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Developing a Novel Attractive Toxic Sugar Bait (ATSB) Device
for Intra-domiciliary Control of *Aedes aegypti*

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A mi madre.

1 **Abstract**

2 On account of vector control being the most successful approach towards prevention of the major
3 arboviral concerns worldwide and the imperative pursuit of alternatives to traditional pesticides, we
4 developed a novel attractive toxic sugar bait (ATSB) device. The device incorporates seemingly
5 inexpensive (cost < 1 USD) olfactory and visual cues attractive to *Aedes aegypti* L. We incorporated 1%
6 boric acid in 10% sucrose solution as toxic component on the devices and this showed to be effective
7 killing female *A.aegypti* in controlled laboratory conditions (0% survival probability after 48h).

8 In addition, we evaluated the biological action of the device and concluded that boric acid acts
9 as a stomach poison. Using transmission electron microscopy we further determined that it disrupts the
10 continuity of the epithelial tissue of the posterior midgut. The device is effective poisoning *A. aegypti*
11 females in two different physiological statuses (recently blood fed and parous). Finally, devices were
12 effective after 180 days of being assembled. These features provide us with information that will be
13 useful for future semi-field and field trials.

14 15 **Introduction**

16 The diseases caused by dengue, chikungunya, and Zika viruses are becoming increasingly
17 important public health concerns around the globe. Dengue virus (DENV) alone presents an estimate of
18 more than 300 million new infections worldwide every year [1]. In the Americas only, dengue renders
19 an average economic burden of USD 2.1 billion per year [2]. Chikungunya virus (CHIKV) and Zika virus
20 (ZIKV) have caused major outbreaks in the Americas in 2013 and late 2015, respectively [3-5].
21 Furthermore, there have even been clinical reports of patients presenting co-infection between the three
22 viruses in the region [6].

1 In Ecuador all three viruses have been reported and, according to health officials, the incidences
2 of both chikungunya and dengue during 2015 (33,621 and 42,505 cases, respectively) more than doubled
3 the incidence of these diseases during any of the previous four years [7].

4 Although there are promising efforts aimed at developing a vaccine against DENV [8-14], at the
5 moment there are no commercially available vaccines or antivirals for any of these diseases leaving
6 patients with the sole option of symptomatic treatment. As a consequence of this lack of direct
7 approaches, vector control has become the main focus towards preventing these diseases.

8 *Aedes aegypti* L. is the principal vector of these three arboviruses [15-17] throughout its
9 geographic distribution range. Originally from Africa, this mosquito species is, at present, widely
10 distributed around the globe [18]. *A. aegypti* is both highly anthropophilic and highly synanthropic [19,
11 20, 21].

12 Female *A. aegypti*'s strong preference towards human blood hosts (i.e. anthropophily) has been
13 vastly studied [20]. It is now known that this behavior is genetically fixed on at least one odorant receptor
14 (*AaegOr4*), located on the antennae, which is highly sensitive to a component (Sulcatone) present in
15 human odor at particularly high levels [19].

16 Synanthropy indicates the tight connection of species to human households. Several aspects of
17 the biology of *A. aegypti* benefit from conditions provided by human habitations [21]. For instance,
18 aquatic stages of their life cycle develop in artificial water containers in or around human habitations
19 [22]. In addition, adults tend to rest on clothing or surfaces inside houses [23, 24]. These ecological and
20 behavioral traits have made intradomicile control a priority [21].

21 Historically, pesticide spraying has been the main approach towards *A. aegypti* control. The
22 intense application of dichlorodiphenyltrichloroethane (DDT), promoted by the PAHO during the

1 decades of the 1940s to the 1960s, achieved mosquito eradication in more than 18 countries in the
2 Americas [25]. This intention to eradicate the mosquito at a continental scale rapidly fell apart after a
3 lack of political interest on continuing eradication programs and strong selection of vectors resistant to
4 DDT and other organochlorine insecticides [26]. This cleared the way for reinfestation on the course of
5 the following decades. At present, long-term overuse of traditional pesticides has widespread selection
6 of resistance alleles in mosquito populations which, consequently, has made the pursuit of new control
7 methods imperative [27].

8 At present, a variety of novel mosquito control methods exist under different phases of
9 development. These methods include, among other, the use of genetically modified mosquito strains,
10 infection of mosquito strains with the intracellular parasite *Wolbachia*, and the use of attractive toxic
11 sugar baits (ATSBs) [27].

12 ATSBs target the sugar feeding behavior of mosquitoes [27-29] by using sugary solutions laced
13 with a toxic component [28, 30]. Boric acid has proven to be highly effective as the toxic component of
14 ATSBs used against different species of mosquitoes, including: *Anopheles gambiae* Meigen [31], *Aedes*
15 *albopictus* Skuse [30], and *Culex quinquefasciatus* Say [30]. In addition, boric acid presents a relatively
16 low toxicity to humans and other vertebrates. To the best of our knowledge, there is no published
17 information evaluating boric acid's toxicity on *A. aegypti*.

18 Most studies published on the use of ATSBs for mosquito control have consisted on aerosol
19 dispersal of toxic sugar solution on outdoor vegetation and have been focused on the control of malaria
20 vectors [31-36]. As far as we are aware, only a few studies have trialed ATSB's in intradomiciliary
21 conditions, and were carried out in Africa to control malaria vectors as well [37,38].

22 The efficiency of ATSBs depends on the sugar feeding behavior of mosquitoes. Some
23 controversy has arisen regarding sugar consumption of female mosquitoes following blood ingestion

1 [39]. Since this would directly affect the performance of the devices, it is worth mentioning that in field
2 conditions, female mosquitoes readily feed on sugar [40, 41]. However, there is a relevant requirement
3 for further information to clarify potential effectiveness of ATSB's in different physiological statuses
4 and for novel means of enhancing ATSB's attractiveness.

5 It is known that olfactory, visual, and thermal cues are key for mosquitoes in detecting potential
6 hosts [42]. Regarding visual cues, black and white contrasts have proven to be strong long-range
7 attractants for *A. aegypti* [43, 44] and have been previously used on successful mosquito traps [45]
8 therefore we consider visual cues to be of vast importance for the development of novel traps and baits.
9 We think that ideally the sum of these cues will play an important role on improving the attractiveness
10 of ATSBs nonetheless simplicity is required for logistical purposes.

11 In this study we present the design of a simple ATSB device, which has the potential to be used
12 as a tool for the reduction of indoor adult populations of *A. aegypti*. We assessed the efficiency of this
13 device under laboratory conditions, provide insight into the biological mode of action of the devices, and
14 evaluated parameters relevant for future field trials. Given the fact that devices will potentially be utilized
15 inside households of vulnerable sectors of the population we feel it is important for the device to be safe
16 for humans and affordable as well.

17 **Methods**

18 **Mosquitoes**

19 Two Ecuadorian strains of *Aedes aegypti* (*Ae. aeg*-2: Ecuador, Guayas province, Guayaquil city,
20 acquired in 2014; T-COCA 02.1: Ecuador, Orellana province, Puerto Francisco de Orellana city, acquired
21 in 2015), generously provided by the National Service for Malaria Eradication (SNEM) and maintained
22 at the Center for Research on Health in Latin America (CISeAL), were used in the experiments.
23 Mosquitoes were reared and maintained under standard insectary conditions: 28 ± 2 °C temperature; 80

1 ± 10 % relative humidity; 12h:12h (L:D) light cycle. Larvae were fed finely ground fish food. When
2 required, mosquitoes were sexed during the pupal stage. Adults were kept in 15 x 15 x 15 cm cages. For
3 maintenance, adult mosquitoes were fed 10% sucrose solution *ad libitum*. For blood feeding, female
4 adult mosquitoes were offered access to a restrained female mouse (*Mus musculus* L.). All mosquitoes
5 were maintained under insectary conditions between 0 and 14 days after adult emergence before they
6 were used for experiments. Mosquitoes referred to as “starved” were deprived of access to sugar during
7 48 hours previous to their use in experiments (but allowed continuous access to water throughout this
8 time).

9 **Attractive Sugar Bait Devices**

10 The devices consisted of two concentric foam sheet circles: an inner white circle (5 cm diameter)
11 and an outer black circle (10 cm diameter). Before assembly, both foam circles were individually
12 submerged for 24 hours in either a non-toxic sugar solution (10% sucrose, prepared using distilled water
13 and brown sugar) or a toxic sugar solution (1% boric acid, prepared using 10% sucrose solution as
14 solvent). Henceforth in this manuscript, devices coated with non-toxic sugar solution will be called
15 “attractive sugar baits” (ASBs) in order to differentiate them from ATSBs, which are coated with the
16 toxic sugar solution.

17 After 24-hour submersion, the foam circles were air-dried for 24 hours and subsequently stapled
18 together. A bamboo stick was fixed to the back of the device, to serve as a stand (Fig. 1B).

19

20 **Fig 1. Attractive Toxic Sugar Bait Devices.** (A) 3D model of the attractive toxic sugar bait devices.
21 Diameters for each of the foam sheet circles are shown. (B) Photography of an attractive sugar bait
22 device. Photographed by M. Neira.

23

24 **Survival Assessment of Mosquitoes Exposed to the Device**

1 To determine whether exposure to the ATSB devices has an influence on adult mosquito survival
2 probability, we conducted an experiment in which groups of 30 adult female mosquitoes, placed in a 15
3 x 15 x 15 cm cage, were exposed during 48 hours to either an ATSB device (for experimental treatments)
4 or an ASB device (for control treatments). Mortality in each cage was recorded every 24 hours. The test
5 was replicated four times. The assessment was repeated using each of the two strains.

6 For each treatment, interval censored survival data and subsequent non-parametric maximum
7 likelihood estimate (NPMLE) was plotted and analyzed using the ‘survival’ package [39] in R version
8 3.2.2 (R Core Team, www.r-project.org). A log-rank hypothesis test was used to compare the survival
9 distributions of the two treatments.

10

11 **Appraisal of the Biological Mode of Action of the Devices**

12 **Uptake Mechanism of the Toxic Component.** To establish whether the toxic component of ATSBs
13 needs to be ingested by the mosquitoes in order to exert its effect, we presented the devices to cohorts of
14 adult females which were unable to ingest food due to the surgical ablation of their mouthparts. To
15 establish these cohorts, individuals were first anesthetized by placing them at 4°C during 10-15 minutes.
16 Anesthetized specimens were individually placed under a dissection microscope and, using a human hair,
17 we tied a knot at the proboscis’ proximal end in order to create a constriction that would impede the flow
18 of food. Subsequently, the part of the proboscis anterior to the knot was removed using micro-dissection
19 scissor (Fig. 2). Following intervention, mosquitoes were left to rest for 24 hours before being used in
20 any experiment.

21

22 **Fig 2. Feeding Disruption Procedure.** (A) Anesthetized individual with whole proboscis. (B) Human
23 hair tied at the proximal end of the proboscis. (C) Micro-dissection scissor removal of proboscis’ segment
24 anterior to the knot. (D) Feeding disrupted individual.

1 To control for the potentially negative effect of the anesthetizing procedure in mosquito survival,
2 non-ablated mosquitoes used in control groups were also placed at 4°C during 10-15 minutes, and
3 allowed to recover during 24 hours before experimental set-up.

4 For each experiment two cages were set up, each containing 20 starved ablated mosquitoes.
5 Individuals in one of these cages were exposed to an ATSB device, and individuals in the other cage
6 were exposed to an ASB device. Two more cages containing 20 non-ablated, starved mosquitoes each
7 were set up likewise, making a total of four cages (a summary of the experimental set-up is shown in
8 Table 1). Mortality in all groups was assessed at 24 and 48 hours of exposure to the devices. The
9 experiment was replicated three times. Only one strain (*Ae.aeg-2*) was tested. Normal distribution of the
10 data was determined with Kolmogorov-Smirnov and Shapiro-Wilk tests. Analysis of variance (ANOVA)
11 was performed to evaluate differences between treatments and a post-hoc Tukey's test was used to
12 determine ranks. These analyses were performed in R version 3.2.2 (R Core Team, www.r-project.org).

13 **Table 1. Names assigned to treatments used to evaluate the mechanism of ingress of the toxic**
14 **component**

	Whole proboscis	Cut proboscis
Toxic Device	“Whole toxic”	“Cut toxic”
Non-toxic device	“Whole non-toxic”	“Cut non-toxic”

15
16 **Histopathological Effects on the Midgut.** Two cages were set up, each containing 30 adult starved
17 female mosquitoes. Specimens in one of these cages were exposed to a toxic device (ATSB), and
18 specimens in the other cage were exposed to a non-toxic device (ASB). Cages were monitored during
19 the next 24 hours, and dead mosquitoes were removed by aspiration every hour from the cages. Using a
20 dissection microscope, the legs, head and wings of every dead specimen were removed on a drop of 70%
21 ethanol. The abdominal cuticle was gently disrupted in order to permit the exposure of internal tissues to

1 the fixative. Afterwards, individuals were fixed in a solution containing 2.5% glutaraldehyde, 2.5%
2 paraformaldehyde in 0.1M cacodylate buffer (pH 7.4), and stored at 4 °C for 72 hours. Specimens were
3 then washed in cacodylate buffer with 0.1M sucrose overnight. Post-fixing was achieved by leaving the
4 specimens for two hours at 4°C in 2% osmium tetroxide in 0.1 cacodylate buffer, pH 7.4. Subsequently,
5 individuals were stained using 2% uranyl acetate and left to rest for three hours in the dark at room
6 temperature. Tissues were later dehydrated through a series of ethanol baths (50%, 70%, 95%, 100%).
7 Afterwards, they were placed in propylene oxide for 30 minutes, then in a 1:1 volume propylene
8 oxide:resin (Epon 812, Araldite 502, dodecenyl succinic anhydride, benzyl dimethylamine) mixture for
9 one hour, and later, 1 more volume of resin was added and left on a rotator overnight. Finally, mosquitoes
10 were embedded in resin and incubated at 60°C for 24 hours. Resin embedded tissues were cut using an
11 ultramicrotome and mounted on copper grids. Later, mosquitoes were stained using 2% uranyl acetate.
12 Specimens were observed using a transmission electron microscope and micrographs of tissues of interest
13 were obtained.

14

15 **Evaluation of Parameters Relevant for Future Field Trials**

16 **Effects of the Physiological Status of the Mosquitoes on the Performance of the Device.** These tests
17 were performed using strain TCOCA 02.1. Two different physiological statuses were evaluated using
18 mated starved female adult mosquitoes: blood fed and parous. Females deemed as “blood fed” were
19 established by selecting blood-engorged individuals immediately after a blood meal. Females deemed as
20 “parous” were first blood fed and subsequently maintained for 7 days under insectary conditions, in order
21 to ensure that they had oviposited before being used for experimentation.

22 Two cages for each of the defined physiological statuses were set up with 30 mosquitoes each.
23 One cage exposing them to an ATSB and the other to an ASB. Survival data was gathered at 24 and 48
24 hours. The test was replicated 3 times. Interval censored survival data was plotted and analyzed using

1 the ‘survival’ package [39] in R version 3.2.2 (R Core Team, www.r-project.org). A log-rank hypothesis
2 test was used to compare the survival distributions of the two treatments.

3

4 **Shelf Life of the Device.** In order to determine the shelf life of ATSB devices, toxicity tests were
5 performed using ATSB and ASB devices which had been stored for 38, 80 and 118 days after their
6 production. For storage, devices were individually wrapped inside a sealed plastic bag and placed inside
7 an incubator at 28 ± 2 °C and $80 \pm 10\%$ relative humidity.

8 The protocol for performing the bioassays was identical to that previously described to assess
9 survival of mosquitoes exposed to the device. For each group of mosquitoes exposed to an ATSB device,
10 we set up a matching control group exposed to an ASB device stored during an equivalent amount of
11 time.

12 For each storage time, three replicates of the experiment were set up. Interval censored survival
13 data was plotted and analyzed using the ‘survival’ package [39] in R version 3.2.2 (R Core Team, www.r-project.org). A log-rank hypothesis test was used to compare the survival distributions of the two
14 treatments.
15

16

17 **Results**

18 **Survival Assessment of Mosquitoes Exposed to the Device**

19 Mosquitoes exposed to toxic devices presented 55% survival probability reduction in the first 24
20 hours post-exposure, and 45% reduction between 24 and 48 hours post-exposure, resulting in a 0%
21 survival probability by the end of the trials. On the other hand, mosquitoes exposed to control devices
22 presented 0.83% survival probability drop during the first interval (0h-24h] and 1.67% reduction during
23 the second interval (24h-48h], resulting in 97.5% survival probability by the end of the experiment (Fig.
24
25

3). Differences between the survival curves of toxic and non-toxic treatments were highly significant ($p < 0.001$).

Fig 3. Survival Assessment of Mosquitoes Exposed to the Device. Survival and NPMLE of individuals exposed to toxic (dotted line; $n=120$) or non-toxic devices (solid line; $n=120$). Interval-censored survival data collected at two time points (24h and 48h).

Appraisal of the Biological Mode of Action of the Devices

Uptake Mechanism of the Toxic Component. After 48 hours, mosquitoes which could still feed (i.e. mosquitoes with an intact proboscis), presented 100% mortality when exposed to the toxic device, and 3.33% mortality when exposed to the non-toxic device. Mosquitoes which were intervened in order to block feeding presented 38.33% mortality regardless of the toxic or non-toxic condition of the devices. Significant differences were found between the four treatments ($p < 0.001$). Post-hoc pairwise comparison determined only intervened treatments were not significantly different between each other (Fig. 4).

Fig 4. Uptake Mechanism of the Toxic Component. Mortality after 48 hours of exposure to devices and mosquito conditions summarized in Table 1. The letters above the bars show ranks of statistical significance. Different letters mean a $p < 0.05$.

Histopathological Effects on the Midgut. Mosquitoes that had ingested toxic sugar solution presented histological abnormalities in the posterior midgut. Electron micrographs depict a disruption of the continuity of the epithelial tissue (Fig. 5A, 5C). Due to the distribution of bacteria in the gut lumen, we suggest this disruption cannot be considered a microscopy artifact. In addition, we found abnormal adipocytes that we hypothesize are undergoing a process of necrosis (Figs. 5E, 5F). We suggest these

1 two affections are the probable cause of death of these individuals. Microscopic images of individuals
2 that were only exposed to sucrose solution presented none of these pathologies on the posterior midgut
3 (Figs. 5B, 5D).

4

5 **Fig 5. Histopathological Effects on the Midgut.** Longitudinal sections of *Aedes aegypti* posterior
6 midgut. (A,C,E,F) Mosquitoes exposed to toxic devices. (B, D) Normal posterior midgut of mosquitoes
7 exposed to non-toxic devices. Abbreviations: LM, gut lumen; AC, adipocyte; ED, epithelial disruption.

8

9 **Evaluation of Parameters Relevant for Future Field Trials**

10 **Effects of the Physiological Status of the Mosquitoes on the Performance of the Device.** Both
11 physiological statuses evaluated (“blood fed” and “parous”) presented a lower survival probability when
12 exposed to toxic devices than when exposed to non-toxic devices.

13 Blood fed females’ survival probability dropped 13.33% during the (0h-24h] interval, 22.22%
14 during the (24h-48h] interval, and 55.56% during the last interval (48h-72h]. This results in 8.89%
15 survival probability by the end of the experiment after 72 hours of exposure. On the other hand, the non-
16 toxic control for this physiological status resulted in 90% survival probability by the end of the 72 hours
17 after having dropped 8.89%, 1.1%, and 0% during the (0h-24h], (24h-48h], and (48h-72h] intervals,
18 respectively. Differences between control and toxic treatment survival curves are highly significant
19 ($p < 0.001$).

20 Parous females presented 65.6% decline on their survival probability during the first interval (0h-
21 24h] and 0% survival probability after 48 hours of being exposed to toxic devices. These results are
22 significantly different ($p < 0.001$) to the non-toxic control, which showed 2.2% survival probability drop
23 during the (0h-24h] interval, resulting in 97.8% survival probability after 48 hours of exposure had passed
24 (Fig. 6B).

1 **Fig 6. Effects of the Physiological Status of the Mosquitoes on the Performance of the Device.**
2 Survival of individuals exposed to toxic (dotted line; n=90) or non-toxic devices (solid line; n=90).
3 Survival curves and NPMLE for (A) blood fed individuals exposed for 72 hours and (B) parous
4 mosquitoes exposed to the device 48 hours.

5
6 **Shelf Life of the Device.** Mosquitoes exposed to toxic devices stored for 38 days showed 0% survival
7 probability after the (0h-24h] interval. On the contrary, non-toxic treatment showed 96% survival
8 probability after the (24h-48h] interval was concluded. Highly significant differences were found
9 between treatments (Fig. 7A).

10 Mosquitoes exposed to toxic devices stored for 80 days showed 16% survival probability after
11 the (0h-24h] interval, and 0% survival probability at the end of the experiment. On the other hand, non-
12 toxic treatment showed 97% survival probability after the (24h-48h] interval was concluded. Highly
13 significant differences were found between treatments (Fig. 7B).

14 Mosquitoes exposed to toxic devices stored for 118 days showed 95% survival probability after
15 the (0h-24h] interval, 64% survival probability during the (24h-48h] interval, and 35% survival
16 probability by the end of the trials. On the contrary, non-toxic treatment showed 96% survival probability
17 after the (24h-48h] interval was concluded. Highly significant differences were found between treatments
18 (Fig. 7C).

19
20 **Fig 7. Shelf Life of the Device.** Survival and NPMLE of individuals exposed to toxic (dotted line; n=90)
21 or non-toxic devices (solid line; n=90) that had been stored for: (A) 38 days, (B) 80 days, and (C) 118
22 days. Interval-censored survival data collected in two time points (24h and 48h).

23

24

1 **Discussion**

2 Our results provide strong evidence that toxic sugar bait devices loaded with boric acid as active
3 component are highly toxic to *A. aegypti* when tested under our experimental conditions.

4 Our appraisal of the biological action of the devices provides an insight into how low
5 concentrations of boric acid (1% boric acid in 10% sucrose solution) affect *A. aegypti* and have the
6 potential of causing mortality in these insects. Although boric acid's toxicity has been previously reported
7 for other mosquito species [28-38], to the best of our knowledge, this is the first report of the effect of
8 this compound on this species of mosquito. By determining that the mechanism by which the toxic
9 component enters the body of the insect is ingestion, we provide further evidence to support the notion
10 that this inorganic pesticide acts as a stomach poison, which has been previously suggested [29, 30].
11 Because the digestive system of insects is well adapted to avoid intoxication and infection, an effective
12 stomach poison needs to be able to cause cellular damage [47, 48]. Our electronic microscopy analysis
13 confirms that insects which ingested boric acid, display tissue abnormalities similar to those previously
14 reported in other insect species which ingested stomach poisons [47, 48]. Based on our experimental
15 results, we suggest that in *A. aegypti* these effects are altering the integrity and normal performance of
16 the midgut, to the point of becoming lethal to the mosquitoes.

17 The evaluation of the influence of the physiological status of the females becomes relevant for
18 the eventual use of the devices in the field, where female mosquitoes are likely to have access to various
19 sources of food, including blood and sugar. There has been some debate about whether female *A. aegypti*
20 do in fact consume sugar when they have blood available as a food source [39, 40]. The fact that
21 significant mortality is observed when recently blood-fed mosquitoes are exposed to the ATSB devices
22 supports the notion that blood feeding does not completely inhibit sugar feeding behavior in this species.

23 Under our experimental conditions, exposure of blood fed females to ATSB devices does result
24 in significant mortality, albeit at a somewhat reduced rate when compared to the mortality observed in

1 starved individuals. Interestingly, the largest drop in survival probability in blood fed females is observed
2 between 48h and 72h post-exposure to the ATSB (Fig. 6), suggesting that after 48 hours females have
3 already used imbibed blood for the development of eggs, and are keen to search for further meals.

4 Based on this evidence, it is plausible to suggest that if deployed in the field, ATSB devices would
5 be efficient in killing female mosquitoes of various physiological statuses, including females which have
6 already ingested blood – a particularly important group from an epidemiological standpoint.

7 One other parameter considered important for future field trials was the shelf life of the devices.
8 The fact that toxic devices are still effective after 118 days storage (in conditions similar to those found
9 in the field), enormously simplifies potential logistical challenges that might occur during future field
10 and semi-field trials. As far as we can tell, the extended duration of the devices (at least 118 days) is
11 probably a consequence of the boric acid's relatively high stability.

12 Further investigation on evaluating the device's attractiveness is suggested. Our research does
13 not quantify how attractive to mosquitoes are the devices. We suggest additional semi-field trails in order
14 to evaluate this and various other parameters.

15 Finally, we would like to remark the need for developing novel control methods which take into
16 consideration geographic, social, and economical challenges affecting those individuals most vulnerable
17 to mosquito-borne infections. The attractive toxic sugar bait devices we developed in this investigation
18 resulted to be relatively inexpensive, having a cost of less than 1 USD. The actual cost might result to be
19 even lower if the devices were to be mass-produced. Furthermore, the toxicity of boric acid to vertebrates
20 is low in the concentrations which were used for the devices. This provides an enormous logistical asset.

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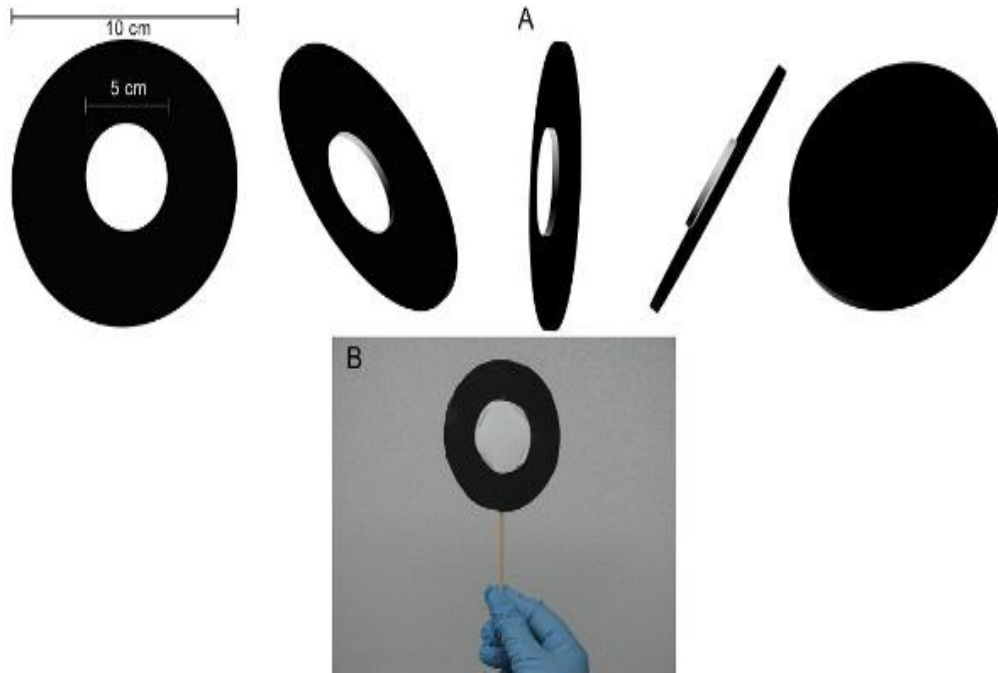
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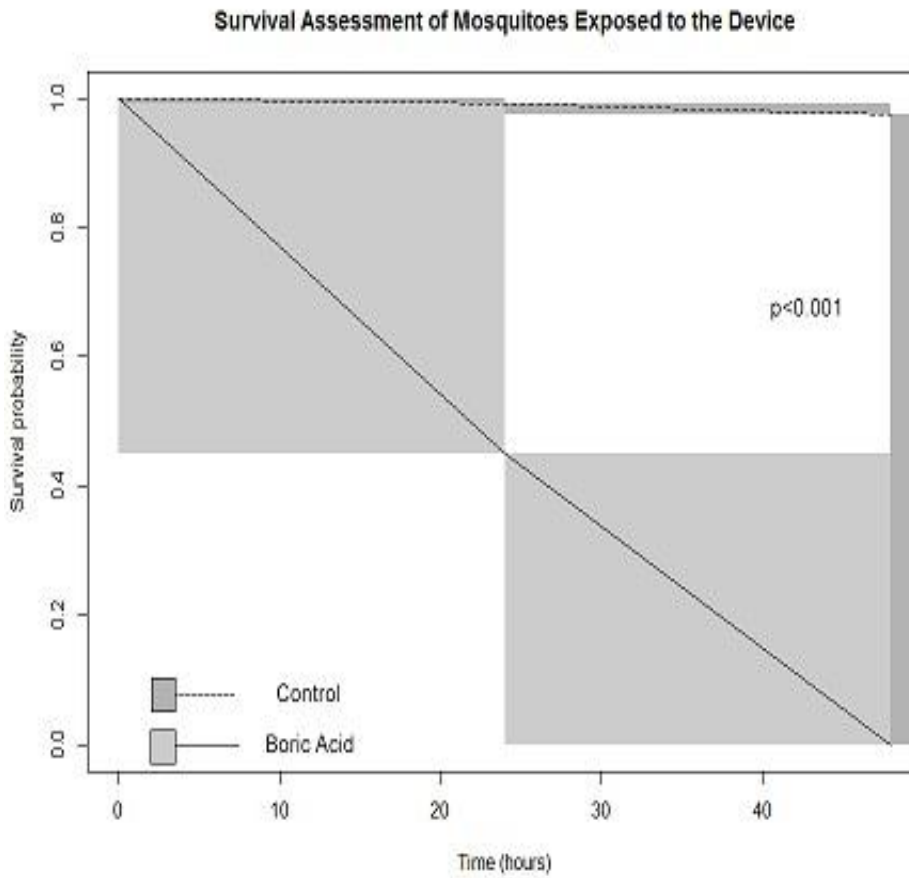
1 **FIGURES**



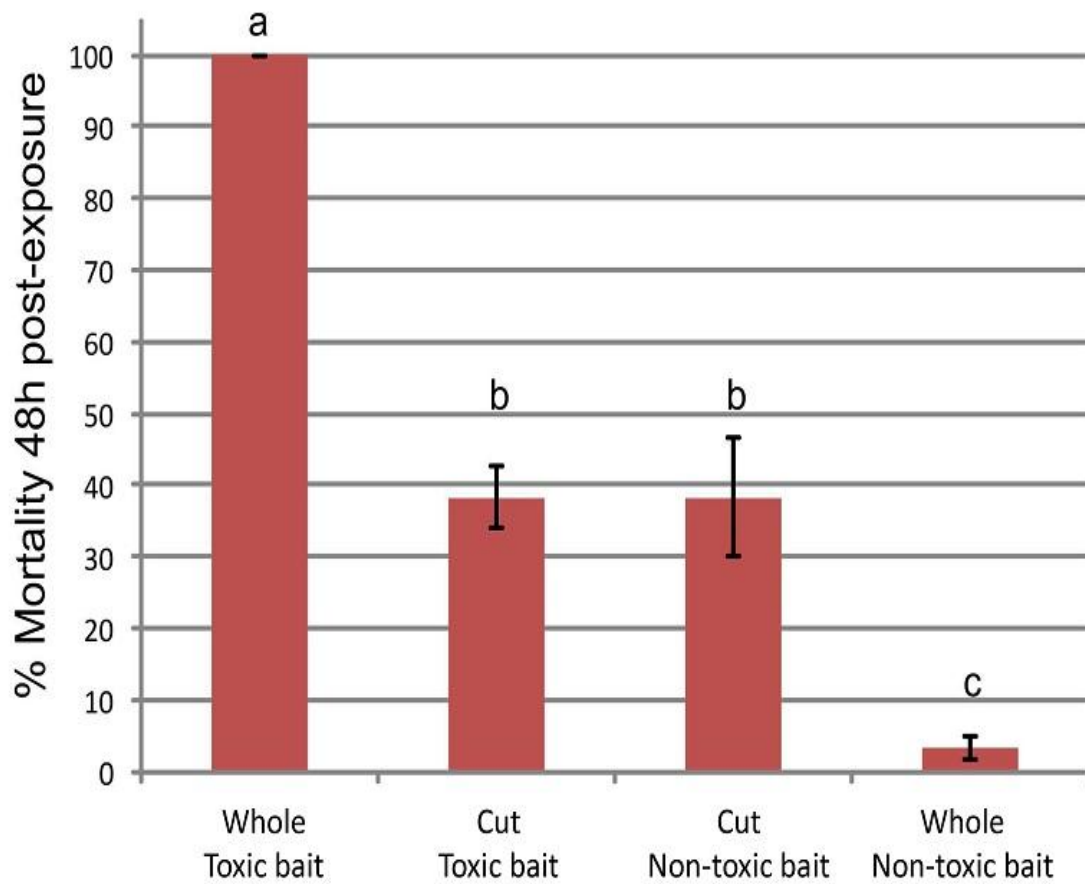
2
3 **Fig 1. Attractive Toxic Sugar Bait Devices.** (A) 3D model of the attractive toxic sugar bait devices.
4 Diameters for each of the foam sheet circles are shown. (B) Photograph of an attractive sugar bait
5 device.



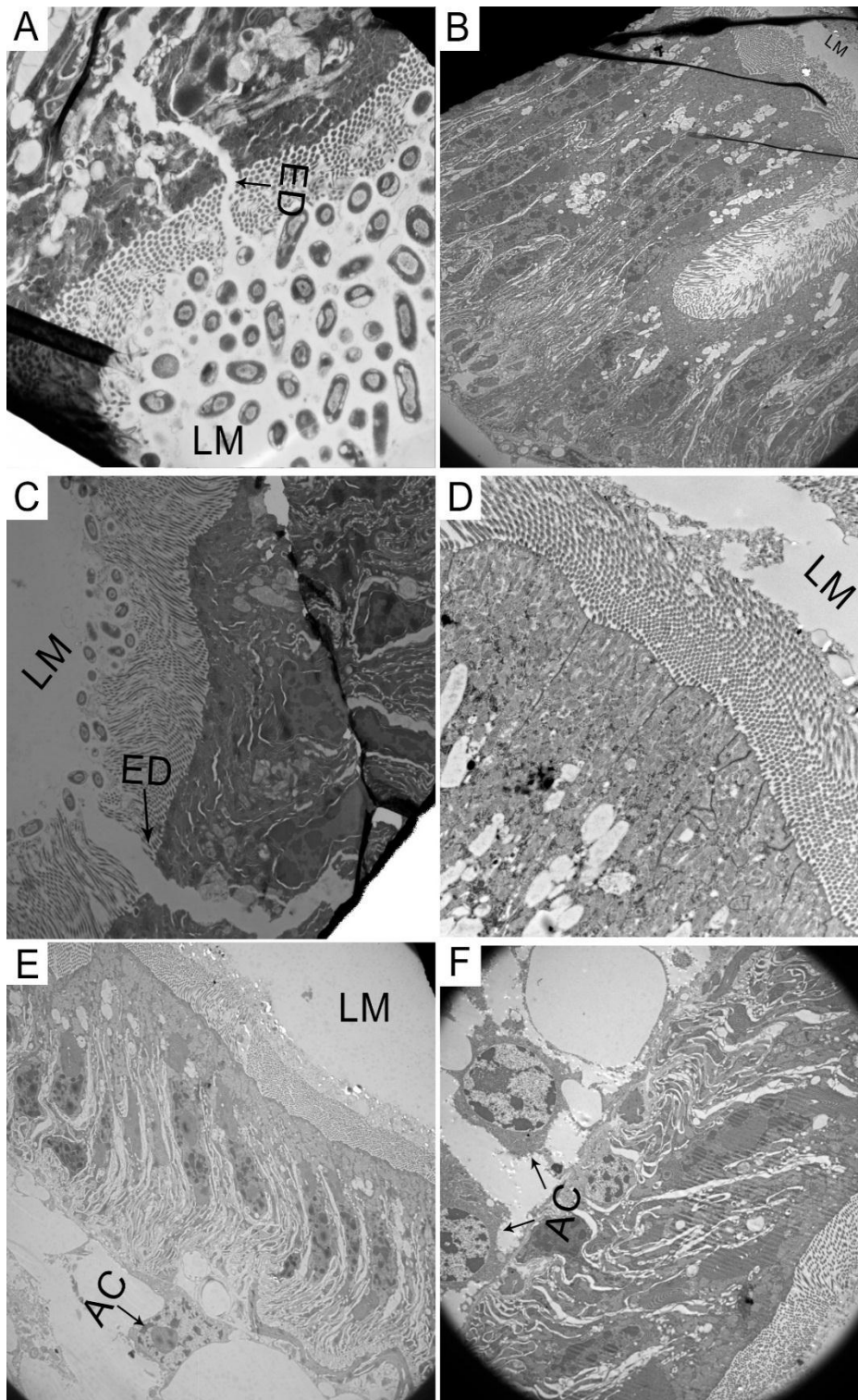
1 **2. Feeding Disruption Procedure.** (A) Anesthetized individual with whole proboscis. (B) Human hair
2 tied at the proximal end of the proboscis. (C) Micro-dissection scissor removal of proboscis segment
3 anterior to the knot. (D) Feeding disrupted individual.



1 **Fig 3. Survival Assessment of Mosquitoes Exposed to the Device.** Survival and NPMLE of
 2 individuals exposed to toxic (dotted line; n=120) or non-toxic devices (solid line; n=120). Interval-
 3 censored survival data collected at two time points (24h and 48h).

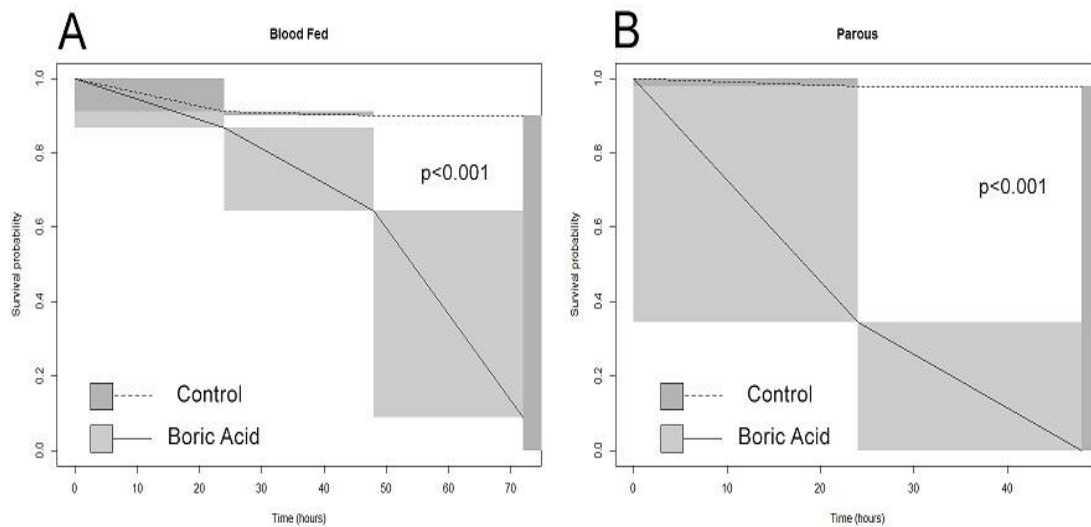


1 **Fig 4. Uptake Mechanism of the Toxic Component.** Mortality after 48 hours of exposure to devices
 2 and mosquito conditions summarized in Table 1.



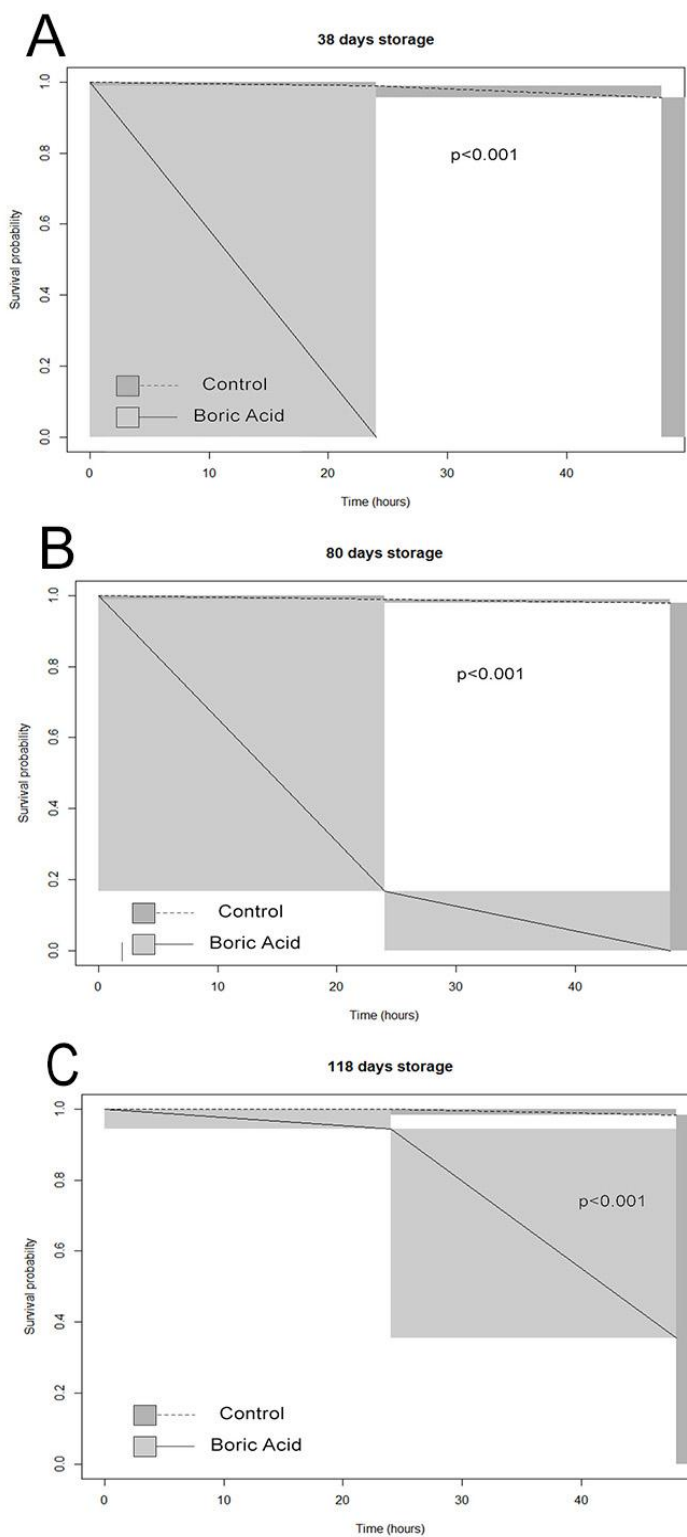
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2 **Fig 5. Histopathological Effects on the Midgut.** Longitudinal sections of *Aedes aegypti* posterior
 3 midgut. (A,C,E,F) Mosquitoes exposed to toxic devices. (B, D) Normal posterior midgut of mosquitoes
 4 exposed to non-toxic devices. Abbreviations: LM, gut lumen; AC, adipocyte; ED, epithelial disruption.



1 **Fig 6. Effects of the Physiological Status of the Mosquitoes on the Performance of the Device.**
 2 Survival of individuals exposed to toxic (dotted line; n=90) or non-toxic devices (solid line; n=90).
 3 Survival curves and NPMLE for (A) blood fed individuals exposed for 72 hours and (B) parous
 4 mosquitoes exposed to the device 48 hours.

5



1

2 **Fig 7. Shelf Life of the Device.** Survival and NPMLE of individuals exposed to toxic (dotted line; $n=90$) or non-toxic devices (solid line; $n=90$) that had been stored for: (A) 38 days, (B) 80 days, and
 3
 4 (C) 118 days. Interval-censored survival data collected in two time points (24h and 48h).