Parasitology Research (2018) 117:611–615
https://doi.org/10.1007/s00436-017-5729-y

SHORT COMMUNICATION

N-substituted methyl maleamates as larvicidal compounds against *Aedes aegypti* (Diptera: Culicidae)

Laura Harburguer[1] · Paula V. Gonzalez[1] · Paola Gonzalez Audino[1,2] · Eduardo Zerba[1,2] · Héctor Masuh[1,2]

Received: 4 August 2017 / Accepted: 20 December 2017 / Published online: 30 December 2017

Abstract

Severe human arboviral diseases can be transmitted by the mosquito *Aedes aegypti* (L.), including dengue, chikungunya, zika, and yellow fever. The use of larvicides in containers that can result as potential breeding places and cannot be eliminated is the main alternative in control programs. However, their continuous and widespread use caused an increase in insecticide-resistant populations of this mosquito. The aim of this study was to evaluate the effect of three *N*-substituted methyl maleamates as larvicides on *Ae. aegypti*, the *N*-propyl methyl maleamate (PMM), *N*-butyl methyl maleamate (BMM), and *N*-hexyl methyl maleamate (HMM). These compounds could have a different mode of action from those larvicides known so far. We evaluated the larva mortality after 1 and 24 h of exposure and we found that mortality was fast and occurs within the first 60 min. HMM was slightly more effective with LC_{50} values of 0.7 and 0.3 ppm for 1 and 24 h of exposure and LC_{95} of 11 and 3 ppm. Our results demonstrate that *N*-substituted methyl maleamates have insecticidal properties for the control of *Ae. aegypti* larvae. These compounds could become useful alternatives to traditional larvicides after studying their insecticidal mechanism as well as their toxicity towards non target organisms.

Keywords *Aedes aegypti* · Larvae · Larvicide · Maleamates

Introduction

Severe human arboviral diseases can be transmitted by the mosquito *Aedes aegypti* (L.), including dengue, chikungunya, zika, and yellow fever, and hence, it is considered a species of international concern. With a 30-fold increment in the last 50 years, dengue is at this time the most important of these diseases and rising in geographic expansion (WHO 2014).

Ae. aegypti control is mostly aimed at the larval stages (removal of breeding sites, larviciding, and community education) to reduce the population of new adults. Also, adult control using spatial sprays with adulticides is recommended when dengue outbreaks occur (Pilger et al. 2010). The use of larvicides in containers that can result as potential breeding places and cannot be eliminated is the main alternative in control programs. Worldwide reports of resistance to the principal larvicide used in the last years, the organophosphorus temephos, point out the need of new larvicides for mosquito control (Macoris et al. 2003; Braga et al. 2004; Seccacini et al. 2008; Tikar et al. 2009). For treating drinking water, WHO recommends, along with others, the biolarvicide *Bacillus thuringiensis* var. *israelensis*, and the insect growth regulators (IGRs) methoprene and pyriproxyfen (WHO 1999, 2008; Patil et al. 2012). However, resistance has also been detected to methoprene in the mosquitoes *Ochlerotatus nigromaculis* and *Aedes taeniorhynchus* (Cornel et al. 2002; Dame et al. 1998). Resistance to pyriproxyfen has been found in agricultural pests like *Bemisia tabaci* where it has been in

✉ Laura Harburguer
lharburguer@citedef.gob.ar; lharburguer@gmail.com

✉ Héctor Masuh
hmasuh@citedef.gob.ar

Paula V. Gonzalez
pgonzalez@citedef.gob.ar

Paola Gonzalez Audino
pgonzalezaudino@citedef.gob.ar

Eduardo Zerba
ezerba@live.com.ar

[1] Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-UNIDEF/CITEDEF/CONICET), J.B. de LaSalle 4397, Villa Martelli, 1603, Buenos Aires, Argentina

[2] Instituto de Investigaciones e Ingeniería Ambiental (3IA), Universidad Nacional de San Martín, San Martín, Buenos Aires, Argentina

use for longer time and its use has been more intensive and it also has been reported for *Musca domestica* (L.) (Ma et al. 2010; Shah et al. 2015).

Chemical compounds like salt, sugar, water, and a variety of odors can be perceived by insects via chemoreceptor organs. Receptor cells are dispersed through all the antennal surface, mouth, legs, or other body parts (Morita and Shiraishi 1985). Various chemicals can inhibit chemically induced behavioral responses by differentially interacting with an insect chemoreceptor (Frazier and Heitz 1985). For example, reagents reacting with sulfhydryl groups can modify the sensitivity of the insect chemoreceptor system. Previous work of our laboratory demonstrated that several *N*-substituted methyl maleamates produce feeding and mating deterrency in *Triatoma infestans* (Klug), a blood-sucking reduviid bug, vector of Chagas disease (Picollo et al. 1993; Gonzalez Audino et al. 1997). Also, the pretreatment of larvae with a similar compound *N*-ethylmaleimide (NEM) causes a suppression of behavioral responses to natural and synthetic chemical stimuli in *Ae. aegypti* (Gonzalez et al. 2015).

Although antimicrobial and antifungal properties of NEM and other *N*-substituted maleimides have been reported (Zentz et al. 2002; López et al. 2005; Sortino et al. 2008) as well as their ability to serve for the construction of a potent antibody-drug conjugate for humans (Nunes et al. 2015), nothing else has been studied about *N*-substituted methyl maleamates except for what was already mentioned above. In the present study, we examined the larvicidal effect of *N*-substituted methyl maleamates on *Ae. aegypti.* These compounds could have a mode of action different from those larvicides known so far.

Materials and methods

Biological material An insecticide-susceptible strain of *Ae. aegypti* (Rockefeller strain, Venezuela) was used for these assays. The laboratory colony was kept in the laboratory, free of exposure to pathogens, insecticides, or repellents, at 27 ± 2 °C and a 12:12-h light to dark photoperiod. To conserve the colony, eggs are collected over a wet cotton; they are allowed to dry at room temperature and then stored for at least 20 days before its use. They are rehydrated in dechlorinated water (about 500 eggs per two liters of water), and 24 h after rehydration, first instar larvae are observed. All larval instars were fed on a mixture of rabbit pellets and yeast in a 3:1 proportion.

Chemicals *N*-substituted maleamic acids were prepared in very good yield according to Mehta et al. (1960) and, as previously reported, purified by recrystallization from ether (Licastro et al. 1993). The *N*-substituted methyl maleamates were prepared according to Gonzalez Audino et al. (1997) and the (Z)-isomers were used as they were the effective isomers as shown in the cited work. The initial reaction rates of (Z)-isomers with GSH decrease with increasing length of the alkyl; this phenomenon was observed for alkyl chains up to C6; for longer alkyl chains, the initial reaction rates become similar. That is why we chose to use *N*-substituted methyl maleamates with chains up to C6 as *N*-propyl methyl maleamate (PMM), *N*-butyl methyl maleamate (BMM), and *N*-hexyl methyl maleamate (HMM) because longer chains will not change the reactivity with respect to GSH.

Chemical analysis GC/MS analysis of the *N*-substituted methyl maleamates was performed on a Shimadzu QP2010 equipped with a DB-5 (J&W Scientific, Agilent Technologies) (30 m × 0.25 mm × 0.25 μm). It was programmed from 60 to 240 °C at a rate of 5 °C per minute. The initial and final temperatures were held for 2 and 5 min, respectively.

Bioassays The larvicidal bioassay was completed following the protocol by Bisset et al. (2005). One milliliter of the insecticide solution to be assayed was added to 224 mL of water in a 500-mL plastic container and then was shaken lightly to ensure a homogeneous test solution. Then, 25 mL of water with 20 third instar *Ae. aegypti* larvae previously acclimated for 1 h was added to the container. Five different concentrations of each *N*-substituted methyl maleamate were tested. One milliliter of solvent (acetone) was added to other cup and used as a control. Each concentration, including the control, was replicated five times. Cups containing the treated larvae were placed in a regulated chamber (27 ± 2 °C, 60–70% RH, and 12:12 h photoperiod) and larva mortality was recorded after 1 and 24 h of exposure.

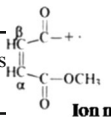
The 50 and 95% lethal concentrations (LC_{50} and LC_{95}) with the corresponding 95% confidence intervals (± CI) were calculated using the probit method (Lichtfield and Wilcoxon 1949).

Results

Qualitative analysis of maleamates Table 1 shows the results of the GC-MS analysis of the maleamates evaluated in this work: PMM, BMM, and HMM. In all cases, the mass spectra of *N*-substituted maleamates revealed the presence of the ion m/z 113, corresponding to the loss of the N-alkyl amine (NH-R) fragment (also shown in Table 1).

Larvicidal bioassay Figure 1A, B shows the results of LC_{50} and LC_{95} (± CI) values for the three maleamates evaluated after 1 and 24 h of exposure. The best larvicidal effect was obtained with HMM (LC_{50} of 0.73 and 0.36 ppm for 1 and 24 h, respectively, and LC_{95} of 11.6 and 3.53 ppm); furthermore, at 24 h of exposure, this treatment was significantly

Table 1 Fragmentation pattern of the *N*-substituted maleamates. The ion m/z 113 structure corresponding to the loss of NH-R fragment from the *N*-alkyl methyl maleamate is also shown

Peak retention time (min)	Compound	Mass weight	Fragmentations  Ion m/z 113	GC purity (%)
17.80	PMM	171	171 (M+), 113 (100), 85,156.	> 95
20.29	BMM	185	185 (M+), 113 (100), 85.	> 95
25.08	HMM	213	213 (M+), 113 (100), 85, 72, 100	> 95

PMM N-propyl methyl maleamate, *BMM N*-butyl methyl maleamate, *HMM N*-hexyl methyl maleamate

different from the treatment with PPM and BMM. No significant differences were found between 1 and 24 h of exposure indicating that the mortality caused by these maleamates was fast and occurs within the first 60 min.

Discussion

Different mosquito species are vectors of important human diseases, as malaria, dengue, zika, chikungunya, yellow fever,

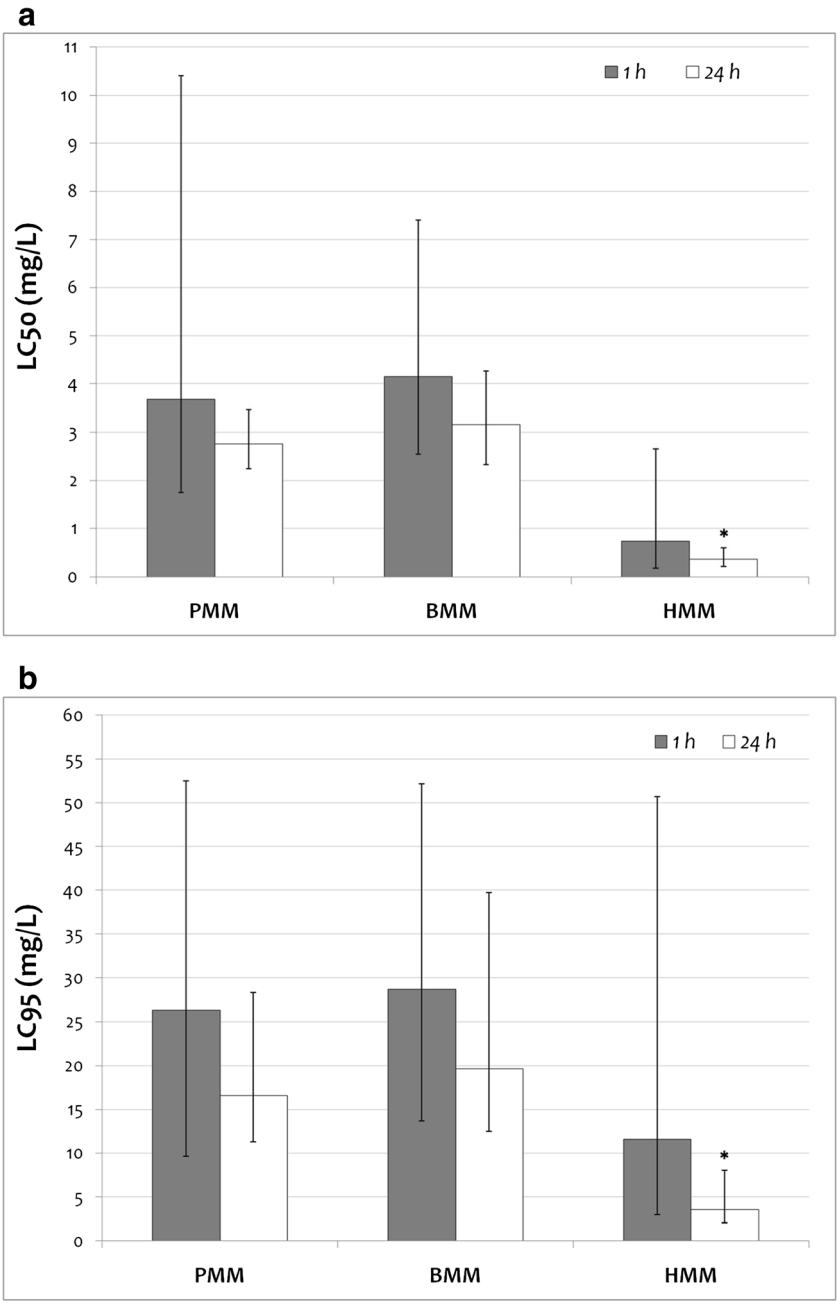


Fig. 1 **a** LC_{50} (± CI) values for the three maleamates evaluated after 1 and 24 h of exposure. **b** LC_{95} (± CI) values for the three maleamates evaluated after 1 and 24 h of exposure. PMM *N*-propyl methyl maleamate, BMM *N*-butyl methyl maleamate, HMM *N*-hexyl methyl maleamate. *This treatment was significantly different from the others within 24 h

and encephalitis. In the 1940s and 1950s, synthetic organic insecticides for vector control began to be massively applied; as a result, in many endemic countries, there was a large decline in the prevalence of these diseases. However, their incidence is nowadays increasing.

In spite of insecticides being the main tools used to control vector populations and to reduce the incidence of these diseases, almost no new insecticides are being developed and commercialized for vector control. This is probably due to the high cost of finding and developing a synthetic insecticide, the latest reduction in the agrochemical industry, and the low market for vector control, namely, the countries most in need of new insecticides for controlling vector-borne diseases have very restricted funds to acquire them (Ayesa et al. 2006). As a result of these factors, together with the occurrence of resistance in these vector species (Ponlawat et al. 2005), few insecticides are available for vector control (Gratz and Jany 1994; Brogdon and McAllister 1998) and very few new insecticides are being proposed as substitutes. Thus, as it was recently pointed out, there is an imperative need for alternative insecticides to control the vectors of human diseases (Zaim and Guillet 2002).

As described before, previous work in our laboratory demonstrated that NEM and other sulfhydryl group (-SH) reagents as *N*-substituted methyl maleamates, when topically applied on fifth instar nymphs and adults of *Triatoma infestans* resulted in a suppression of food intake, attributed to a chemoreceptor blockage produced by -SH reagents (Picollo et al. 1993; Gonzalez Audino et al. 1997). The larvicidal effect on *Ae. aegypti* observed in this work is probably related to the inhibition of sulfhydryl groups in various key receptors for the survival of the mosquito larva. On the other hand, biological thiol-dependent enzymes in insects are involved in a variety of critical physiological process. These enzyme active thiol groups are derived from the cysteine residues present. Therefore, the selective reversible or irreversible inhibition of these enzyme activities by modification of the thiol moiety may potentially lead to insect death (Stenersen 2004). More research is needed to know the mode of action of these compounds as well as their toxicity on non target organisms. The results obtained in our work represent a contribution to the search for new targets for the control of the *Ae. aegypti* mosquito.

Compliance with ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

References

Ayesa P, Harrington L, Scott J (2006) Evaluation of novel insecticides for control of dengue vector *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 43(1):55–60

Bisset J, Blanco S, Braga I, Coto H, Masuh H, Moncayo A, Nathan M, Orellano P, Vázquez J, Zerba E (2005) Protocolo para determinar la susceptibilidad o resistencia a insecticidas de mosquitos de la especie *Aedes aegypti*. (Protocol to evaluate the susceptibility or resistance to insecticides by *Aedes aegypti* mosquitoes). Documento propuesto por la Red Latinoamericana de Control de Vectores (RELCOV). Fundación Mundo Sano *Eds.* Buenos Aires, Argentina 13 pp

Braga IA, Lima JBP, Soares SS, Valle D (2004) *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe and Alagoas, Brazil. Mem Inst Oswaldo Cruz 99(2):199–203. https://doi.org/10.1590/S0074-02762004000200015

Brogdon WG, McAllister JC (1998) Insecticide resistance and vector control. Emerg Infect Dis 4(4):605–613. https://doi.org/10.3201/eid0404.980410

Cornel AJ, Stanich MA, McAbee RD, Mulligan SF III (2002) High level methoprene resistance in the mosquito *Ochlerotatus nigromaculis* (Ludlow) in Central California. Pest Manag Sci 58(8):791–798. https://doi.org/10.1002/ps.521

Dame DA, Wichterman GJ, Hornby JA (1998) Mosquito (*Aedes taeniorhynchus*) resistance to methoprene in an isolated habitat. J Am Mosq Control Assoc 14(2):200–203

Frazier JL, Heitz JR (1985) Inhibition of olfaction in the moth *Heliothis virescens* by the sulfhydryl reagent fluorescein mercuric acetate. Chem Senses Flavor 1:271–281

Gonzalez Audino P, Masuh H, de Licastro SA, Vassena C, Picollo MI, Zerba E (1997) Suppression of food intake in *Triatoma infestans* by *N*-substituted methyl maleamates. Pestic Sci 49(4):339–343. https://doi.org/10.1002/(SICI)1096-9063(199704)49:4<339::AID-PS523>3.0.CO;2-R

Gonzalez PV, Gonzalez Audino PA, Masuh HM (2015) Behavioral response of *Aedes aegypti* (Diptera: Culicidae) larvae to synthetic and natural attractants and repellents. J Med Entomol 52(6):1315–1321. https://doi.org/10.1093/jme/tjv136

Gratz NG, Jany WC (1994) What role for insecticides in vector control programs? Am J Trop Med Hyg 50(6):11–20

Licastro SA, Wallace G, Seccacini E, Gonzalez Audino P, Zerba E (1993) Synthesis and bioactivity of new phosphorodithioates derived from *N*-substituted maleamic esters. Comp Biochem Physiol 104:43–46

Lichtfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 2:99–113

López SN, Castelli MV, de Campos F, Corrêa R, Cechinel Filho V, Yunes RA, Zamora MA, Enriz RD, Ribas JC, Furlán RL, Zacchino SA (2005) In vitro antifungal properties structure-activity relationships and studies on the mode of action of *N*-phenyl, *N*-aryl, *N*-phenylalkyl maleimides and related compounds. Arzneim Forsch 55:123–132

Ma W, Li X, Dennehy TJ, Lei C, Wang M, Degain BA, Nichols RL (2010) Pyriproxyfen resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) biotype B: metabolic mechanism. J Econ Entomol 103(1):158–165. https://doi.org/10.1603/EC09122

Macoris MLG, Andrighetti MTM, Takaku L, Glasser CM, Garbeloto VC, Bracco JC (2003) Resistance of *Aedes aegypti* from the state of S. Paulo, Brazil, to organophosphates insecticides. Mem Inst Oswaldo Cruz 98(5):703–708. https://doi.org/10.1590/S0074-02762003000500020

Mehta K, Phillips AP, Lui F, Brooks RE (1960) Maleamic and citraconamic acids, methyl esters and imides. J Org Chem 25(6):1012–1015. https://doi.org/10.1021/jo01076a038

Morita H, Shiraishi A (1985) Chemoreception physiology. In: Kerkut GA, Gilbert LJ (eds) Comprehensive insect physiology, biochemistry and pharmacology, vol 6: Nervous system sensory. Pergamon Press, pp 133–170

Nunes JPM, Morais M, Vassileva V, Robinson E, Rajkumar VS, Smith MEB, Pedley BR, Caddick S, Baker JR, Chudasama (2015) Functional native disulfide bridging enables delivery of a potent, stable and targeted antibody–drug conjugate (ADC). Chem

Commun 51(53):10624–10627. https://doi.org/10.1039/C5CC03557K

Patil CD, Patil SV, Salunke BK, Salunkhe RB (2012) Insecticidal potency of bacterial species *Bacillus thuringiensis* SV2 and *Serratia nematodiphila* SV6 against larvae of mosquito species *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. Parasitol Res 110(5):1841–1847. https://doi.org/10.1007/s00436-011-2708-6

Picollo MI, Seccacini E, Vassena C, Zerba E (1993) Feeding and mating deterrency by sulfhdryl reagents in *Triatoma infestans*. Acta Trop 53:297–307

Pilger D, De Maesschalckm M, Horstick O, San Martin JL (2010) Dengue outbreak response: documented effective interventions and evidence gaps. TropIKA 1. TropIKA.net. (http://journal.tropika.net/pdf/tropika/v1n1/a02v1n1.pdf). [Accessed 12 July 2017]

Ponlawat A, Scott JG, Harrington LC (2005) Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. J Med Entomol 42(5):821–825. https://doi.org/10.1093/jmedent/42.5.821

Seccacini E, Lucia A, Zerba E, Licastro S, Masuh H (2008) *Aedes aegypti* (L.) resistance to temephos in Argentina. J Am Mosq Control Assoc 24(4):608–609. https://doi.org/10.2987/5738.1

Shah RM, Shad SA, Abbas N (2015) Mechanism, stability and fitness cost of resistance to pyriproxyfen in the house fly, *Musca domestica* L. (Diptera: Muscidae). Pestic Biochem Physiol 119:67–73. https://doi.org/10.1016/j.pestbp.2015.02.003

Sortino M, Cechinel Filho V, Corrêa R, Zacchino S (2008) *N*-Phenyl and *N*-phenylalkyl-maleimides acting against *Candida* spp.: time to-kill, stability, interaction with maleamic acids. Bioorg Med Chem 16(1): 560–568. https://doi.org/10.1016/j.bmc.2007.08.030

Stenersen J (2004) Chemical pesticides mode of action and toxicology—chapter two: why is a toxicant poisonous? CRC Press, p 16

Tikar SN, Kumar A, Prasad GBKS, Prakash S (2009) Temephos-induced resistance in *Aedes aegypti* and its cross-resistance studies to certain insecticides from India. Parasitol Res 105(1):57–63. https://doi.org/10.1007/s00436-009-1362-8

World Health Organization (WHO) (1999) *Bacillus thuringiensis*. Environmental health criteria 217. WHO, Geneva, Switzerland http://www.who.int/ipcs/publications/ehc/en/EHC217.pdf. [Accessed 12 July 2017]

World Health Organization (WHO) (2008) Pyriproxyfen in drinking-water: use for vector control in drinking-water sources and containers—background document for development of WHO guidelines for drinking-water quality

World Health Organization (WHO) (2014) A global brief on vector-borne diseases. http://apps.who.int/iris/bitstream/10665/111008/1/WHO_DCO_WHD_2014.1_eng.pdf. [Accessed 12 July 2017]

Zaim M, Guillet P (2002) Alternative insecticides: an urgent need. Trends Parasitol 18(4):161–163. https://doi.org/10.1016/S1471-4922(01)02220-6

Zentz F, Valla A, Le Guillou R, Labia R, Mathotb AG, Sirot D (2002) Synthesis and antimicrobial activities of *N*-substituted imides. Il. Farmaco 57(5):421–426. https://doi.org/10.1016/S0014-827X(02)01217-X