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ORIGINAL ARTICLE

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Aedes aegypti control in breeding sites through an insecticidal coating with dual effect: Laboratory trials and safety assessment

Control de *Aedes aegypti* en los sitios de cría mediante un recubrimiento insecticida de efecto dual: ensayos de laboratorio y evaluación de la seguridad

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Abstract

Ground water tanks are known to be preferred *Aedes aegypti* oviposition places providing opportunities for adult and larvae control. Therefore, a dual-effect insecticidal coating (IC) (alphacypermethrin/ pyriproxyfen) with a slow-release mechanism and safe for users could be applied within *Aedes* spp. breeding sites, representing a promising option. Bioassays were designed to determine the mortality and sterilizing effects on gravid mosquitoes exposed to IC. The effect of inhibition of emergence was evaluated in eggs, larvae and pupae exposed in different containers. For the water safety assessment concentrations of active ingredients were determined by reverse phase high performance liquid chromatography (RP-HPLC) and the health risk was calculated. The IC applied to the interior walls of water-holding containers showed efficacy against *Ae. aegypti* in terms of high gravid-female mortality (81% at 24 h, $p < 0.01$), sterilizing effect (inhibition of oviposition by 63%, $p < 0.01$) and emergence inhibition (100% in eggs, L3 and L4; 97% in pupae). The offspring rate was reduced [only 0.15 (38/250) new adults emerged per exposed gravid females as against 11.90 per unexposed female (2976/250) at

baseline]. Emergence inhibition was recorded up to 12 months and adult mortality >80% up to 6 months. The use of water stored in treated containers, either for washing or drinking, is not expected to pose a health risk to users. IC applied to domestic water containers has dual and complementary action that reduces *Ae. aegypti* densities (immature and adult stages). This represents baseline information for a cluster randomized efficacy trial in Colombia.

KEYWORDS
Aedes aegypti, Colombia, Cucuta, dengue, insecticidal coating, pyriproxyfen, vector control, water container

INTRODUCTION

Dengue and other emerging viral diseases, such as chikungunya and Zika, are causing epidemics at intervals of 2–4 years, which overburden healthcare systems and disrupt the economies of tropical and subtropical countries (WHO, 2009). Recent epidemics have led to growing concern and alarm about the rapid spread of arboviruses, mainly in urbanized settings where humans are amplification hosts and *Aedes (Stegomyia) aegypti* (Linnaeus) (Diptera: Culicidae) maintains person-to-person transmission (Paz-Bailey et al., 2024; Weaver et al., 2018). Colombia is a tropical country with the presence of the dengue vector in its 32 administrative divisions. The region of Norte de Santander and its metropolitan area of Cucuta are at risk for the transmission of dengue/Zika/chikungunya (Carrillo et al., 2023).

To reduce or interrupt vector-borne transmission of arboviruses, decisions are made based on Integrated Vector Management (IVM), and in this framework insecticides and growth regulators remain one of the most widely used pillars, particularly larvicide with chemical insecticides, indoor residual spraying, outdoor spraying and insecticide treated materials (WHO, 2009; WHO, 2012). However, the use of vector control against dengue is limited and it is uncertain which of the currently available interventions work (Bowman et al., 2016; Horstick et al., 2018). This situation could have repercussions on the increase in arboviruses throughout the world.

Recent meta-analyses motivate the need for rigorous design in vector control studies, suggest the implementation of new approaches and indicate that optimal results are achieved when targeting both larvae and adults simultaneously (Horstick et al., 2018; Paz-Bailey et al., 2024). Following this guidance, the IVM recommends the synergistic combination of formulations, for example, treatment of water containers together with the spraying of adulticides, or the separate application of two or more formulations with active ingredients of different mechanisms of action applied during the same time and space. However, it is important to note that these formulations have different application logistics and residual effect that does not exceed 6 months in all cases.

Microencapsulated insecticide formulations have been contributing to the reduction of these gaps. These formulations employ strategies that can combine insecticides of diverse action in the same presentation and facilitate the slow release of their active ingredients. Gradually, laboratory and field evidence suggests that microencapsulation of active ingredients in the matrix of paints or lacquers is durable and effective against insect vectors for a considerably long period of time (Mateo, 2009); applied on walls of dwellings they are receiving special attention for their potential use against several insect vectors (Alim et al., 2023; Banjara et al., 2019; Gómez et al., 2024; Huda et al., 2019; Maloney et al., 2013; Mosqueira et al., 2010; Mosqueira et al., 2013; Tilak et al., 2022).

Despite technological advances with insecticidal paints, they are not routinely used for dengue vector control, partly because of the lack of evidence of effects on *Aedes* spp. populations, the limited dissemination of safety studies associated with their application and the cost to health programmes of treating the walls of many homes in arboviruses-endemic communities. To address these challenges, a coating-type formulation with microencapsulated active ingredients, of larvicidal and adulticidal action is proposed for application in sites identified as having high mosquito productivity. Ground tanks used to store water for household cleaning are among the main breeding sites for *Ae. aegypti* in Latin America (Alcalá et al., 2015; Carrillo et al., 2023; Diéguez Fernández *et al*., 2010; Quintero et al., 2009; Wright et al., 2023). These containers, common in middle and low socio-economic households, are constantly filled with water, are used daily and lack lids to prevent mosquito entry, posing an enormous challenge for public health programmes.

Despite water treatment with insecticides, biologicals or growth regulators (effervescent tablets, granules, liquids or powders), the larvicidal effect is lost with water renewal, but a residual action coating could solve this situation if it proves to be safe for human populations and environmentally acceptable. The preference of mosquitoes for this type of breeding site represents an opportunity for targeting adult and immature stages through an insecticidal coating (IC) applied on the internal walls of these containers. The IC SP Coating-SATIS® (Inesfly Corporation S.L., Paiporta, Spain) is a water-based polymeric transparent coating that contains microencapsulated pyriproxyfen (PPF) (0.063%) and alpha-cypermethrin (ACM) (0.7%) without interaction between them (Mateo, 2009). Larvae and adults can be affected simultaneously by combining an insect growth regulator (IGR) and a pyrethroid in an insecticide formulation. The PPF and ACM, widely employed in public health, have very low toxicity to humans and most non-target terrestrial wildlife (JMPR, 1999; JMPR, 2006; Schiøler et al., 2016; WHO, 2019).

Considering that IC with PPF and ACM applied in ground tanks may contribute to the prevention of arboviruses transmission with a

reduced cost and limited insecticide use, this study aims to evaluate the entomological efficacy of IC under controlled conditions and safety related to the use of the water contained in these ground tanks. In addition, it complements a baseline for a cluster-randomized controlled trial of this novel vector control tool for water containers (Carrillo et al., 2023).

MATERIALS AND METHODS

Study areas

The experiments were carried out in the Laboratory of Entomology from the National Health Institute in Bogotá (Instituto Nacional de Salud-INS), Colombia, and the Entomology Unit Departmental Health Institute in Cucuta, Colombia (Instituto Departamental de Salud de Norte de Santander – IDS). The follow-up assessment of the safety of the water in the treated tanks was carried out at the Workstation of the University of Freiburg in Cucuta, Colombia, and the chemical analyses were performed at the Laboratory of the Department of Vector Biology, Liverpool School of Tropical Medicine, UK.

Biological material

A colony of insecticide-susceptible Rockefeller strain of *Ae. aegypti* mosquitoes maintained in the laboratory were used in the bioassays. The field populations were collected as larvae from Norte de Santander, Colombia (7°54′21.0″ N-72°28′22.4″ W). The CDC test revealed these populations to be highly resistant to ACM with mortality of 16% and 10% to diagnostic dose of 10 µL/mL/bottle (Centres for Disease Control and Prevention methodology-CDC, 2010); consequently, it was assumed as the wild strain in this study. The reference and wild strains were kept under constant conditions of temperature and relative humidity (28°C, HR 50%), 10:14 h photoperiod (light vs. darkness) and reared following internal protocols free of exposure to insecticides. Mosquitoes selected for bioassays were gravid females, 48 h after their first bloodmeal. Female mosquitoes were fed through an in vitro feeding system as described in Gunathilaka et al., 2017. During the trials, the mosquitoes were fed *ad libitum* with a supply of water and sucrose solution (10%). Larvae were fed with dry cat food.

Treatment of containers for bioassays

The IC was applied according to a standardized cleaning, painting and drying procedure (Technical Data Sheet INESFLY, 2020). The inner walls of experimental concrete pots, plastic containers and tanks were treated by brushing in a single layer at a 15 m^2/L dosing, the drying time was 24 h. The containers were coated only once and were used for bioassays for up to 12 months. The concrete pots were kept without water and protected from light when not in use. Five containers were set up for the residual effect study, two plastic containers, two

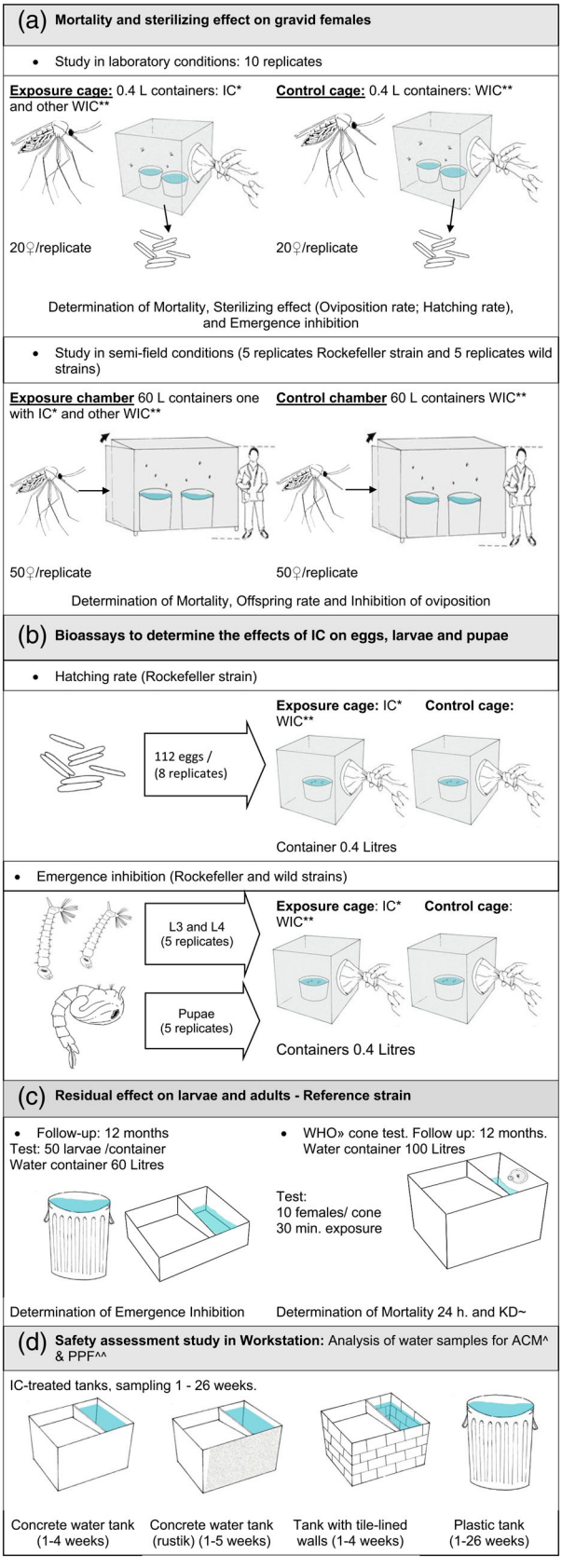


FIGURE 1 Experiments designed for this study. *IC: insecticidal coating; **WIC: without insecticidal coating; ^ACM: alpha-cypermethrin; ^^PPF: pyriproxyfen; ~KD: knock-down effect; WHO» World Health Organization.

small concrete tanks and a medium-size concrete tank (65 × 65 × 40 cm) (Figure 1c). They were kept at the working station (28 ± 2°C, 80%–90% RH and 12:12 h photoperiod). During the 12-month follow-up, the contents of each tank—controls and treatments—were changed weekly. Other five tanks for the water safety assessment were comprised of different materials on their inner walls: rusty concrete, concrete (under shadow and partial sunlight), tile and plastic (Figure 1d). These tanks measured 65 × 65 × 40 cm and held 100 L of water. Application procedure and dosage were the same as described above. These tanks were not subject to regular use and only enough water was replenished to maintain a constant volume, not fully replaced.

Bioassays of mortality and sterilizing effect on gravid females exposed to insecticidal coating

Ten replicates in groups of 20 *Ae. aegypti* females and 10 males of the Rockefeller strain were exposed in cages (30 × 30 × 30 cm): (a) Exposure cage including two concrete containers (0.4 L) partially filled with tap water as oviposition sites, one of them treated with IC and the other one without insecticide coating (WIC), and (b) Control cage including two concrete containers free of insecticide coating and partially filled with water (Figure 1a). Mosquitoes were recaptured after 24 h of its release with insect aspirator (pick-up straw Ø12 mm) and mortality was recorded. Alive females were observed at 24 h in the paper cups for delayed mortality recording. To establish the effect on eggs and larvae (inhibition of hatching), the development of eggs oviposited-bioassay containers was monitored for up to 15 days (Figure 1a). The eggs were counted using a magnifying glass. The 10 replicates of these trials were conducted for 15 weeks at 10 days' time intervals. Hatching rates were estimated using the following formula: n hatched eggs (cracked)/ n egg-laying ×100.

In two study chambers (1.6 m × 1 m × 1.6 m) five replicates in groups of 50 *Ae. aegypti* gravid females and 10–20 males were exposed simultaneously: (a) Exposure chamber and (b) Control chamber. Each chamber included two plastic water containers. In the treatment chamber, one of these containers was coated with IC while the other one remained untreated (WIC), and in the control chamber the two containers were untreated (WIC) (Figure 1a). The floor was covered using new white paper to allow the observation of fallen mosquitoes. Dead females were collected and examined under the stereomicroscope to record mortality and morphological conditions of the material, 48, 72 and 96 h after mosquito release. Females that remained alive until 96 h were also recaptured with insect aspirator and cold killed to be observed under the stereomicroscope. Furthermore, the gravid females post-exposure were examined and classified according with abdominal distention (carrying eggs), related to inhibition of oviposition, A: no inhibition; B: partial inhibition; C: complete inhibition. Approximately 10% of the mosquitoes were dissected to check the eggs under the stereomicroscope (4X), as verification of the abdominal contents. The adult emergence in containers was follow-up for 7–8 days and larval food was provided daily. Five replicates of this assay were conducted with intervals of 8 days between each repetition, taking 10 weeks for this study with the Rockefeller strain. The number of adults emerged per chamber was counted and offspring were estimated as the number of adults emerged per female released. In addition, with the wild strain the bioassay was carried out in the same conditions, for the next 10 weeks.

Bioassays to determine the effects of IC on eggs, larvae and pupae (offspring of female mosquitoes not exposed to IC)

An average of 14 viable eggs from the colony (Rockefeller strain) were placed one by one on the surface of the water with the help of a fine brush in insecticide-coated containers (0.4 L) and non-insecticide-coated containers as a control. Larvae food was added during the 15-day assay follow-up and the experiment was repeated eight times (WHO, 2005; WHO, 2022) (Figure 1b). The number of hatched eggs, live and dead larvae, was recorded until the emergence of adults. Groups of 15 *Ae. aegypti* larvae (late L3 to early L4) and pupae from the Rockefeller strain and ACM-resistant strain were exposed to the 0.4 L water-filled concrete containers treated and untreated with IC up to 10 months after the coating application. Monitoring consisted of the two strains being monitored for up to 9 days, including larval mortality and adult emergence (WHO, 2005; WHO, 2022). Five replicates were made for each stage and *Ae. aegypti* strain (Figure 1b).

Residual effect study in larvae

Fifty larvae (late L3 to early L4) were introduced into each container 48 h after renewing the water ($n = 100$ exposed larvae and 100 control larvae) (Figure 1c). The tanks were examined daily for 15 days to record the cumulative mortality (of larvae—pupae) and emergence of adults. Food was provided daily to the larvae and the pupae were removed to observation containers.

Residual effect study in adults

For the evaluation, 2-day-old, insecticide susceptible, non-blood-fed, females were exposed to the surface in WHO cone bioassay for 30 min (WHO, 1998) (Figure 1c). Tests were done in 10 repeats using 10 females per cone (five cones on treated surface and five cones on control surface). Mosquito knock-down (KD) was recorded after 60 min, whereas mortality was observed after 24 h of exposure (Mosqueira et al., 2010); females were left at a temperature of 27 ± 1°C and a relative humidity of 80%. The mortality was estimated as number of knock-down/ (number of mosquitoes used) X100 (WHO, 1998) and with the Abbot formula, mortality was corrected according to control rates. These tests were run one month post-treatment and every 3 months for 1 year.

Determination of alphacypermethrin and pyriproxyfen in water

For sampling 500 mL of water were taken from the IC-treated containers for 1–26 weeks post-treatment. Water samples, considered as the control, were taken before the coating-application (Figure 1d). The method for the simultaneous determination of PPF and ACM insecticides in water samples was developed by Liverpool School of Tropical Medicine (LSTM) to trace the leaching of both insecticides in drinking water. Filtered water samples were extracted for insecticides using solid-phase extraction (SPE) cartridge with no additional clean-up steps and subsequently analysed with reverse phase high performance liquid chromatography (RP-HPLC). This method was suitable for determining the ACM and PPF, at microgram-per-litre (part per billion 'ppb') concentrations in water. The calculated detection limit of the HPLC method for ACM is 2.4 µg/L (2.4 ppb) and PPF as 0.312 µg/L (0.3 ppb). The estimated recovery of the method was 69% for PPF and 58% for ACM. This was estimated by spiking 500 mL dd-water with 5 µg (10 µg/L"10 ppb) of both insecticides.

Exposures and health risks

The International Programme on Chemical Safety's (IPCS) recommendation is that at the first-tier risk assessment, dose-additivity should be applied even to chemicals for which the mode of action is not known to be similar (Meek et al., 2011). Therefore, in this assessment the dose-additivity model is applied to the combination of ACM and PPF. The exposure and health risks were assessed using the WHO Generic Models for Risk Assessment of Insecticides used for Larviciding and Molluscicid-ing (WHO, 2018) and the models were fed with the experimental concentrations. The model sets that larviciding is limited to 6 months a year. In the case of IC, however, it is assumed that water is stored in treated containers throughout the year, that is, the exposure is daily.

Statistical analysis

To determine differences in mortality of gravid females exposed in the control group and IC treatment, a Student's t-test for independent samples was applied. An analysis of variance (ANOVA) was performed to determine whether there was a statistically significant difference between the number of eggs and hatching percentage between treatment groups. A Scheffe's multiple comparison test of averages was applied for the cases where significant statistical differences were found between the different trial conditions. To identify statistical differences between percentages of egg hatching, larval mortality and inhibition of adult emergence *Ae. aegypti* between the control group and IC treatment, a Student's *t*-test for independent samples was used. When mortality rates in control assays were between 5% and 20%, Abbott's mortality correction formula was applied.

Emergence inhibition (EI) was calculated for the third and fourth larval instars and pupae of the susceptible strain (Rockefeller) and the resistant strain of *Ae. aegypti* exposed to insecticide-coated or untreated (control) containers at different exposure times. EI was calculated for each stage using the following formula (WHO, 2005): $EI = (1 - [Etr/Eco]) \times 100$. Where Etr represents the average percentage of adult emergence in the strain exposed to the container with insecticide or treatment, and Eco is the average percentage of adult emergence of the same strain in the control container. The emergence rate was calculated dividing the number of emerged individuals by the total exposed. The difference in the percentage of adult emergence (DE) was calculated using the formula: $DE = Etr - Eco$. The DE was accompanied by its 95% confidence interval. The z-test was applied for the difference between two proportions, assuming that the differences were statistically significant for $p < 0.05$. The percentage of mortality was calculated dividing the number of individuals that died in each time period by the total exposed.

Ethical considerations

This study was approved by the Ethics Committee of the National Institute of Health of Colombia (approved by agreement No. 21/2018, April 27, 2019). Favourable vote from the Ethics Committee of Albert-Ludwigs-Universität Freiburg Number 141/19 (approved 3 May 2019).

RESULTS

Mortality effect and sterilizing effect on gravid females

The average percentage of dead females after 24 h post-exposure to the PPF-ACM coating was 79.4% higher than the control ($p < 0.01$), resulting in a mortality of 81.2% in the treated cage, and 1.9% in the control. Most of the dead females 62.1% (95/153) were found inside the container treated with IC in the exposure cage. Other dead females were collected from inside the untreated container 29.4% (45/153) and from the floor of the cage 8.5% (13/153). Regarding the oviposition rate 7619 eggs were laid, 72.4% (5519/7619) in the control cage containers and 27.6% (2100/7619) in the exposure cage containers (container IC and WIC). The average number of eggs deposited per replicate was similar in both containers of the treatment cage ($\overline{X}$ WIC = 107.1 and $\overline{X}$ IC = 102.9 eggs, $p = 0.07$), but significantly lower compared to the control group ($\overline{X} = 276$ eggs, $p < 0.01$). The oviposition rate was 10.1 eggs per female mosquitoes (2100/207) in the treated cage, compared to 26.5 eggs per female mosquitoes (5519/208) in the control. Therefore, gravid females exposed to CI approach the treated surface without repellency and exhibit mortality and inhibition of oviposition.

In the chambers, the mortality effect was also evident with the susceptible-strain *Aedes* females, because after 24 h post-exposure, an average mortality of 60.4% (151/250) was obtained, reaching 93.2% (233/250) at 48 h (Figure 2). In contrast, the mortality of the

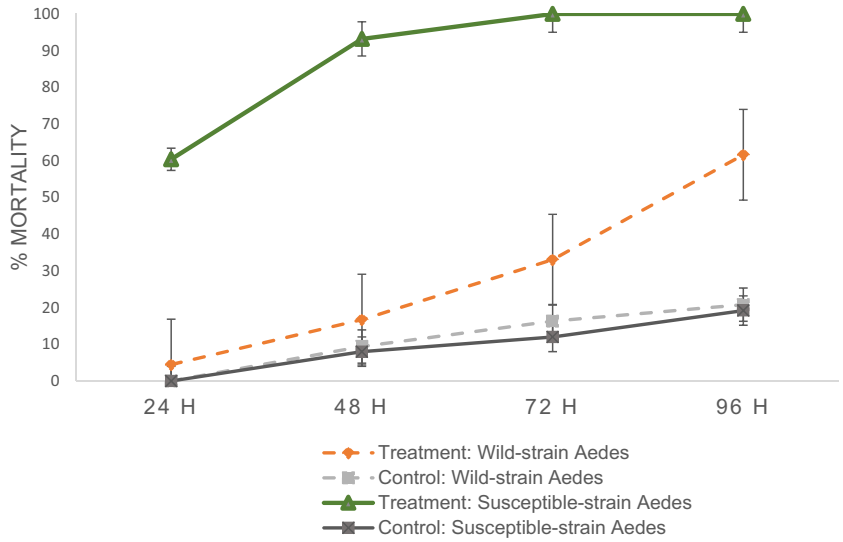
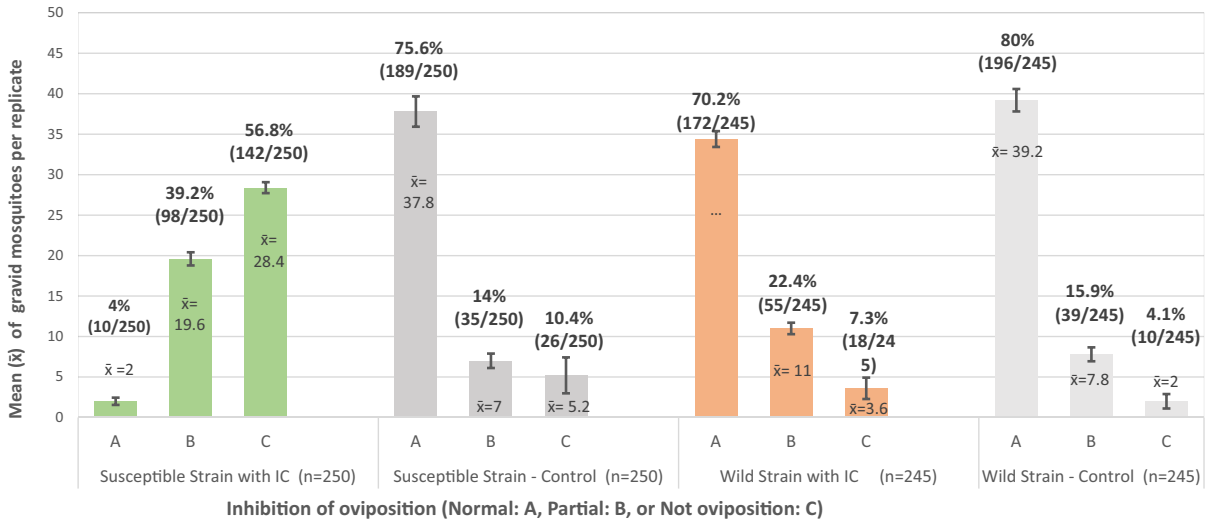


FIGURE 2 Cumulative mortality of gravid *Aedes aegypti* females when exposed to scenarios with the presence of insecticidal coating-treated and untreated containers, results with susceptible strain (green line), wild strain (orange line) and controls (grey line).

(a)



(b)

FIGURE 3 Effects on oviposition of gravid females (carrying eggs) of *Aedes aegypti* exposed to surfaces treated with the insecticidal coating (IC). (a) Gravid mosquitoes with different grades of inhibition of oviposition (A: no inhibition; B: partial inhibition; C: complete inhibition). (b) Percent inhibition of oviposition % (*n* per grade/ *n* exposed) and Mean ± SE of gravid mosquitoes per replicate (total = 5 replicates) of susceptible (green bars), wild-strain *Aedes* females (orange bars) and controls (grey bars).

wild strain was 16.7% (41/245) at 48 h and 61.6% (151/245) at 96 h (Figure 2). The inhibition of oviposition was observed in susceptible *Aedes* females (Rockefeller strain) with 56.8% (142/250), and 39.2% (98/250) of females with complete (C group) and partial (B group) inhibition respectively; oviposition was not affected in only 4% (10/250) (A group) (Figure 3 a-b). By comparison, this trend of inhibition of oviposition was not observed in the wild strain (Figure 3b). In summary, the coating caused mortality and inhibition of oviposition

TABLE 1 Cumulative mortality due to the effect of an insecticidal coating (IC) on the immature stages of *Aedes aegypti*.

			Observation time					
			24 h		48 h		72 h	
Instar	Strain	Variable	IC	Control	IC	Control	IC	Control
L3	Rockefeller susceptible ($N = 12$)	N° exposed larvae	180	180	NA		NA	NA
		N° dead larvae	177	0	178		180	0
		Percentage of mortality	98.3	0	98.9		100.0	0
		CI 95%	(95.2–9.6)		(96.0–99.7)		(98.0–100.0)	
	Wild strain ($N = 6$)	N° exposed larvae	90	90	NA	NA	NA	NA
		N° dead larvae	33	0	51	0	76	0
		Percentage of mortality (IC 95%)	36.7	0	56.7	0	84.4	0
		CI 95%	(26.8–7.5)		(45.8–67.1)		(75.3–91.2)	
	Wild strain ($N = 6$)	N° exposed larvae	90	90	NA	NA	NA	NA
		N° dead larvae	19	0	28	0	58	0
		Percetage of mortality	21,1	0	31,1	0	64,4	0
		CI 95%	(13.2–1.0)		(21.8–41.7)		(53.7–74.3)	
L4	Rockefeller –susceptible ($N = 12$)	N° exposed larvae	240	240	NA	NA		
		N° dead larvae	197	0	240	0		
		Percentage of mortality	82,1	0	100	0		
		CI 95%	(76.6–6.7)		(98.5–100.0)			
	Wild strain ($N = 5$)	N° exposed larvae	75	75	NA	NA	NA	NA
		N° dead larvae	41	0	61	0	72	0
		Percentage ofmortality	54,7	0	81,3	0	96,0	0
		CI 95%	(42.7–6.2)		(70.7–89.4)		(88.8–99.2)	
	Wild strain (N = 6)	N° exposed larvae	90	90	NA	NA	NA	NA
		N° dead larvae	21	0	71	0	81	0
		Percentage of mortality	23.3	0	78,9	0	90,0	0
		CI 95%	(15.1–3.4)		(69.0–86.8)		(81.9–95.3)	
Pupae	Rockefeller susceptible ($N = 16$)	N° exposed pupae	240	240	NA		NA	NA
		N° dead pupae	68	0	157		233	0
		Percentage of mortality	28.3	0	65.4		97.1	0
		CI 95%	(22.7–4.5)		(59.0–71.4)		(94.1–98.8)	
	Wild strain ($N = 6$)	N° exposed pupa	90	90	NA	NA	NA	NA
		N° dead pupae	0	0	11	0	36	0
		Percentage of mortality	0	0	12.2	0	40.0	0

in gravid females of the reference strain, consistently across the five replicates of the bioassays (Figure 3). But in the wild-type strain with ACM resistance these effects are not elicited.

No larval development was observed in the treated container of the bioassay chamber with both strains of *Ae. aegypti*. In the untreated container of this chamber, larval development, pupae and adult emergence were observed, resulting in 38 adults of the susceptible strain and 460 adults of the wild strain. In the control chambers, 2976 adults of the Rockefeller strain and 3810 adults of the wild strains were recorded. With susceptible *Ae. aegypti*, the offspring rate in the treatment chamber was 0.15 (38/250), and in the control chamber 11.90 (2976/250). Likewise, the offspring rates were slightly

TABLE 2 Emergence inhibition of larvae exposed to insecticidal coating (IC): (a) on larvae L3 y (b) on larvae L4 of *Aedes aegypti* strain Rockefeller.

(a)

		Observation time							
		Day 1		Day 7		Day 8		Day 9	
Instar	Variable	IC	Control	IC	Control	IC	Control	IC	Control
L3 ($N = 16$)	N°exposed individuals	90	90	NA	NA	NA	NA	NA	NA
	N°of adults emerging			0	20	0	67	0	88
	% individuals emerged			0	22.2	0	74.4	0	97.8
	Difference in % individuals emerged in relation to the control			−22.2 (−30.8 a −13.6)		−74.4 (−83.4 a−65.5)		−97.8 (−100 a−94.8)	
	% emergency inhibition			100		100		100	

(b)

		Observation time					
		Day 4		**Day 5**		**Day 6**	
Instar	**Variable**	**IC**	**Control**	**IC**	**Control**	**IC**	**Control**
L4 ($N = 10$)	N°exposed individuals	150	150	NA	NA	NA	NA
	N° of adults emerging	0	58	0	142	0	150
	% individuals emerged	0	38.7	0	94.7	0	100
	Difference in % individuals emerged in relation to the control	−38.7 (−46.5 a−30.9)		−94.7 (−98.3 a−91.1)		−100 (−100 a − 100)	
	% emergency inhibition	100		100		100	

higher with wild *Ae. aegypti*, 1.88 (460/245) in the treatment chamber and 15.55 (3810/245) in the control chamber. Thus, in IC-exposed egg laying, adult emergence is inhibited and offspring rates are reduced for both susceptible and wild strains.

Effect on egg hatching and inhibition of adult emergence (eggs from mosquitoes not exposed to IC)

The mean percentage of egg hatching in the control was 80.8%, compared to 52% in eggs exposed in IC containers. In addition, 100% L1 mortality was observed, compared to only 1% mortality in the control. No pupae were found in the treated container, while 97.5% of the eggs deposited gave rise to the adult stage in the control container. There were highly significant differences in all cases ($p < 0.01$). Overall, L3 and L4 mortality was 100% in the reference strain 48–72 h post-exposure, but fluctuated between 80% and 90% with the wild-type strain (Table 1).

Mortality was also observed in pupae exposed to IC, at 72 h the reference strain recorded 97.1% mortality, while the wild strain showed 40% mortality (Table 1). Adult emergence inhibition was 100% for L3 and L4 of the Rockefeller strain exposed in containers with IC (Table 2). The inhibition of emergence on pupae ($n = 480$; reference strain) was 100% after day 3 post-exposure (Table 3). Pupae of the wild strain ($n = 180$) exhibited 70.4% and 77.8% inhibition of emergence after 2 and 3 days post-exposure, respectively (Table 3). Individuals that emerged partially or completely from pupae in IC-treated containers were not viable and died within 24 h, in contrast to 100% viability of the control (data not included in the table). Exposure of different stages of *Ae. aegypti* to the insecticidal coating inhibits emergence of eggs, larvae and pupae, descendants of females not previously exposed to the product.

Residual effect study in *Ae. aegypti* larvae

Overall, EI of 100% (300/300) was observed in exposed larvae up to 6 months. By 9 and 12 months, this figure dropped to 98% (49/50) – 96% (48/50) and 80% (40/50) – 82% (41/50) respectively. During the trials at 1–3 months, 100% mortality of exposed larvae was achieved in both plastic (50/50) and concrete (50/50) containers. At 6 months follow-up, L4 stage mortality rates reduced to 92% (46/50) and 78% (39/50) for the plastic and concrete containers, respectively. After 6 months pupae development was observed, with four pupae out of 50 larvae exposed in plastic and 11 pupae out of 50 larvae exposed in concrete water containers, however all pupae died during this phase. In the ninth month there was emergence inhibition of pupae (plastic: 28 pupae out of 50 larvae exposed and concrete: 30 pupae out of 50 larvae exposed). From the emerged adults, only three adults survived for 48 h (one from the plastic container and two from the concrete container). At 12 months the emergence inhibition in the plastic container was 80% (40/50), and in the concrete container 82% (41/50) with 19 adults surviving for 48 h post-emergence (10 in the plastic container and nine in the concrete container) (Figure S1).

TABLE 3 Emergence inhibition of *Aedes aegypti* pupae exposed to insecticide coating (IC).

			Observation time					
			24 h		48 h		72 h	
Instar	Strain	Variable	IC	Control	IC	Control	IC	Control
Pupae		N° exposed individuals	240	240	NA	NA	NA	NA
	Rockefeller Susceptible ($N = 16$)	N° individuals emerged	0	0	0	62	0	213
		% individuals emerged			0	25.8	0	88.8
		Difference in % individuals emerged in relation to the control			−25.8 (−31.3 a−20.3)		−88.8 (−92.8 a−84.8)	
		% Emergence inhibition			100		100	
	Resistant strain ($N = 6$)	N° exposed individuals	90	90	NA	NA	NA	NA
		N° individuals emerged	0	0	16	54	20	90
		% individuals emerged			17.8	60.0	22.2	100.0
		Difference in % individuals emerged in relation to the control			−42.2 (−55.0 a − 29.4)		−77.8 (−86.4 a − 69.2)	
		% Emergence inhibition			70.4		77.8	

Mortality was not higher than 16% in the control groups with different types of containers, plastic ($\overline{X} = 7.6\% \pm 4–12$) and concrete ($\overline{X} = 13.6\% \pm 10–18$). In the plastic container controls the emergence rate was 92% (231 adults/250 larvae exposed), and in the concrete container controls it was 87% (218/250), significantly different from the exposure tests ($p < 0.01$).

Residual effect in *Ae. aegypti* adults

The KD effect was observed to be 86% (43/50) in the first month, 82% (41/50) in the third month and 80% (40/50) in the sixth month. However, during the later months of follow-up, the KD dropped to 70% (35/50) at ninth month and further decreased to 56% (28/50) at 12 months. In the initial residual effect tests, the 24-h mortality was 90% (45/50), reducing to 84% (42/50) by the third month. Mortalities experienced a decline, with rates of 62%, 48% and 24% recorded for months 6, 9 and 12, respectively. KD and mortality rates of the control group remained consistently below 10% through the study period. In summary, the IC demonstrated efficacy in inhibiting emergence for 12 months after intervention and adult mortality for up to 6 months.

Assessment of exposure and health risks

The concentration of ACM in the water of treated containers with IC was undetectable in all samples, with the limit of detection in this analytical series being 2.4 μg/L. PPF concentrations ranged from 0.02 to 1.7 μg/L during 26 weeks after treatment (Table 4). PPF was detected in all samples from the rusty concrete containers, with concentrations ranging from 0.13 to 0.49 μg/L. It was found in water samples from containers with tile-lined walls in both sun-exposed positions (0.08–0.72 μg/L), and shaded areas (1.15–1.69 μg/L), as well as in containers made of concrete, with concentrations ranging from 0.09 to 0.72 μg/L. Plastic containers exhibited concentrations from non-detected to 0.02 μg/L. There were no differences between the values obtained in the tanks exposed to the sun and those in the shade (Table 4).

For the risk assessment, the limit of detection in the main study (2.4 μg/L) was used to represent the concentration of ACM in the contained water. For PPF the highest concentration measured in the container with most leaching, made of concrete (1.69 μg/L), was used for the safety assessment. The model predicts that the dose of ACM for adults, children, toddlers and bottle-fed infants from drinking water stored in the treated containers is less than 0.05, 0.12, 0.29 and 0.26 μg/kg bw, and for breast-fed infants, 0.002 μg/kg bw. These figures represent 0.4%, 1%, 2.4%, 2.1% and 0.01% of the time-weighted average Tolerable Systemic Dose (TSD) (12 μg/kg bw d). As the exposure is continuous, it also represents maximal daily exposure, and this is equivalent to a lower proportion of the short-term tolerable systemic dose TSDAC (24 μg/kg bw). The predicted systemic dose from bathing and washing using water stored in the container remains below 0.001 μg/kg bw for all age groups.

The model predicts that exposure to PPF for adults, children, toddlers and bottle-fed infants from drinking water stored in the treated containers is no more than 2.9, 7.3, 17 and 15 μg/kg bw per day respectively, and for breast-fed infants, 0.5 μg/kg bw per day. These represent 7%, 18%, 43%, 38% and 1.3% of the TSD (40 μg/kg bw per day). The predicted systemic dose from bathing and washing using water stored in the container remains below 0.1 μg/kg bw in all age groups. Assuming dose-additivity, the proportion of the predicted maximal systemic dose for the two components is less than 8%, 19%, 44%, 39% and 1.4% of the TSD for adults, children, toddlers, bottle-fed infants and breast-fed infants. Assuming that: (i) the product and the active ingredients ACM, and PPF comply with impurity profiles established in the WHO specification; (ii) the coating of storage tanks is performed in compliance with the prescribed standard operating procedure. It is predicted that using water stored in metal, plastic or

TABLE 4 Analysis of alphacypermethrin and pyriproxyfen in water stored in containers with insecticidal coating (security assessment).

Sample info	Weeks post-treatment	ACM concentration (μg/L)	PPF concentration (μg/L)
Concrete water tank (rustic walls)	Control	nd	nd
	1	nd	0.134
	2	nd	0.463
	3	nd	0.459
	4	nd	0.234
	5	nd	0.491
Tank with tile-lined walls (partial sunlight)	Control	nd	nd
	1	nd	1.256
	2	nd	1.545
	3	nd	0.701
	4	nd	1.281
Concrete water tank	Control	nd	nd
	1	nd	0.721
	2	nd	0.098
	3	nd	0.152
	4	nd	0.087
Plastic tank	Control	nd	nd
	2	nd	0.02
	4	nd	nd
	26	nd	0.003
Tank with tile-lined walls (under shadow)	Control	nd	nd
	1	nd	1.154
	2	nd	1.562
	3	nd	1.236
	4	nd	1.687

Abbreviation: nd, not detectable.
*The calculated detection limit of the HPLC method for ACM is 2.4 μg/L (2.4 ppb).

concrete containers coated with IC, for washing or drinking does not pose a health risk to the users (adults, children, toddlers, newborns).

DISCUSSION

We investigated the effects of IC exposure on gravid females of *Ae. aegypti*, including mortality, sterilization and larval development. The concentrations of accumulated ACM and PPF in the water of the containers remained well below their acceptable TSD limits, confirming the safety of IC application in water tanks. These findings underscore the potential of IC for dengue vector control in breeding sites. While previous studies have reported improved operational efficiency in vector control using insecticide mixtures (Alim et al., 2023; Madgwick & Kanitz, 2023), IC uniquely demonstrates a novel application approach with dual action against both adults and immature stages, providing a 12-month residual effect. The high mortality observed among exposed gravid female *Ae. aegypti* mosquitoes suggests that they had direct contact with IC without any repellent effect. This mortality likely results from minimal but sufficient exposure to ACM while resting near the water or during oviposition (Figure 2) (Zeichner & Perich, 1999). Furthermore, female mosquitoes exposed to IC exhibited reduced fecundity, as evidenced by inhibition of oviposition and hatching in the IC containers.

Another notable effect was the reduction in offspring rate. Specifically, only 0.15 to 1.88 adults per gravid female exposed to IC were observed in the susceptible and wild strains, respectively (vs. 12 and 15.5 adults per gravid female in controls). Consequently, if >80% mortality was not achieved in a wild strain, a substantial decrease in offspring could be anticipated (Hustedt et al., 2020). The emergence inhibition was 100% in L3-L4 and 97% in pupae exposed to IC containers (as shown in Tables 2 and 3). Remarkably, this effect persisted for 12 months under semi-field conditions, aligning with previous studies on microencapsulated insecticides known for their long-lasting effects in vector control (Alim et al., 2023; Banjara et al., 2019; Gómez et al., 2024; Mosqueira et al., 2013). Therefore, IC demonstrated a more pronounced impact than other PPF formulations (Berti et al., 2013; Caputo et al., 2012; Tilak et al., 2022). Although ACM and PPF in aqueous solutions rapidly degrade due to sunlight exposure (with half-lives of 3.4–6.3 days and 3.72–6.36 days, respectively,

according to ECHA assessment reports), our results indicate that the IC coating remained effective in terms of mortality and adult emergence inhibition for several months. This persistence may be attributed to the absence of direct sunlight exposure and the protective effect of the coating.

Exposure of *Ae. aegypti* females to PPF results in sterilizing effects, which depend on their blood-fed status and time, leading to declines in fertility and fecundity (Yadav et al., 2019). In our study, IC exposure resulted in nearly a 60% reduction in the number of eggs oviposited. The inhibition of oviposition of gravid females exposed to IC underscores its significant impact on fertility. The primary cause of reduced egg hatching was direct exposure of gravid females to IC (Figure 3). Further, this also inhibits the development of eggs from females that have had no previous contact with coating, albeit at a lower rate (hatch rate = 52%). The remaining proportion of unhatched eggs (48%) likely experienced an ovicidal effect (Hustedt et al., 2020; Suman et al., 2013). These findings suggest that applying IC to *Ae. aegypti* breeding sites could effectively reduce mosquito densities inside homes by decreasing the offspring rate. Furthermore, IC-treated containers would simultaneously impact gravid females, immature stages and newly emerged imagos. No oviposition deterrence was observed due to IC, as evidenced by the absence of differences in the number of eggs laid in both treated and untreated containers. Consequently, IC treatment is unlikely to create new *Ae. aegypti* breeding sites, as it allows gravid females to rest and competes with untreated containers for selection as oviposition sites.

The efficacy and safety of IC support its application in domestic water containers, overcoming barriers often associated with breeding site control. These barriers include short residual effect times and the high cost of repeated applications. While larvicides in granular, liquid or tablet formulations lose their effectiveness upon water replacement, lasting no more than 3 months (Bowman et al., 2016), devices may exhibit residual activity for up to 6 months. However, their efficacy diminishes when objects are removed from inside tanks, often due to user carelessness (Oo et al., 2018). In particular, no existing larvicidal formulation takes advantage of the breeding site scenario to directly affect adult mosquitoes. IC uniquely addresses this gap, as gravid females, which frequently visit these surfaces for oviposition, are directly affected by IC. Furthermore, IC demonstrates entomological effects even with an annual application at the recommended dosage (15 m^2/L) per container. This performance is attractive for community implementation due to the reduced coating usage per container.

For instance, in Cúcuta, Colombia, ground tanks serve as the primary sites for *Ae. aegypti* pupae development, typically one tank per dwelling. These tanks have average dimensions of approximately 1 m × 0.7 m × 1 m (Carrillo et al., 2023). Treating the internal faces of one tank with IC would require 233 mL of the formulation, resulting in a cost of $0.5 USD per ground tank (house) per year.

Vector control continues to be a challenge and the ideal strategy has yet to be encountered. We know from systematic literature reviews that the quality of the implementation plays an overarching role (Horstick et al., 2018) but there are other aspects to be considered, like cost-effectiveness, user-friendliness and long-lasting effect. It has been shown in Yucatan, Mexico, that insecticide treated window screening is highly effective in reducing *Aedes* spp. mosquito densities and is sustainable for a long time (Che-Mendoza et al., 2018). However, when retested in Brazil is was too costly to be financed by the public health system (Quintero et al., 2017). Likewise, the painting of walls in bedrooms of endemic areas for visceral leishmaniasis in Bangladesh and Nepal: It was highly effective in reducing vector densities for at least 2 years, but the national programme could not afford the massive application in ten thousands of houses (Alim et al., 2023). Also the use of BG traps for *Aedes* spp. control has a considerable cost and is not suitable in large urban areas.

The use of insecticide-treated window curtains against *Aedes* mosquitoes (Kroeger et al., 2006; Rizzo et al., 2012), vectors of Chagas disease (Kroeger et al., 2003) and cutaneous leishmaniasis (Vanlerberghe et al., 2011) was highly efficacious in reducing vector densities. However, people felt 'imprisoned' in their houses because they did not perceive proper ventilation during hot days. Similarly, water container covers, tested in various places, were not appreciated by users due to their complexity in covering the containers after use. Additionally, the use of biological methods such as dragonflies or copepods in Southeast Asia (Wai et al., 2012), or larvivorous fish, is limited by container type (which should be large) and user acceptance. In comparison, the insecticidal coating (IC) formulation tested in this study offers long-lasting effects, low cost, user-friendliness, and once applied, it does not require maintenance from homeowners. Based on the previously described findings, it is likely that IC applied in the main *Ae. aegypti* breeding sites would sustainably reduce populations for 9–12 months and contribute to reducing the transmission of dengue, Zika and chikungunya without posing risks to user health.

AUTHOR CONTRIBUTIONS

Rocio Cárdenas: Conceptualization; investigation; funding acquisition; writing – original draft; project administration; methodology; visualization; formal analysis; writing – review and editing; supervision; resources. **Olga L. Cabrera:** Methodology; writing – review and editing; writing – original draft; investigation; formal analysis; validation; data curation; visualization. **Maria A. Carrillo:** Investigation; writing – review and editing; methodology; formal analysis; validation; supervision; resources; project administration. **Alejandra Pineda:** Writing – review and editing; investigation; methodology; visualization. **Martha L. Ahumada:** Validation; methodology; supervision; formal analysis; investigation; writing – review and editing. **Yohana Yañez:** Investigation; validation; data curation; supervision; project administration; writing – review and editing. **Hanafy Ismail:** Investigation; validation; supervision; formal analysis; writing – review and editing; methodology. **Mark Paine:** Investigation; validation; formal analysis; supervision; writing – review and editing; methodology. **Tatiana Rivera:** Investigation; writing – review and editing; visualization; data curation; formal analysis; software. **Axel Kroeger:** Conceptualization; investigation; funding acquisition; writing – original draft;

writing – review and editing; formal analysis; project administration; supervision; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there was not any conflict of interest in conducting this study.

DATA AVAILABILITY STATEMENT

The data is available in doi:10.5061/dryad.2z34tmpwb.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

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