypti* OF BOTH SUSCEPTIBLE AND RESISTANT STRAINS SPRAYED WITH FOUR COMMERCIAL AEROSOL INSECTICIDES (A, B, C, AND D), AND LETHAL CONCENTRATIONS (LC) IN GRAMS FOR RESISTANT MOSQUITOES. THE EVALUATION WAS UNDERTAKEN IN SEMI-FIELD CONDITIONS IN A 56 m³ ROOM OF A TYPICAL MIDDLE-CLASS HOUSE FROM TAPACHULA, CHIAPAS, MEXICO, FROM JULY TO NOVEMBER, 2016

	Type of exposure	Mortality percentages under different concentrations (g) measured per seconds of spray									LC50 (fiducial limits)	LC90
		0.012 1 s	0.024 2 s	0.049 4 s	0.098 8 s	0.147 12 s	0.196 16 s	0.245 20 s	0.343 28 s	0.441 36 s		
A	Exposed	95.83	73.33	75.42	100							
	Sheltered	71.11	31.67	69.44	100							
	Res. Exposed	19.58	0	0.830	7.08	23.33	48.33	44.58	47.08	77.08	0.308 (0.155-1.075)	2.423
	Res. Sheltered	6.67	2.77	0	2.22	23.89	28.89	53.89	36.66	57.77	0.453 (0.218-2.017)	3.565
B	Exposed	99.62	93.88	100	100							
	Sheltered	82.69	90.28	97.22	100							
	Res. Exposed	23.77	30.83	14.16	31.20	71.43	87.76	99.59			0.016 (0.008-0.033)	0.072
	Res. Sheltered	32.79	15.55	2.70	18.00	63.33	90.05	98.33			0.020 (0.010-0.043)	0.089
C	Exposed	87.14	87.03	100	100							
	Sheltered	54.7	83.89	80.56	100							
	Res. Exposed	9.58	17.50	40.42	62.50	64.28	77.08	87.56	95.00	99.17	0.039 (0.027-0.053)	.136
	Res. Sheltered	5.59	12.78	12.78	37.78	28.42	32.78	81.11	72.78	100	0.075 (0.056-0.103)	.264
D	Exposed	70.00	100	100	100	100	100					
	Sheltered	62.78	100	99.56	94.44	99.44	100					
	Res. Exposed	10.42	21.250	9.17	13.48	31.25	35.00	50.21	91.67	97.92	0.018 (0.010-0.036)	.082
	Res. Sheltered	6.11	7.22	2.78	2.22	18.89	24.44	32.78	22.78	82.78	0.039 (0.021-0.116)	.180

Susc.: susceptible strain; Res.: resistant strain. All figures in italics are mortality percentages with n=240 mosquitoes for each figure, undertaken in three repetitions of 80 mosquitoes each, distributed in four mesh-cages tested.

The total number of mosquitoes used per concentration in the three replicates of each commercial aerosol insecticide was 240, which were distributed in 12 hanging mesh-cages to represent the exposed mosquitoes, and an equal amount of mosquitoes were placed in cages in covered areas to represent the sheltered mosquitoes, making a total of 2 160 mosquitoes in 108 cages for the aerosol insecticides that required nine concentrations

for the resistant exposed, and 2 160 for the resistant sheltered mosquitoes. While 960 susceptible mosquitoes in 48 cages were used in four concentrations for exposed and 960 for sheltered ones.

Comparisons on the LC₅₀ values along with fiducial confidence intervals for the type of exposure, and between the different aerosol insecticides for the resistant mosquitoes, were obtained requesting the relative

median potency table from a Probit analysis.* Where concentration functioned as a covariate and the factor was the type of exposure or the different aerosol insecticides. All Probits converged as the right analysis for the data. Lethal concentrations (50 and 90%) were obtained from the "Confidence Limits for Effective Doses" table of the Probit output. All covariates were transformed to Log base 10. When the estimate for relative median potency (RMP) was 1.00, LC₅₀ values for comparison of two were then statistically identical.

Potential number of houses covered per commercial aerosol insecticide

According to the rate of discharge, the number of three-room houses that can be sprayed with the concentration that kills 100% of the mosquito population for each aerosol insecticide can be calculated, depending on whether the mosquitoes to be controlled were susceptible or resistant (table III).

Results

Comparisons of lethal concentrations

Mortality results for type of exposure in resistant and susceptible mosquitoes for each commercial aerosol tested are shown in table II. Lethal concentrations

were calculated only for resistant mosquitoes, since the Probit model could not fit for the susceptible strain because of the short range of concentrations that produced high mortalities with any of the four aerosol insecticides. An individual Probit regression for each aerosol insecticide showed significant differences between LC50 of sheltered mosquitoes compared to exposed (table II), where the former mosquitoes always needed higher concentrations (RMP ≠ 1). Another Probit regression for each type of exposure where the LC50 between the four aerosols were compared, showed that aerosol A needed statistically higher concentrations than the others (LC50 = 0.261, fiducial limits (FL) 0.133 - 0.603), followed by C, then D (RMP ≠ 1) for both exposed and sheltered mosquitoes.

Potential number of houses covered per commercial aerosol

A comparison for both susceptible and resistant mosquitoes, which could be used as recommendation to householder's usage, is provided in table III, where numbers are based on the seconds of spray required to kill near or the total population of both exposed and sheltered mosquitoes. However, without knowing the insecticide resistance status of a mosquito population, the best choice is to spray as if dealing with a resistant population, which eliminates the advantages that heterozygotes may have when applying low dosages.

* IBM SPSS Statistics Version 24

Table III

COMPARISON OF FOUR COMMERCIAL INSECTICIDE AEROSOLS TO CONTROL 100% (OR NEARLY 100%) OF SUSCEPTIBLE AND RESISTANT EXPOSED OR SHELTERED *Aedes Aegypti* MOSQUITOES IN A 56 m³ ROOM OF A TYPICAL MIDDLE-CLASS HOUSE FROM TAPACHULA, CHIAPAS, MEXICO, AND ESTIMATION OF THE POTENTIAL NUMBER OF THREE-ROOM HOUSES THAT CAN BE TREATED PER CAN. THE PERCENTAGE LISTED FOR EACH COMMERCIAL PRODUCT IS THE TOTAL OF THEIR ACTIVE INGREDIENTS. TESTS WERE PERFORMED FROM JULY TO NOVEMBER 2016

Strain	Commercial aerosol insecticides															
	A (0.55%)			B (0.399%)			C (1.018%)			D (0.1915%)						
LT	Net content (g)	Total discharge (s)	Houses covered	LT	Net content (g)	Total discharge (s)	Houses covered	LT	Net content (g)	Total discharge (s)	Houses covered	LT	Net content (g)	Total discharge (s)	Houses covered	
Susc.	8 s	407.86	614.95	26	2 s	483.86	728.73	121	2 s	452.46	682.2	114	4 s	453.6	683.91	57
Res.	36s*	407.86	614.95	6*	12s	483.86	728.73	20	9 s	452.46	682.2	25	10s	453.6	683.91	23

Susc.: susceptible strain, Res.: resistant strain, LT: lethal time.

* Only 77% (58% for sheltered mosquitoes) mortality obtained at 36 s of discharge exposure to product A, and its net content covers only 6 three-room houses of 56 m³

Discussion

ULV application of insecticides to control adult mosquitoes is more effective when applied indoors⁵ as compared to outdoor applications. Since people do not open doors and windows when ULV outdoor applications are taking place, the desired dose does not reach the places where mosquitoes are more likely to be resting indoors.¹⁹ Therefore, it is expected that canned aerosols could be a useful tool for mosquito vector control if indications provided from studies like this one are followed to apply the proper and required dosage needed. The current use of aerosol insecticides by the inhabitants of Tapachula is not known, but in a dengue-endemic area in the state of Yucatán, Mexico, a high percentage of the population interviewed (73.6%) uses aerosol insecticides to kill mosquitoes in their homes.³ Which spray insecticide consumers buy is likely to be based on the variety on the market and/or may be further influenced by price. However, the use of domestic aerosols has not been evaluated in terms of the type of formulation and amount of AI needed to achieve a given percent mortality in susceptible and resistant mosquitoes, as well as the impact that this application may have on vector control. Whereas resistant mosquito populations in urban areas are easily found when activities are commonly undertaken by the control programs,¹⁶ susceptible mosquitoes are more likely found in rural areas. However, both populations often contain a percentage of mosquitoes carrying recessive alleles. It is known that dominance can be dose-dependent,²⁰ that is, heterozygotes behave as dominant phenotypes when lower dosages are applied. Therefore, a higher dose is preferred if we assume that untreated areas are left as refuges for susceptible genes. In this case, since aerosol products are used exclusively in domestic settings, mosquitoes resting outdoors might escape exposure. Also, the proper use of canned aerosols to control domestic mosquitoes has been scarcely documented.¹⁷ In fact, the same companies producing the aerosol insecticide cans fail to give a complete and clear recommendation for spraying on the label. Our results showed that commercial sprays killed both susceptible and resistant mosquitoes, and that applying concentrations that also kill sheltered mosquitoes, the method would represent an option for recommendation to be implemented allowing sustainable mosquito control by the same community, backed up with the required assistance.

The usefulness and acceptability of commercial aerosols has been documented previously for the prevention of diseases like malaria²¹⁻²³ and dengue.³⁻⁴ In addition, the effect of 13 commercial aerosols was recently evaluated against an *Ae. aegypti* field strain

in Morelos, showing that only three killed 100% of the exposed mosquitoes.¹⁷ They concluded that the location of the mosquitoes (sheltered or exposed) was the main factor for such variable results. However, they also mentioned that no dose for most of the products could be considered for the analysis of the results. In our study, it was evident that sheltered resistant mosquitoes needed higher amounts of insecticide than the exposed ones. However, both mosquito exposure types responded equally to the four different aerosol insecticides tested, requiring higher time of spraying from the aerosol A, followed by C, then D. Aerosol B demonstrated better control of this resistant strain. It is therefore suggested that marketing of domestic insecticide aerosols should consider the geographical patterns of insecticide resistance in their guiding recommendations.

Results of our study with this pyrethroid resistant strain suggest that it would be preferable to use products B, D, and C, over product A, since there appears to be more resistance to the latter product, as indicated by the lower mortality and higher LC₅₀ and LC₉₀. All four aerosols cans contain at least two pyrethroids, but aerosol B additionally contains two synergists in its formulation, which are enhancing the effectiveness of its pyrethroids, by blocking its metabolic pathway carried out by enzymes. This is also indication of cross-resistance between bifenthrin (a pyrethroid to which this strain is resistant) and the pyrethroids present in aerosol formulations. Resistance to pyrethroids in Mexico has been mostly due to its high use by the programs for dengue control.¹³⁻¹⁶ Aerosols C and D, besides containing two classes of pyrethroids (type I and II), also contains propoxur, an insecticide targeting another site, which could explain why they performed better with the resistant mosquito strain.

The number of houses with three rooms that we calculated could be treated by a can of aerosol A (when the target mosquitoes are pyrethroid resistant) is only six, while for the other aerosols ranged from 20-25 houses. Household commercial aerosol insecticides are marketed either for crawling or flying insects, and although differences in AI concentrations are dependent on the suggested use, the most important characteristic is the formulation. The water-based formulations are frequently recommended for "flying insects" while the oil-based for "crawling insects" (according to label descriptions), the latter perhaps based on the residual efficacy once applied to surfaces. In our study, we tested two commercial products for "flying insects" which are aerosols (A and B), both of which gave the most extreme results among the four tested products. Considering that the droplet sizes for both the water- and oil-based formulations were similar, our results indicate that the

content of the formulation, rather than recommendation for flying versus crawling insects, is an important factor to consider for controlling field resistant mosquitoes.

One interesting concern about the use of aerosols is their potential to be included as a tool for community participation intervention. Results reported by several authors^{3,21,23} indicate that the purchase of household commercial insecticides is primarily to protect against mosquito bites. Furthermore, in a study from Taiwan,⁴ household members preferred the use of aerosol cans over the government fogging interventions. Therefore, estimating the number of houses or rooms in terms of cubic meters, acquires relevance. For example, in our study, we estimated that the maximum number of three-room houses of 56 m³ that can achieve an average of 100% mortality in a field resistant mosquito population was 25 when using product C. On the other hand, in tests made with sprays of 13 aerosol products in resistant field mosquitoes from Morelos placed inside rooms, only three of the products were successful.¹⁷ Which teaches us that, before give any recommendation of usage, we must do tests with the spray to be used, and with the local mosquitoes where the control is to be carried out, to calculate the optimal effective lethal concentrations. This concentration would of course, vary according to the size of the room treated and, consequently it will affect the number of rooms to be covered.

The chance of a successful intervention is greatly increased when communities assist in the indoor applications of insecticides. To obtain the desired results as we did, a recommendation is that the sprayer should wear a mask, shirt with long sleeves and pants. Spraying must be done by holding the aerosol can with the nozzle pointed upwards at a 45 degree angle, with the arm extended at head height and spraying the can in a sweeping motion from left to right across the room. To control pyrethroid resistant *Ae. aegypti* mosquitoes, spray for a minimum time of 36 s if using a formula similar to aerosol A, or 12 s if it is a formula similar to aerosol B. The spray time would be 9 and 10 s, if using formulas similar to aerosols C and D, respectively. For example, the risk of poisoning that a person weighing 75 kg would experience if they are not protected when spraying the concentration of aerosol A (36 s), there would be 72 g of that formulation containing tetramethrin (0.35%), permethrin (0.10%) and allethrin (0.10%). Which will make a net dose of tetramethrin=3.36 mg, permethrin=0.96 mg, and allethrin=0.96 mg / kg of weight. When compared to the LD₅₀ of tetramethrin 5 000 mg, permethrin 4 000 mg, and allethrin 685 mg / kg of weight reported for rats,²⁴ it will probably not have significant effects on that person. However, particular attention should be paid to prevent exposure of aerosol

A to cats, since they are glucuronidase deficient, which is the enzyme responsible for breaking down permethrin and other synthetic pyrethroids.²⁵

In summary, commercial aerosol insecticides could be a good tool to control mosquitoes. They are able to be applied and targeted towards indoor areas where mosquitoes are most likely to be at rest. As with any insecticide, the efficacy depends on the insecticide resistance observed in local populations, the concentration of insecticide used, application technique, and class of active ingredients. The sprays that best killed pyrethroid-resistant mosquitoes were those containing the synergists piperonyl butoxide and N-octyl bicycloheptene dicarboximide (aerosol B), or those containing propoxur in their formulation (aerosols C and D). It is strongly recommended to make maps of the geographical distribution of insecticide resistance patterns available to the public. Such strategies would need further studies to evaluate the economic feasibility of their implementation.

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References

1. Achmadi UF, Pauluhn J. Household insecticides: evaluation and assessment of inhalation toxicity: a workshop summary. *Exp Toxicol Pathol.* 1998;50(1):67-72.
2. Osaka K, Ha DQ, Sakakihara Y, Khiem HB, Umenai T. Control of dengue fever with active surveillance and the use of insecticidal aerosol cans. *Southeast Asian J Trop Med Public Health.* 1999;30:484-8.
3. Loroño-Pino MA, Chan-Dzul YN, Zapata-Gil R, Carrillo-Solis C, Uitz-Mena A, García-Rejón JE, et al. Household use of insecticide consumer products in a dengue-endemic area in México. *Trop Med Int Health.* 2014;19(10):1267-75. <https://doi.org/10.1111/tmi.12364>
4. Pai HH, Hsu EL. Effectiveness and acceptance of total release insecticidal aerosol cans as a control measure in reducing dengue vectors. *J Environ Health.* 2014;76(6):68-74.
5. Ordóñez-González JG, Thirion J, García A, Rodríguez AD. Effectiveness of indoor ultra-low volume application of Aqua Reslin® Super during an emergency. *J Am Mosq Control Assoc.* 2011;27(2):162-4. <https://doi.org/10.2987/10-6065.1>

6. Ordoñez-González JG, Cisneros-Vázquez LA, Danis-Lozano R, Valdés-Delgado KM, Fernández-Salas I, Penilla-Navarro RP, et al. Nebulización térmica intradomiciliar de la mezcla de flupyradifurona y transflutrina en mosquitos *Aedes aegypti* susceptibles y resistentes a piretroides en el Sur de México. *Salud Publica Mex.* 2020;62(4):432-8. <https://doi.org/10.21149/11142>
7. Paredes-Esquivel C, Lenhart A, del Río R, Leza MM, Estrugo M, Chalco E, et al. The impact of indoor residual spraying of deltamethrin on dengue vector populations in the Peruvian Amazon. *Acta Trop.* 2016;154:139-44. <https://doi.org/10.1016/j.actatropica.2015.10.020>
8. Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol.* 2000;45:371-91.
9. Marcombe S, Mathieu RB, Pocquet N, Riaz MA, Poupardin R, Sélion S, et al. Insecticide resistance in the dengue vector *Aedes aegypti* from Martinique: distribution, mechanisms and relations with environmental factors. *PLoS One.* 2012;7(2): e30989. <https://doi.org/10.1371/journal.pone.0030989>
10. Kawada H, Oo SZ, Thuang S, Kawashima E, Maung YN, Thu HM, et al. Co-occurrence of point mutations in the voltage-gated sodium channel of pyrethroid-resistant *Aedes aegypti* populations in Myanmar. *PLoS Negl Trop Dis* 2014;8:3-10. <https://doi.org/10.1371/journal.pntd.0003032>
11. Linss JG, Brito LP, Garcia GA, Araki AS, Bruno RV, Lima JB, et al. Distribution and dissemination of the Val1016Ile and Phe1534Cys Kdr mutations in *Aedes aegypti* Brazilian natural populations. *Parasit Vectors.* 2014;7:25. <https://doi.org/10.1186/1756-3305-7-25>
12. Saavedra-Rodríguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores AE, Fernandez-Salas I, et al. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. *Insect Mol Biol* 2007;16(6): 785-98. <https://doi.org/10.1111/j.1365-2583.2007.00774.x>
13. García GP, Flores AE, Fernández-Salas I, Saavedra-Rodríguez K, Reyes-Solis G, Lozano-Fuentes S, et al. Recent rapid rise of a permethrin knock down resistance allele in *Aedes aegypti* in México. *PLoS Negl Trop Dis.* 2009;3(10):e531. <https://doi.org/10.1371/journal.pntd.0000531>
14. Aponte HA, Penilla RP, Dzul-Manzanilla F, Che-Mendoza A, López AD, Solis F, et al. The pyrethroid resistance status and mechanisms in *Aedes aegypti* from the Guerrero state, Mexico. *Pest Biochem and Physiol.* 2013;107:226-34. <https://doi.org/10.1016/j.pestbp.2013.07.005>
15. Saavedra-Rodríguez K, Beaty M, Lozano-Fuentes S, Denham S, Garcia-Rejon J, Reyes-Solis G, et al. Local evolution of pyrethroid resistance offsets gene flow among *Aedes aegypti* collections in Yucatan State, Mexico. *Am J Trop Med Hyg.* 2015;92(1): 201-9. <https://doi.org/10.4269/ajtmh.14-0277>
16. Saavedra-Rodríguez K, Maloof FV, Campbell CL, Garcia-Rejon J, Lenhart A, Penilla P, et al. Parallel evolution of vgsc mutations at domains IS6, IIS6 and IIIS6 in pyrethroid resistant *Aedes aegypti* from Mexico. *Sci Rep.* 2018;8(1):6747. <https://doi.org/10.1038/s41598-018-25222-0>
17. Kuri-Morales PA, Correa-Morales F, González-Acosta C, Moreno-García M, Dávalos-Becerril E, Benitez-Alva JI, et al. Efficacy of 13 commercial household aerosol insecticides against *Aedes aegypti* (Diptera: Culicidae) from Morelos, Mexico. *J Med Entomol.* 2018;55(2):417-22. <https://doi.org/10.1093/jme/tjx212>
18. Moreno J, Falcó JV, Oltra MT, Jiménez R. The requirement for the inclusion of formulation efficacy trials in pesticide preregistration evaluations. *Pest Manag Sci.* 2008;64(5): 527-35. <https://doi.org/10.1002/ps.1536>
19. Perich MJ, Davila G, Turner A, Garcia A, Nelson M. Behavior of resting *Aedes aegypti* (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. *J Med Entomol.* 2000;37(4):541-6. <https://doi.org/10.1603/0022-2585-37.4.541>
20. Georghiou GP, Taylor CE. Operational influences in the evolution of insecticide resistance. *J Econ Entomol.* 1977;70: 653-658. <https://doi.org/10.1093/jee/70.5.653>
21. Rodríguez AD, Penilla RP, Henry-Rodríguez M, Hemingway J, Francisco Betanzos A, Hernández-Avila JE. Knowledge and beliefs about malaria transmission and practices for vector control in southern Mexico. *Salud Publica Mex.* 2003;45(2):110-6.
22. Astatkie A. Knowledge and practice of malaria prevention methods among residents of Arba Minch town and Arba Minch Zuria district, southern Ethiopia. *Ethiop J Health Sci.* 2010;20(3):185-93. <https://doi.org/10.4314/ejhs.v20i3.69448>
23. Mora-Ruiz M, Penilla RP, Ordóñez JG, López AD, Solis F, Torres-Estrada JL, Rodríguez A. Socioeconomic factors, attitudes and practices associated with malaria prevention in the coastal plain of Chiapas, Mexico. *Malaria J.* 2014;13:157. <https://doi.org/10.1186/1475-2875-13-157>
24. Instituto Regional de Estudios en Sustancias Tóxicas, Universidad Nacional de Costa Rica. Manual de Plaguicidas de Centroamérica. Costa Rica, 2022 [cited July, 2022]. Available from: <http://www.plaguicidasdecentroamerica.una.ac.cr/index.php/>
25. Richardson JA. Permethrin spot-on toxicoses in cats. *J Vet Emerg Crit Care.* 2000;10:103-6 [cited July, 2022]. Available from: https://www.aspcapro.org/sites/default/files/d-veccs_april00_0.pdf