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Alfamurom Efficacy Study with ULV Spatial Application against the *Aedes aegypti* Mosquitoes

Herbert Nacke[1]
https://orcid.org/0009-0006-4498-5608

Eduardo da Silva Ceciliano[2*]
https://orcid.org/0009-0003-6794-8568

[1]Forquímica Agrociência Ltda., Cambira, Paraná, Brasil; [2]UniCesumar, Jardim Aclimação, Maringá, Paraná, Brasil

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*Correspondence: du_ceciliano@hotmail.com; Tel.: +55-43-999777096 (E.S.C.)

HIGHLIGHTS

- The efficacy of Alfamurom + Natuinset against *Aedes aegypti* mosquitoes was proved.
- The adulticidal effect of Alfamurom demonstrated 100% mortality.
- Formulation was considered suitable, and it can be used in vector control actions.

Abstract: This study was performed to evaluate the efficacy of Alfamurom (alpha-cypermethrin) against *Aedes aegypti* mosquitoes under spatial application using a portable ultra-low volume (ULV) nebulizer. The study consisted of two treatments: the treatment with four repetitions using two cages at each application distance of 10, 15, 20, and 25 meters, and a control group. Twenty-five female mosquitoes were used per repetition (cage). The sample was diluted to obtain a formulation containing 300 mL of Alfamurom + 100 mL of water + 100 mL of Natuinset. A total of 37.5 mL of this solution was applied to a final area of 750 m² (equivalent to 500 mL per hectare). Knockdown assessment was conducted after 60 minutes, and mortality evaluation was performed 24 hours after insecticide application. The results showed 100% *A*. *aegypti* mortality at distances of 10, 15, 20, and 25 meters. The study was considered remarkable appropriate since there was no mortality in the control group. The *A*. *aegypti* female mosquitoes' mortality in this experiment with alpha-cypermethrin (Alfamurom) using the ULV formulation was proved, and this formulation can be used in vector control actions alongside the adjuvant Natuinset.

Keywords: Alpha-cypermethrin; Insecticide; Mosquito control; Ultra-low volume nebulizer.

INTRODUCTION

Aedes aegypti is the most widespread arbovirus vector globally, found on every continent except Europe [1]. It is responsible for transmitting diseases such as Dengue, Chikungunya, Zika, and yellow fever, posing a significant global public health challenge due to its unique capacity for viral dissemination. This mosquito thrives in domestic or peri-domestic environments closely associated with human habitation. Its widespread presence means that over one-third of the world's population is potentially exposed to this vector [2].

The preferred oviposition sites of *A*. *aegypti* are aquatic, often found in artificial containers like domestic water storage tanks, rainwater collection receptacles, tires, plant pots with standing water, uncovered tanks,

and tree hollows [3]. Water conditions conducive to the development of its immature stages include clarity, low levels of decomposing organic matter, shaded environments, containers with dark surfaces, and low salinity [4].

Historically, *A*. *aegypti* was introduced to Brazil during colonization and was effectively controlled by Oswaldo Cruz between 1903 and 1909, halting yellow fever transmission in Rio de Janeiro. Despite these efforts, *A*. *aegypti* reappeared in Pará in 1967 and Maranhão in 1969. Subsequent reintroductions occurred in Bahia in 1976 and Rio de Janeiro in 1977. From these coastal states, the mosquito spread to inland regions of Brazil, facilitated by urban growth and the proliferation of suitable breeding sites, thereby exposing diverse geographic areas to diseases transmitted by this vector [5].

The resurgence of dengue in recent decades has emerged as a critical public health concern, particularly in tropical countries like Brazil. Control efforts have historically relied on chemical insecticides targeting both adult and larval stages of the dipteran population [6]. However, the development of resistance in vector insects due to prolonged exposure to these chemicals has necessitated the development of new formulations capable of effectively combating *A*. *aegypti* mosquitoes.

In this context, this study evaluates the chemical composition, safety, and efficacy of a novel formulation containing Alfamurom and Natuinset against *A*. *aegypti* mosquitoes, aiming to overcome insecticide resistance and enhance control measures.

MATERIAL AND METHODS

Material

Alfamurom and Natuinset were used according to the facts presented in their datasheet. In brief, Alfamurom is an insecticide based on pyrethroid (alpha-cypermethrin 30 g/kg; 3% w/w) associated with benzoylurea (an insect growth regulator, IGR, commercially named Triflumuron, 30 g/kg; 3% w/w) used for the control of adults and larvae insects' vectors of *Aedes* spp.*, Anopheles* spp., and *Culex* spp., which are responsible for the transmission of dengue, yellow fever, malaria, filariasis, and encephalitis [7]. Natuinset is an adjuvant composed by natural oils that can act as an emulsifier for the tank mix application by enhancing the performance of the product application. It possesses a dispersive action by decreasing the droplet in order to provide a better distribution at the environment as well as a repellent effect caused by the presence of citronella essential oil, which dislodges the mosquito from its hiding places and inhibits its local reappearance.

Due to its liquid formula, Alfamurom must be applied in mist form through ultra-low volume (ULV) atomizers at internal and external areas, including residential, institutional, recreational, and industrial areas. *A. aegypti* is the main target since it is the predominant vector of dengue, Chikungunya, yellow fever, and Zika [7].

The product is register at the Ministry of Health from Brazil with the following number: 333080051. The suggested use requirement of Alfamuron to control *A. aegypti* is diluting the commercial product as following: 300 mL of Alfamurom + 100 mL of water + 100 mL of Natuinsect. A final volume of 37,5 mL per tank mix containing 750 m² provides a suitable dose, which is equivalent to 500 mL per hectare. The diluted product can be applied at a flow rate of 50 mL/min with a speed of 5,4 km/h using an ULV application equipment [7].

Evaluation of physical and chemical properties of Alfamurom

The physical and chemical characteristics of the commercial product were determined by the following companies: Dominus Química and ASR Analytical & Scientific Research, according to the technical procedures stablished by the National Health Regulatory Agency [8].

Determination of purity degree of Alpha-Cypermethrin and Triflumuron

The determination of the purity degree of each ingredient from Alfamurom was conducted through High Performance Liquid Chromatography (HPLC) compared to standards of alpha-cypermethrin and Triflumuron using the experimental conditions described in Table 1. This analytical method was previously validated according to the usual parameters for obtaining the recovery values.

Table 1. Experimental conditions for testing the Alfamurom product by HPLC-DAD method

Parameters	**Description**		
Equipment:	HPLC 1260 Infinity G1311C		
Chromatography Column:	Eclipse Plus c18 (4.6 mm x 100 mm x 3.5 µm)		
Oven Temperature (°C):	25	Injection volume:	5
Mobile Phase Flow (mL/min):	1.5	Time (min):	12
Wavelength (nm):	230		
Detector:	UV/DAD (Diode Array Detector)		
Mobile Phase:	Solvent A	Solvent B	
	Acetonitrile (%)	Ultrapure water with 0.1 phosporic acid (%)	
	70	30	

Reference: National Health Regulatory Agency [8].

Evaluation of toxicological data about Alfamurom

Additional acute studies (mammalian and ecotoxicity) were performed by Laboratório Ecolyzer Ltda. (São Paulo, Brazil) by following the Organization for Economic Cooperation and Development (OECD) guidelines, which is a guide for testing of chemicals in conformity with the good laboratory practice (GLP) patterns. Comprehensive analyses about the safety of alpha-cypermethrin and Triflumuron were also provided in this paper.

Efficacy evaluation against *A. aegypti*

The main aim of the pesticide use is to control a plague or a vector, whereas resistant or susceptible, and not managing its resistance. Different tools could be used as a part of a resistance management plan. The sum of those factors is an element of the Integrated Vector Management depending on a series of interventions as well as the use of more effective patterns to manage the vectors and its chemical and behavioral resistance. Alfamurom is compound by two active substances with different action routes, Group 3A and 15 [9]. The premise of combining two distinct mechanisms of action is to reduce the probability of a simultaneous resistance to both compounds since alpha-cypermethrin and Triflumuron present non-related methods of action. The product do not also present any sign of synergy or additional effect towards the combination of the two active ingredients.

In this paper, *A. aegypti* female adults were used within the age of 2 to 5 days, raised in an insectary with controlled temperature and humidity (27±2°C and 80±10%, respectively), fed with a specific diet and acclimatized for minimum of 12 hours before the study.

The evaluated product was Alfamurom containing 3% alpha-cypermethrin + 3% Triflumurom. This commercial product was diluted as following: 300 mL of Alfamurom + 100 mL of water + 100 mL of Natuinsect, an adjuvant composed by D-limonene and citronella essential oil, using a volume of 37,5 mL per tank mix of 750 m² (equivalent of 500 mL per hectare) and a flow rate of 50 mL/min (grey nozzle) with a drop size of 23.3 µm and a speed of 5.4 km/h.

For the efficacy experiment, the Alfamurom treatment was replicated four times and compared to the control group. The formulation was applied at four different application distances (Figure 1) using two cages containing 25 female mosquitoes per each cage. Only one cage was used for the control group.

Each trial was carried out in an open area with no vegetation following the diagram depicted in Figure 1 for the Alfamurom treatment. Insects were collected using an insect collector and were placed into the cages. About 15 minutes before the beginning of application, the cages were suspended at a high of approximately 1.5 m above ground level. Beside the cages, sensitive papers were inserted in a perpendicular manner from the spray.

The ultra-low volume (ULV) atomizer was adjusted before the application. It was positioned in a perpendicular line to wind and in favor of it. The equipment was turned on 10 meters prior and turned off 10 meters after the study area. Subsequently to the application, the cages were maintained for 15 minutes on the field. The presence of insecticides was then measured through the sensitive paper analysis for validating the application range. The study was conducted identically for the control group using water.

After 60 minutes from the Alfamurom application, the total of insects in knockdown was determined. The insects were also gathered in 500 mL plastic containers and storage at a experimental room with a temperature of 27±2°C, relative humidity of 80±10%, and a photophase of 12 hours. The insects' mortality was evaluated in the following 24 hours after Alfamurom application. The mortality was calculated for each investigated distance [10].

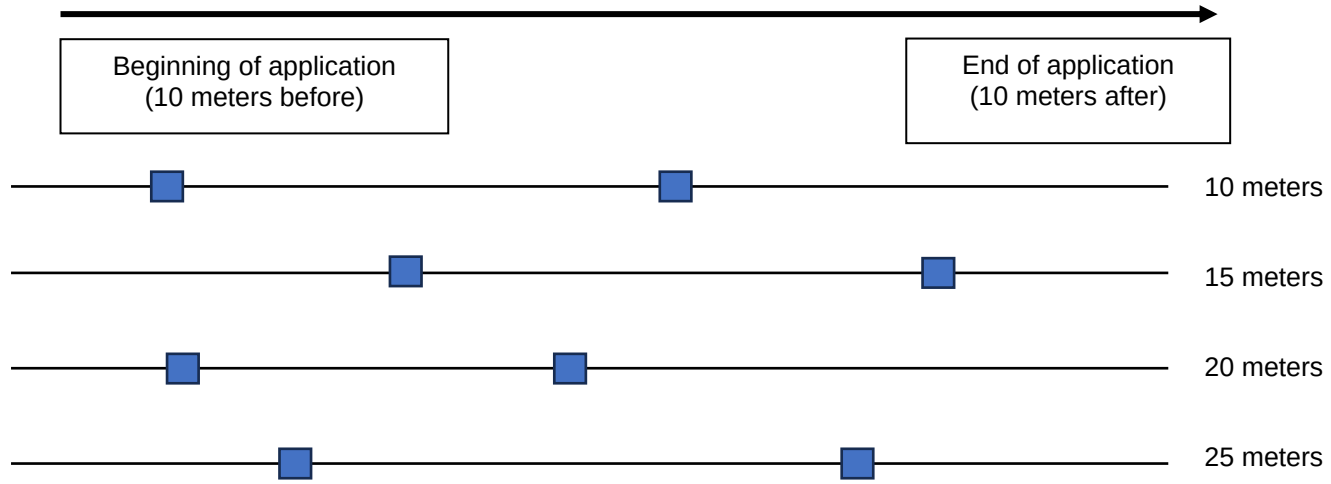

Figure 1. Diagram used for Alfamurom application and cages disposal

RESULTS AND DISCUSSION

Physical and chemical properties of Alfamurom are summarized in Table 2. These data were obtained throughout studies performed in accordance with the good laboratory practice (GLP) [8].

Table 2. Experimental chemical and physical properties of Alfamurom

Property	Study Methodology	Analytical Institution	Result
Appearance	BRASIL. Agência Nacional de Vigilância Sanitária. RDC n° 59, December 17th, 2010. It disposes regarding the procedures and technical requirements to the notification and register of sanitizer products, summing other measurements. Official Diary of the Union, Executive Power, Brasília, Brazil, December 22nd, 2010	Dominus Química	Liquid
pH		Dominus Química	6.00 – 8.00
Density		Dominus Química	1.195 g/cm³
Stability at room temperature for a 24-month period		ASR Analytical & Scientific Research	Considered stable at room temperature during the 24-month period
Thermal and air stability	Organization for Economic Co-operation and Development (OECD), 1981. Guidelines for testing chemical, OECD 113. Screening Test for Thermal and Air Stability	ASR Analytical & Scientific Research	Active ingredient degradation ≤ 5%, considered satisfactory

Validation and recovery data are shown in Table 3 and Table 4 for alpha-cypermethrin and Triflumuron, respectively. The HPLC-DAD method was suitable for the determination of the active ingredients.

Table 3. Validation data and recovery for Alpha-cypermethrin

Parameter	Result		
Specificity / Selectivity	No interference with the analyte		
Detection Limit (DL)	0.22 $mg.L^{-1}$		
Quantification Limit (QL)	0.73 $mg.L^{-1}$		
Signal Noise	256.2		
Sensibility	12.62493		
Linearity	Linearity range ($mg.L^{-1}$): 18.07893 to 4427300 Interception (b): 2.34112 Inclination (m): 12.62493 Correlation coefficient: 0.99978		
Precision (repeatability)	Average (% w/w): 2.994 Variation coefficient (% RSD): 2.53 Grubbs Test: $G_{n\ High}$ = 1.150 $G_{n\ Low}$ = 1.436	% RSD acceptable: ≤ 2.27 % RSD (modified Horwitz): 2.27 Acceptable Grubbs value ≤ 1.715	
Recovery	Level 1 ($mg.L^{-1}$): 23.6289 Recovery (%): 98.9 Level 2 ($mg.L^{-1}$): 28.6139 Recovery (%): 98.4 Level 3 ($mg.L^{-1}$): 37.3875 Recovery (%): 97.8	Acceptable (%): 90 to 107	
	Grubbs test (level 1): $G_{n\ High}$ = 1.713 /$G_{1\ Low}$ = 1.295		Acceptable ≤ 2.020
	Grubbs test (level 2): $G_{n\ High}$ = 0.936 /$G_{1\ Low}$ = 1.435		
	Grubbs test (level 3): $G_{n\ High}$ = 1.284 /$G_{1\ Low}$ = 1.375		

Table 4. Validation data and recovery for Triflumurom

Parameter	Result		
Specificity / Selectivity	No interference with the analyte		
Detection Limit (DL)	0.11 $mg.L^{-1}$		
Quantification Limit (QL)	0.38 $mg.L^{-1}$		
Signal Noise	466.4		
Sensibility	6.99831		
Linearity	Linearity range ($mg.L^{-1}$): 17.13700 to 48.95300 Interception (b): 5.38064 Inclination (m): 6.99831 Correlation coefficient: 0.99815		
Precision (repeatability)	Average (% w/w): 2.897 Variation coefficient (% RSD): 1.32 Grubbs Test: $G_{n\ High}$ = 0.595 $G_{n\ Low}$ = 1.779	% RSD acceptable: ≤ 2.28 % RSD (modified Horwitz): 2.28 Acceptable Grubbs value ≤ 1.715	
Recovery	Level 1 ($mg.L^{-1}$): 23.7886 Recovery (%): 99.3 Level 2 ($mg.L^{-1}$): 30.2764 Recovery (%): 100.7 Level 3 ($mg.L^{-1}$): 38.4353 Recovery (%): 99.1	Acceptable (%) 90 to 107	
	Grubbs test (level 1): $G_{n\ High\ =}$ 1.627 /$G_{1\ Low}$ = 1.058		Acceptable ≤ 2.020
	Grubbs test (level 2): $G_{n\ High\ =}$ 0.909 /$G_{1\ Low}$ = 1.416		
	Grubbs test (level 3): $G_{n\ High\ =}$ 1.431 /$G_{1\ Low}$ = 1.516		

Toxicological Concerns and Impurity

Regarding to the ASR report, there was any impurity of toxicological concern at the active substances and the inert ingredients. Besides, according to studies presented by ASR, the chemical and physical characteristics of the product were according to its requirements. In conclusion, a pre-approval of this product is supported by the analysis of the presented information.

Specific Toxicity Data of Alfamurom – Acute Toxicity

In this perspective, a resume of the acute toxicity data is provided in Table 5. The acute studies using mammalian and ecotoxicity data were conducted by Laboratório Ecolyzer Ltda. (São Paulo, Brazil) by following the OECD guidelines, guideline for testing of chemicals.

Table 5. Toxicity data for Alfamurom

Exhibition Route	Species	Result	GHS Classification	Study Reference
Oral	*Rattus norvegicus*	DL_{50} > 2000 mg/kg	Category 5	Laboratório Ecolyzer Ltda.
Dermal	*Rattus norvegicus*	DL_{50} > 2000 mg/kg	Category 5	Laboratório Ecolyzer Ltda.
Serious ocular injuries/ocular irritation	*Oryctolagus cuniculus*	Ocular irritation 0.0, considered non-irritant	Non-classified	Laboratório Ecolyzer Ltda.
Corrosion/skin irritation	*Oryctolagus cuniculus*	Cutaneous irritation 0.0, considered non-irritant	Non-classified	Laboratório Ecolyzer Ltda.
Skin sensitization	*Cavia porcellus*	Not sensitizing to the skin	Non-classified	Laboratório Ecolyzer Ltda.

Acute toxicity data summarized for Alfamurom show that the combination of both insecticides, alpha-cypermethrin and Triflumurom, did not enhance the toxicological profile of the active substances. Therefore, it can be inferred that the combination of these two insecticides at the same product do not have impact on the human risk assessment.

Toxicity in mammals

Acute toxicity, irritation and sensitization of alpha-cypermethrin

Alpha-cypermethrin has a relatively low toxicity through, not only oral, but also dermal and inhalational means. The Environmental Protection Authority (EPA) [11] suggests the alpha-cypermethrin is classified as acute oral toxicity level 3 and level 4 for acute inhalation toxicity. Whereas no classification was proposed for dermal exposure effects.

Data described by the EPA [12] present seven studies for acute oral toxicity, five studies for dermal acute toxicity, and other six studies for acute inhalation toxicity in rats. For acute oral toxicity, LD_{50} values varied from the reported values of 40–80 mg/kg of body weight to > 5,000 mg/kg of body weight. Likewise, for acute dermal toxicity a LD_{50} > 2,000 mg/kg of body weight was obtained in all the studies without any casualty. In three studies, clinical signs, such as salivation, sensitivity to stimuli, and hyperactivity were only observed using 2,000 mg/kg of body weight in one of those papers. For acute inhalation toxicity of powder, the lowest reported LC_{50} value was 0.510 mg/L, although limited details were provided for this study. Similarly, other reported study demonstrated a LC_{50} > 0.593 mg/L (no casualty was observed). All other papers presented a LC_{50} > 1 mg/L. For dermal sensitization, a topical induction dose prepared at 50%, additionally to an intradermal induction at 5%, was used.

Mutagenicity of Alpha-Cypermethrin

During the *in vitro* tests, negative results were obtained for any genetic mutations on bacterium and mammal cells, as any chromosomal changes on mammal cells with or without metabolic activation. Other *in vivo* trials showed negative results on rodents for chromosomal changes, as well as in bone marrow micronuclei and liver unscheduled DNA synthesis (UDS) assays, in which were performed a partial hepatectomy on treated animals. Negative result was additionally obtained in a lethal assay using male mice, despite the study sensitivity was limited. Although a slightly mutagenic change was reported in literature, this

conclusion was observed in papers using non-standard testing methods and/or presenting study limitations. Therefore, no reliable evidence of any mutagenic potential was demonstrated. In addition, based on the available studies for guidance, it was proposed the mutagenicity classification should not be necessary [12].

Carcinogenicity of Alpha-Cypermethrin

Available data suggests that a classification for carcinogenicity is not necessary. Regardless there was no study of carcinogenicity for rodents, it was observed that Alpha-Cypermethrin was not genotoxic [11].

Development and reproductive toxicity of Alpha-Cypermethrin

During a study using three generation of rats, there was no effect on the rat reproduction. In addition, no male reproductive toxicity and no toxicity on its succession was achieved using the highest tested dose (20 mg/kg of body weight/day) [12]. In this context, a slightly decrease in the size and the weight was observed in the offspring generation (F1) during a test using the highest tested dose (500 ppm equivalent to 50 mg/kg of body weight/day), which produced male reproductive toxicity in all subsequent generations. This study was not considered to demonstrate the potential reproductive toxicity of Alpha-Cypermethrin. Throughout the development study in rats, a slightly decrease at fetal body weights in a level of dose in which produced maternal toxicity, was considered a non-specific secondary consequence of it. Therefore, based on the available data, it is seen as not necessary to classify the development and reproductive toxicity [11].

Neurotoxicity and target organ toxicity of Alpha-Cypermethrin

Dietary studies performed for 90 days considered dogs as the most sensitive specimen. At 270 ppm (approximately 6.75 mg/kg of body weight/day), body trembling, head shake, lips licking, ataxy or moderated agitation, and march in high steps were observed on both male and female dogs. Considering the severity of its clinical toxicity, a female needed to be sacrificed. These results were also observed in rodents, despite the use of higher dose. Considering a 6-week dietary study in rats, males were similarly sacrificed at a dose of 800 ppm (approximately 80 mg/kg of body weight/day) and both genders were sacrificed at 1,200 ppm (approximately 120 mg/kg of body weight/day) [12].

Regarding a 4-week dietary study in rats, a female was mercy sacrificed at 1,200 ppm (equivalent to 212 mg/kg of body weight/day in this gender) and male at 1,600 ppm (equivalent to 241 mg/kg of body weight/day in this gender). The severity of clinical neurotoxicity signs presented on both genders at 90 days in canine study, which demanded a mercy sacrifice of a female at a dietary dosage of 6.75 mg/kg of body weight/day, supports its classification as level 1 category proposed by EPA [11] for specifics target organ (nervous system) after repeatedly oral exposure.

At the most important study of acute inhalation using Alpha-Cypermethrin, there was the presence of gases, nasal secretions, an increase on saliva production at a 2.47 mg/L dose. All those signs were representative of breathlessness and an irritability effect on rats. Besides, it was observed a darker discoloration in the pulmonary lobes of two died females at that concentration, as well as in all the autopsied animals at the end of the study. Consequently, EPA [11] proposed that Alpha-Cypermethrin is classified as a level 3 category in terms of target organ toxicity (respiratory irritation) after a single exposure to inhalation.

Triflumurom

Acute toxicity, cutaneous irritation and sensitization of Triflumurom

Triflumurom presents a low oral toxicity on rodents. After oral administration, the animals showed transient symptoms (general health disturbances, such as fatigue). Moreover, oral studies with other mammal species (rat, sheep, and dog) also indicated an extremely low acute oral toxicity with no adverse clinical signs related to the treatment. A relatively low acute toxicity was observed after intraperitoneal, as well as subcutaneous administration in rats and mice with correspondent symptoms to those observed after oral administration, besides there was no mortality within a dose of 5,000 mg/kg of body weight. There was also no sign of dermal acute toxicity or mortality in rats, even though with the highest tested dosage (5,000 mg/kg of body weight). The tests revealed low inhalation toxicity since Triflumurom was administered at the highest possible dose and only occurred transient symptoms of general health disturbance and nasal secretion in rats. LC_{50} exceeded the levels of limited dose. Therefore, results of sub-acute inhalation supported the unique test results, indicating an extremely low inhalation acute toxicity. Triflumurom did not exhibit dermal irritation

properties on rabbit skin, apart from mild and transitional ocular reactions, none of those exceeding the limits for a classification as ocular irritant [13].

Genotoxicity of Triflumurom

There was no evidence of genotoxic potential in any of the *in vitro* or *in vivo* experiments [13].

Development and reproductive toxicity of Triflumurom

Triflumurom is not toxic for rat nor rabbit development at doses for providing maternal toxicity. Nonetheless, embryotoxic effects were only observed with the highest dose (1,000 mg/kg of body weight) in which resulted in maternal toxicity. At elevated dose, splenic changes associated with erythropoiesis, frequently observed in other studies, were also observed in rodents' and rabbits' mothers. The incidence, as well as its type observed on fetuses, was not affected by maternal treatment. In addition, significant fertility-related effects were not evident on reproductive parameters as development and offspring viability in a study of multiple rat generation [13].

Neurotoxicity of Triflumurom

There was no indication of acute neurotoxicity on either mice and rats through different paths, including oral and percutaneous application, as well as subcutaneous and intraperitoneal injection. In spite of its investigation at the highest possible dose, no study highlighted any observation of clinical selective neurotoxicity. To conclude, a series of studies of short- and long-term toxicity was carried out in different species and there was no indication of effects attributed to systemic or delayed neurotoxicity [13].

Chronic/carcinogenicity of Triflumurom

Non-neoplastic chronic effects were identical to those reported after the subchronic toxicity studies. The erythropoietic system was disturbed and enlarged spleens were evident with pronounced hemosiderosis, as well as extramedullary hematopoiesis, indicating compensatory mechanism in which disagrees with the induced anemia. At the highest dose, there was an increase in the spleen weights and the pigment deposition. Whereas, the hematological changes at medium dose, represented as the lowest observed adverse effect level (LOAEL), were slightly, although consistent with the effects observed using elevated dose. The resulting no observed adverse effect level (NOAEL) of 0.82 and 1.1 mg/kg of body weight/day was observed in male and female, respectively, by applying a conservative approach with a 10 times higher LOAEL. Besides, it was observed an increase in the weight of rat livers and kidneys treated with the highest dose. These results were considered secondary effects of the Triflumurom induced by anemia. A higher NOAEL (5.19 mg/kg of body weight/day) was obtained through a long-term toxicity study using rats. Although an enhance of fluorine levels were determined in rodent bones and teeth by chronic studies, no microscopic, nor macroscopic, changes were detected in these tissues. Therefore, the quantity of fluorine incorporated at Triflumurom was inadequate to produce fluorosis, even though on elevated doses used during chronic studies in rats [13].

Triflumurom was also tested twice, regarding its oncological potential during long-term investigations, with no indication of such potential. During the experiments using rats, any tumor at target organs (spleens) was observed, with other tumors within its normal incidence [13].

Risk evaluation: danger identification

The aim of this stage was to identify the danger of the substances considering their toxicological properties. Experimental data from animal studies were detailed for describing all possible toxicological effects on human health. Usually, it was determined in which level it was not expected this effect occurred.

Alpha-Cypermethrin toxicokinetics

Alpha-cypermethrin is well absorbed by gastrointestinal tract as through inhalation means, whereas is poorly absorbed by intact skin [14]. As its biotransformation occurs rapidly, the distribution of the pyrethroid molecules is relatively meaningless. Mammals are commonly capable to metabolize synthetic pyrethroids by hydrolysis, oxidation, and conjugation. Its biotransformation promptly happens in the intestinal tract; therefore, its oral toxicity is low. While the metabolites are conjugated, Alpha-Cypermethrin is excreted by urine and feces.

Alpha-Cypermethrin toxicodynamics

The Alpha-Cypermethrin is a type II pyrethroid, consequently, it has a cyano group in the alfa position. The proposed mechanism of action for pyrethroids involves the changes of sodium channels in neuron membranes, which cause repeatedly neuronal discharges and a longer repolarization period. Type II pyrethroids preferably acts at the central nervous system. For this reason, the intoxication symptoms in rodents are hypersensitivity, increased salivation, restless paws, and tremors as chronic repetitive movements. Signs and symptoms of acute intoxication for pyrethroids are similar and show local or systemic responses, such as dermal reactions, itching and heat sensations, sneezing, cough, throat irritation, and dyspnea. The most frequent symptom reported in the occupational exposure studies is paresthesia characterized by numbness, itching, burning or tingling sensations after dermal exposure to the pyrethroids. However, this result is considered a transitional and local effect, limited to exposure site [15].

Triflumurom toxicokinetics

Triflumurom is an insect growth regulator. It acts inhibiting the chitin synthesis, an important exoskeleton component. Thus, it directly interferes at the molting and results in insect death at its larval stage. In animals, the main adverse effects were hematological changes in all tested species: erythrocyte damage with a consequent increase of hematopoiesis, elevated metabolic activity in spleen, as well as hemosiderosis in spleens, liver, and kidneys [13].

Triflumurom toxicodynamics

Triflumurom is well absorbed through digestive and respiratory means, although less absorbed through dermal route. Performed tests in rats showed dermal absorption of 1% without dilution and 5% for the diluted product (product containing Triflumurom at 480 g/L). Triflumurom is distributed mostly in adipose tissue and reaches the maximum concentration between 8 and 24 hours after oral administration. The most common metabolic routes are hydrolysis, conjugation, and hydroxylation. However, it is rapidly excreted by urinary and fecal means (89 to 95% within 48 hours), suggesting no bioaccumulation on tissues nor organs [16].

Exposure evaluation

Exposure evaluation involves assessing the extent to which a population comes into contact with a specific substance, including the frequency and duration of such exposure, whether occupational or environmental. Primary exposure pathways include inhalation, dermal contact, and ingestion, influenced by various factors such as the nature of the substance, its application and use, and the exposure context.

Exposure scenarios depending on the product use indication

For a product predominantly used by institutions or specialized companies, the handling, preparation, and solution obtention are assumed to be conducted by a trained professional. This individual is theoretically knowledgeable about product preparation and adherence to safety and protection protocols. The worst-case scenario assumes that the professional fails to use the recommended personal protective equipment (PPE) during the entire process. Both dermal and inhalation exposure pathways were evaluated. However, dermal exposure to the active ingredient Alpha-Cypermethrin was excluded from consideration, as animal studies exposed to zeta-cypermethrin through dermal contact did not exhibit toxicity [17].

In the post-application scenario, the exposure of inhabitants (adults and children) to the product is assessed. Oral exposure is considered due to incidental hand-to-mouth contact, leading to ingestion of contaminants. However, dermal exposure to the active ingredient Alpha-Cypermethrin post-application is not considered, following the rationale previously outlined. Similarly, inhalation exposure is deemed negligible due to the extremely low volatility of the active ingredients, making it an insignificant pathway for absorption in the post-application context [14, 18, 19].

Ecotoxicity data of active ingredients

Alpha-Cypermethrin

Alpha-Cypermethrin is not considered readily biodegradable in aquatic environments. In aquatic-sediment anaerobic systems, it is classified as moderately persistent, with a degradation half-life (TD_{50}) of 96.3 days. In aerobic soil conditions, Alpha-Cypermethrin demonstrates medium persistence, with a

TD_{50} of 86.9 days. This compound has a significant potential for bioaccumulation, evidenced by a bioconcentration factor (BCF) greater than 500 and an *n*-octanol/water partition coefficient (log K_{ow}) higher than 4. In fish, the log K_{ow} is 5.8, with BCF values ranging from 579 to 910. Additionally, Alpha-Cypermethrin is considered immobile in soil due to its low soil organic carbon-water partition coefficient (Koc) of 228,622 mL/g in non-sandy soils [20].

Triflumurom

Conversely, Triflumuron degrades rapidly under aerobic conditions, with a half-life (TD_{50}) ranging from 4.6 to 40.8 days, averaging 16.3 days across four soil types at 20°C. The degradation rate of Triflumuron appears to be independent of soil type and pH. Under anaerobic conditions, degradation of Triflumuron results in the formation of two significant metabolites: M02, with peak formation of 5.9% and 3.9% at 3 to 7 days, subsequently degrading to less than 1% after 30 to 56 days; and M08, with peak formation of 13.5% and 12.3% at 3 to 7 days, decreasing to 0.3% to 2.8% by day 120. The TD_{50} values for metabolites M02 and M08 range from 0.4 to 3.3 days (averaging 1.9 days across five soils) and from 1.3 to 20.5 days (averaging 7.0 days across four soils), respectively. The degradation of Triflumuron accelerates significantly when aerobic conditions follow anaerobic conditions. Additionally, Triflumuron is not photodegradable on soil surfaces, with an average TD_{50} of 16.3 days. Degradation test results, based on nominal concentrations, were recalculated to account for its time-weighted average (TWA) concentration.

Safe conclusion

The risk evaluation for the Alpha-Cypermethrin + Triflumuron product, intended for professional use, employed conservative assumptions across all scenarios and stages. The analysis yielded acceptable margins of exposure (MOE) for all assessed populations, suggesting a low probability of risk to applicators and users when adhering to the labeled precautions and usage conditions.

Efficacy evaluation against *A. aegypti*

The experimental day was characterized by low humidity and temperature, wind speeds below 5.1 km/h, and no precipitation, as detailed in Table 6.

Table 6. Environmental conditions during the field experiment

Parameter	Results			
	1st assay	**2nd assay**	**3rd assay**	**4th assay**
Temperature	22.8°C	22.9°C	26.5°C	26.4°C
Humidity	70% RH	72% RH	59% RH	59% RH
Wind speed	5.1 km/h	4.7 km/h	5.1 km/h	4.7 km/h
Presence of rain	No	No	No	No

Table 7 presents the number of knocked-down insects 60 minutes after the product application for both the treatment and control groups. Table 8 shows the numbers of live and dead insects 24 hours post-application for both the control and study groups.

Table 7. Number of knockdown insects for the treatment using Alfamurom and Natuinset by ultra-low volume (ULV) atomizer and the control after 60 minutes from the product application

Assay (replication)	Distance for treatment group				Control
	10 Meters	**15 Meters**	**20 Meters**	**25 Meters**	
A	100	100	100	100	0
B	100	100	100	100	0
C	100	100	100	100	0
D	100	100	100	100	0
Mean	**100**	**100**	**100**	**100**	**0.0**

Table 8. Number of dead insects for the treatment using Alfamurom and Natuinset by ultra-low volume (ULV) atomizer and the control in a 24-hour period after the product application

Assay (replication)	Distance for treatment group				Control
	10 Meters	15 Meters	20 Meters	25 Meters	
A	100	100	100	100	0
B	100	100	100	100	0
C	100	100	100	100	0
D	100	100	100	100	0
Mean	**100**	**100**	**100**	**100**	**0.0**

The *A*. *aegypti* adulticidal action [21] of Alfamurom demonstrated 100% mortality of adult mosquitoes within 60 minutes at a 60% concentration (equivalent to 500 mL of solution per hectare) when used in association with a 20% concentration of the Natuinset adjuvant .

CONCLUSION

In conclusion, the experiment using Alpha-Cypermethrin (Alfamurom) in a ULV formulation demonstrated 100% mortality of adult *Aedes aegypti* mosquitoes within one hour, in combination with the Natuinset adjuvant.

Based on the comprehensive efficacy data presented in this study, the combination of Alfamurom and Natuinset, when applied according to labeled instructions, meets the efficacy standards required for pre-qualification. This combination is suitable for use in various environments for controlling *A*. *aegypti* mosquitoes using ULV applications.

In summary, the results indicate that the use of Alfamurom insecticide in combination with the Natuinset adjuvant exhibits a synergistic action in controlling *A*. *aegypti*.

Conflicts of Interest: The authors declare no conflict of interest.

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