VALENT BIOSCIENCES®

TECHNICAL USE BROCHURE

ReMoa Tri™

Triple-Action Insecticide Space Spray

A&S Public Health

Triple-action innovation for complete resistance management

ReMoa Tri™, the world's first mosquito space spray based on a bacterium, is a novel combination of three active ingredients (abamectin, fenpropathrin, C8910 fatty acid blend) with three different modes of action for complete mosquito resistance management. ReMoa Tri's patented biorational technology assures consistent application and efficacy against both metabolic resistance and knockdown resistance (*kdr*).

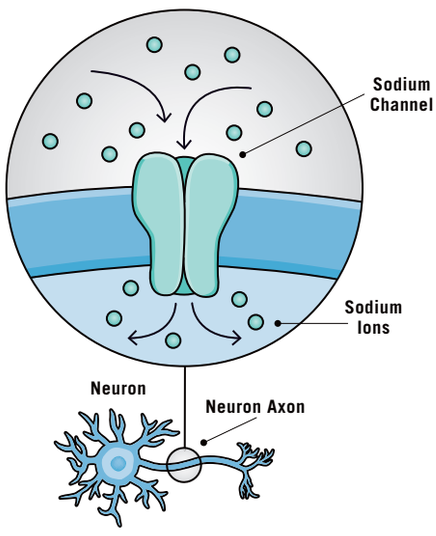
What is Insecticide Resistance?

In biological terms, resistance can be defined as the natural ability of an organism to withstand a damaging agent or an adverse condition. Animals, plants, and microbes have all demonstrated the ability to develop resistance, with either positive or negative outcomes, depending on the interaction. In terms of mosquito insecticide resistance, it can be defined as the ability of a mosquito to survive exposure to a standard dose of insecticide[1].

Types of Resistance

Mosquitoes that are heavily selected with pesticides tend to evolve two major types of resistance: 1) metabolic resistance and 2) knockdown mutation resistance (*kdr*)[1]. Species that show metabolic resistance express higher than normal enzymes in the P-450 area to neutralize/digest the pesticide molecules before they can bind to the target site (see note). In *kdr* species, they develop mechanisms that prevent binding of pyrethroids to sodium channels (see note). Chances of evolving both resistance mechanisms have been reported, but often one mechanism becomes the principal mechanism of defense for a genus. For example, species of the *Culex* genus predominantly evolve metabolic resistance while genus of *Aedes* evolve *kdr* mutation[2,3]. In general, *kdr* mutation is said to be more complicated to overcome because while adding a synergist like piperonyl butoxide (PBO), which aids in blocking enzymes from digesting pesticides, helps with metabolic resistance, it does not help with *kdr* mutation[1].

Fig. 1: Normal Sodium Channel Function



NOTES

Cytochrome P-450: *These are a family of enzymes that are present in many organisms including mammals and insects. This group of enzymes removes unwanted toxins that enter the body of these organisms. They also play a role in biosynthesis of hormones and other compounds. In insects, genetic mutations favor the development of this group of enzymes that break down pesticide molecules that enter the body which leads to development of resistance. Pharmaceutical drug makers for humans pay special attention to this group as they also breakdown drugs that enter the body[4].*

Sodium Channels: *Sodium channels (Fig. 1) are part of transmembrane proteins that are found in most organisms. These communication channels are referred to as voltage gated channels since they achieve the intercellular communication by ion exchange and electrical conductivity. Those channels that conduct sodium (Na) ions are called as sodium channels, which aid in communication between neurons, muscles, blood vessels, etc. The movement of Na ions, by a process called depolarization, aids in sensory communication such as pain or muscle contraction for body movements. Interestingly, most animal venoms (scorpion, spiders, sea anemones, etc.) target the sodium channels for intracellular communication disruption[5]. Similarly, pyrethroid molecules interfere with the sodium channel communication.*

1. Liu, N. Insecticide Resistance in Mosquitoes: Impact, Mechanisms, and Research Directions. *Annual Review of Entomology* 60, 537–559 (2015).
2. Shi, L. *et al.* Development of resistance to pyrethroid in culex pipiens pallens population under different insecticide selection pressures. *PLoS Neglected Tropical Diseases* 9, 1–20 (2015).
3. Kasai, S. *et al.* Mechanisms of Pyrethroid Resistance in the Dengue Mosquito Vector, Aedes aegypti: Target Site Insensitivity, Penetration, and Metabolism. *PLoS Neglected Tropical Diseases* 8, (2014).
4. Yang, T. *et al.* Multiple cytochrome P450 genes: conferring high levels of permethrin resistance in mosquitoes, Culex quinquefasciatus. *Scientific Reports* 11, 1–10 (2021).
5. Nauen, R., Bass, C., Feyereisen, R. & Vontas, J. The Role of Cytochrome P450s in Insect Toxicology and Resistance. *Annual Review of Entomology* vol. 67 105–124 (2022).
6. de Lera Ruiz, M. & Kraus, R. L. Voltage-Gated Sodium Channels: Structure, Function, Pharmacology, and Clinical Indications. *Journal of Medicinal Chemistry* vol. 58 (2015).
7. Liu, N. Insecticide Resistance in Mosquitoes: Impact, Mechanisms, and Research Directions. *Annual Review of Entomology* 60, 537–559 (2015).
8. Plernsub, S. *et al.* Additive effect of knockdown resistance mutations, S989P, V1016G and F1534C, in a heterozygous genotype conferring pyrethroid resistance in Aedes aegypti in Thailand. *Parasites and Vectors* 9, 1–7 (2016).
9. Leong, C. S. *et al.* Enzymatic and molecular characterization of insecticide resistance mechanisms in field populations of Aedes aegypti from Selangor, Malaysia. *Parasites and Vectors* 12, (2019).
10. Kawada, H. *et al.* Co-occurrence of Point Mutations in the Voltage-Gated Sodium Channel of Pyrethroid-Resistant Aedes aegypti Populations in Myanmar. *PLoS Neglected Tropical Diseases* 8, (2014).

Metabolic Pyrethroid Resistance

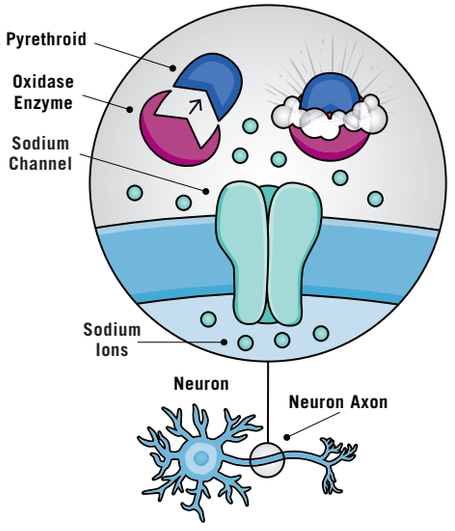
In metabolic resistance, the cytochrome P-450 (see note) area of mosquitoes produces oxidase and esterase enzymes that bind with pesticide molecules and breaks them down[6] (Fig. 2). Typically, metabolic resistant mosquitoes show high knockdown rate (incapacitated) 15 minutes post-application, but gradually recover and start flying normally at 1-, 24-, and 48-hours post-application. This gradual recovery post- application is usually due to gradual breaking down of pesticide molecules. Although there are many genera that show metabolic resistance, members of the *Culex* genus show high metabolic resistance. Studies show that successive selection of *Cx. quinquefasciatus/pipiens* mosquitoes favors metabolic resistance rather than knockdown resistance.

NOTE

Oxidase and Esterase Enzymes: *Both are different classes of enzymes classified based on their mode of chemical action. They convert different compounds as per the metabolic need in the body of a particular organism. In the case of mosquitoes, the oxidase group of enzymes help in the breakdown of pyrethroid molecules, hence PBO is added to many pyrethroids for prevention of oxidase enzymes acting on the pyrethroid molecules that enter the mosquito body. Oxidase enzymes are mostly controlled by the P-450 region. Esterase enzymes in mosquitoes help with organophosphate-based pesticide molecule breakdown. Although there have been identified esterase enzyme inhibitors, organophosphate pesticides with esterase blockers are rare.*

Fig. 2: MPR Enzymes

Enzymes break down pesticide molecules before they reach the sodium channel.



Knock Down Resistance *(kdr)*

Pyrethroids bind to the sodium channel ions and prevent them from normally working thus causing death (Fig. 3). In *kdr*, the target sites where pyrethroid molecules bind are modified, and thus prevent the binding of pyrethroid molecules. When this happens, the sodium channels function normally. Although there are many genera that show *kdr*, *Aedes aegypti* show high levels of *kdr*. Studies show that successive pyrethroid selection of *Ae. aegypti* mosquitoes results in knock down resistance rather than metabolic resistance. There are several different types of mutations that have been documented for *Ae. aegypti*, but the number of *kdr* mutations present determines the level of resistance[3]. Simultaneous occurrence of three mutations has so far been documented to provide the most resistance in *Ae. aegypti* (often referred to as "triple mutation *kdr*" or "*super-kdr*")[3] (Fig. 4).

Fig. 3: *kdr* Mutation

The kdr mutation prevents pyrethroids from binding to the sodium channel, allowing it to open and close normally.

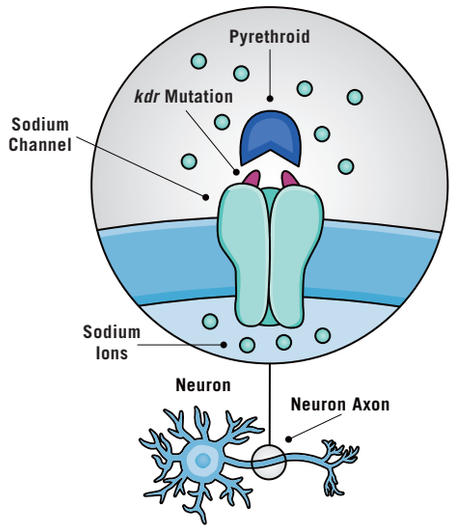


Fig. 4: *kdr* Mutation Distribution

No. Of Mutations	Distribution
Single	Global [7]
Double	Global [7]
Triple (*super-kdr*)	Southeast Asia [8-10]

What is ReMoa Tri?

ReMoa Tri, is the world's first adulticide space spray to control resistant mosquitoes based on the fermentation of a soil bacterium (*Streptomyces avermitilis*). This soil bacterium produces a metabolite called abamectin that is used as the core active ingredient in ReMoa Tri. In addition to abamectin, ReMoa Tri also contains fenpropathrin, which is a pyrethroid and C8910, which are a group of fatty acids (C8, C9, C10). This novel combination of active ingredients that have three different modes of action is delivered with a novel formulation that can penetrate the mosquito cuticle to deliver this active ingredient matrix. This non-corrosive combination adulticide space spray can be sprayed from ground* Ultra Low Volume (ULV) equipment.

FEATURES	BENEFITS
World's first mosquito adulticide space spray based on a soil bacterium	Resistance management
	Sustainability
Contains soil bacterium metabolite that has historically been used in pharmaceuticals (discoverers awarded Nobel Prize)	Reduced risk
Combination of three active ingredients (abamectin, fenpropathrin, C8910) novel to mosquito control with three different modes of action	Broad spectrum efficacy to manage both metabolic and *kdr* resistance
	No PBO required
Ready-to-use formulation	Operational speed
High efficacy of an organophosphate with the safety profile of a pyrethroid	Saves time on training staff to manage multiple active ingredient types (e.g. PPE, pre-cautions, etc.)
Only space spray in market that lists resistant mosquito control on the label	Peace of mind
Multiple application platforms and uses*	Operational flexibility
Non-corrosive formulation	No need to replace expensive valves and fixtures in equipment
Oil-based space spray	Works well with existing ULV equipment in the field

*For aerial and crop use, U.S. Environmental Protection Agency is still reviewing these applications

11. Papich, M. G. Ivermectin. in (ed. Papich, M. G. B. T.-P. H. of V. D. (Fifth E.) 484–488 (W.B. Saunders, 2021). doi:https://doi.org/10.1016/B978-0-323-70957-6.00283-1.
12. Du, Y. *et al.* Molecular determinants on the insect sodium channel for the specific action of type II pyrethroid insecticides. *Toxicol Appl Pharmacol* 234, 266–272 (2009).
13. Vais, H., Williamson, M. S., Devonshire, A. L. & Usherwood, P. N. R. The molecular interactions of pyrethroid insecticides with insect and mammalian sodium channels. *Pest Management Science* 57, 877–888 (2001).
14. Skinner, W. A. *et al.* Influence of human skin surface lipids on protection time of topical mosquito repellent. *Journal of Pharmaceutical Sciences* 66, 1764–1766 (1977).
15. Dunford, J. C., Falconer, A., Leite, L. N., Wirtz, R. A. & Brogdon, W. G. Determination of Insecticidal Effect (LC50 and LC90) of Organic Fatty Acids Mixture (C8910+Silicone) Against Aedes aegypti and Aedes albopictus (Diptera: Culicidae). *J Med Entomol* 53, 699–702 (2016).
16. Samuel, M., Oliver, S. V, Wood, O. R., Coetzee, M. & Brooke, B. D. Evaluation of the toxicity and repellence of an organic fatty acids mixture (C8910) against insecticide susceptible and resistant strains of the major malaria vector Anopheles funestus Giles (Diptera: Culicidae). *Parasit Vectors* 8, 321 (2015).

Active Ingredients

ReMoa Tri contains the following active ingredients: **abamectin, fenpropathrin,** and **C8910.**

Abamectin

Avermectins are used as both pharmaceuticals and pesticides and belong to the group of compounds referred to as macrocyclic lactones. They are fermented from the naturally occurring soil bacterium *Streptomyces avermitilis*. Discovery of avermectin was awarded a Nobel prize in 2015 in recognition of its positive impacts on human and veterinary health. The most popular pharmaceutical from this group is ivermectin which is an antiparasitic in humans and animals. This group of naturally occurring metabolites does not cross the blood brain barrier of higher order animals including mammals[11]. Abamectin is another naturally occurring avermectin compound that is fermented from the same soil bacterium as ivermectin. Abamectin is also used as an antiparasitic by veterinarians and insecticide in agriculture.

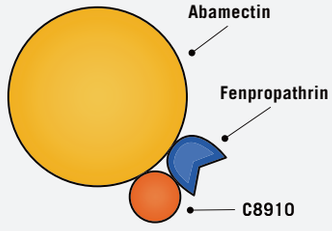
Fenpropathrin

There are two types of synthetic pyrethroids that are used as insecticides referred to as 'Type 1' and 'Type 2'. Permethrin, d-phenothrin (commercially referred to as 'Sumithrin'), etc. belong to the Type 1 pyrethroid class while pyrethroids such as deltamethrin belong to the Type 2 pyrethroid class[12]. Structurally, Type 2 pyrethroids have an extra alpha-cyano group in its chemical structure. Physiological impacts of Type 1 pyrethroids are slightly different from that of Type 2 pyrethroids. The combination of physiological impacts and chemical structure form the basis for pyrethroid classification. Fenpropathrin belongs to the Type 2 group of pyrethroids. Type 2 pyrethroids have shown to be more efficient in interfering with voltage-gated sodium channels[13]. The exact mechanism on how Type 2 pyrethroids achieve efficiency is still not clear[12].

C8910

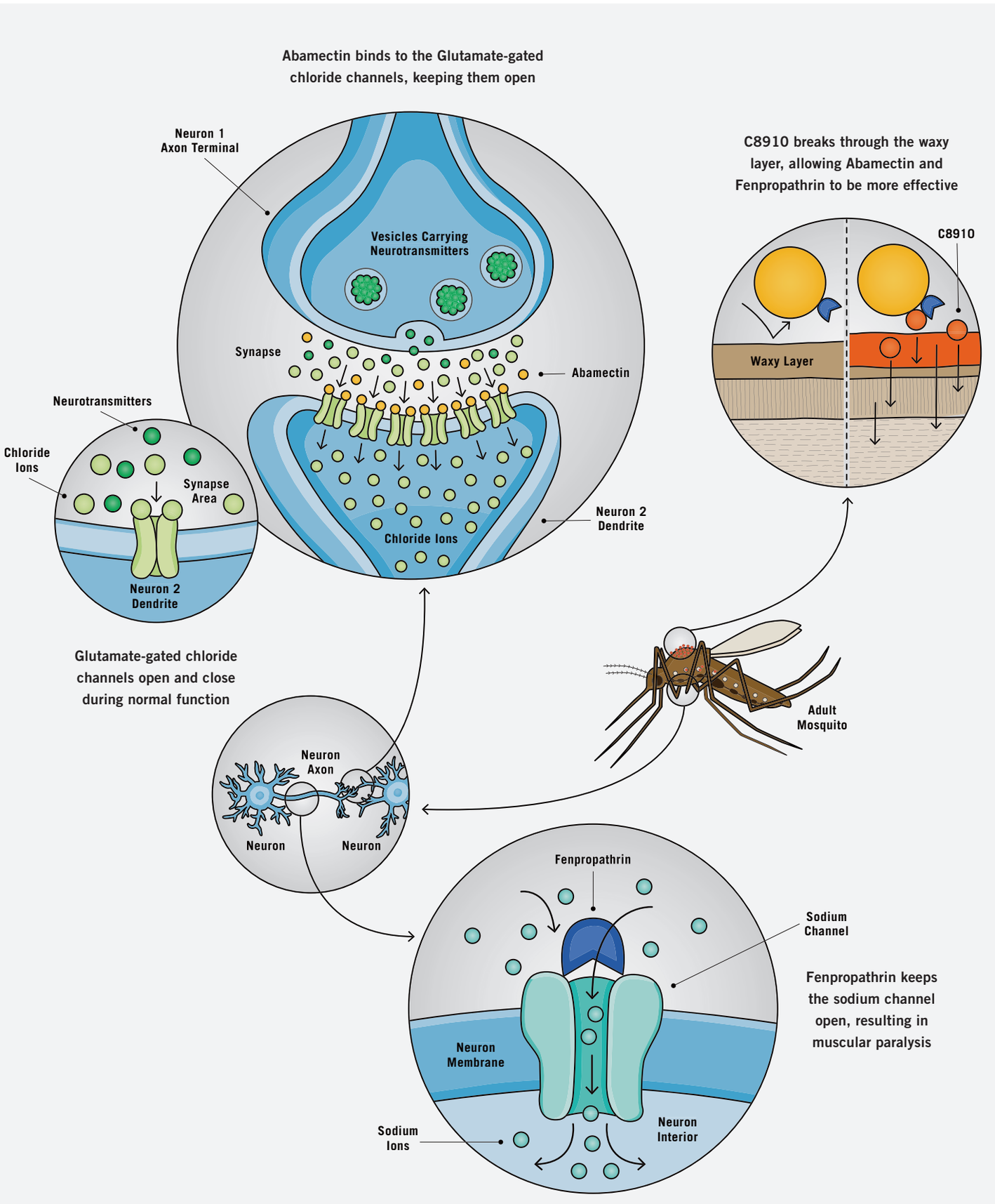
Some fatty acids found naturally on human skin have been shown to have insect repellent properties[14]. Fatty acids are long carbon chain molecules; often abbreviations are used to designate fatty acid length (e.g., an eight-carbon long fatty acid is short-handed to "C8"). It was discovered that a specific combination of octanoic (C8), nonanoic (C9) and decanoic (C10) fatty acids (collectively called C8910[15]) had a synergistic effect in regard to repellency when compared to each of these fatty acids alone. This unique combination of C8910 was patented in 2001. This patented fatty acid combination has a repellent effect at lower doses and an insecticidal effect at higher doses[16]. When combined with pyrethroids, C8910 enhances efficacy by delivering a synergistic effect. Although the original source of discovery was from the observation of differences between human fatty acid emissions, commercial sources have been historically used to manufacture these fatty acids. For example, C8 and C10 are made from palm oil and coconut oil respectively, whereas C9 can be made from tallow.

Unique Formulation: Matrix of Three Different Active Ingredients



The molecular weight of abamectin is 1732.1 g/mol which is 3-4 times larger compared to several other pyrethroids like permethrin (391.28 g/mol) and deltamethrin (505.21 g/mol), or organo-phosphates like Naled (380.784 g/mol) and malathion (330.358 g/mol). Hence, a unique formulation is key in achieving absorption of Abamectin and other active ingredients in a matrix through the cuticle of mosquitoes.

Modes of Action



Abamectin

This naturally occurring compound produced by fermentation of a soil bacterium, interferes with the glutamate-gated chloride channels (GluCls) (see note) found only in the invertebrate neuromuscular system[17]. Binding to these channels causes irreversible conductance, hyperpolarization, and paralysis. GluCls are not found in mammals, and moreover, the semi-permeable membrane in mammals that prevents particles from the blood entering the central nervous system (also known as the blood brain barrier) prevents these compounds from entering the brain there by giving them a wider margin of safety.

NOTE

Glutamate-gated Chloride Channel: *Glutamate-gated chloride channels are transmembrane proteins found only in invertebrates. These proteins help in the communication between two cells. Similar to sodium channels, they also aid in intercellular communication by ion exchange and electrical conductivity. Since the ions involved in these proteins are chloride based, they are called as glutamate-gated chloride channels. They aid in locomotion, feeding, and other behaviors. Their main role is to transfer sensory signal inputs from antennae and other structures to the nervous system and then carry behavioral response messages back to respective body parts. Compounds of the macrocyclic lactone family interfere with the communication of these channels thus causing mortality. These compounds seem to also have roles in reproduction and fecundity since macrocyclic lactones seem to also interfere with these functions.*

Fenpropathrin

Pyrethroids, like pyrethrins, affect the voltage-gated sodium channels by binding to specific receptors and creating persistently open channels which results in muscular paralysis and death of insects[18].

C8910

The exact mode of action for the blend of fatty acids known as C8, C9 and C10 has not been fully expounded, however, fatty acids are thought to have similar effects to that of organic insecticidal soaps by which they either asphyxiate the respiratory system and/or break down the cuticle protection of pest insects[19]. This theory is strengthened by evidence of fatty acids synergizing common pesticides such as pyrethroids (U.S. Patent 2014, US9826742B2). It has been documented that the incapacitating and toxic effects of fatty acids on mosquitoes are highly dependent on the type of fatty acids and the formulation being used. Higher level vapor exposure or physical contact with C8910 causes a sensory overload to exposed insects, resulting in an ‘agitated state’ (U.S. Patent 2009/WO 2010121142A2).

17. Batiha, G. E. S. *et al.* Avermectin derivatives, pharmacokinetics, therapeutic and toxic dosages, mechanism of action, and their biological effects. *Pharmaceuticals* 13, 1–37 (2020).
18. Ensley, S. M. Chapter 39 - Pyrethrins and Pyrethroids. in (ed. Gupta, R. C. B. T.-V. T. (Third E.) 515–520 (Academic Press, 2018). doi:https://doi.org/10.1016/B978-0-12-811410-0.00039-8.
19. Cline, R. E. Lethal Effects of Aqueous Formulations Containing Fatty Amines or Acids Against Eggs and Larvae of Aedes aegypti12. *Journal of Economic Entomology* 65, 177–181 (1972).

Application, Diluents, and Bioassays

Application Rate

ReMoa Tri's novel triple mode of action adulticide can be sprayed between 0.341 to 1 ounce per acre. Nonresistant or susceptible mosquito species can be sprayed at 0.341 ounce/acre. If spray volume needs to be increased, then VBC's special ReMoa Diluent™ can be added to increase the number of droplets per acre. Resistant mosquitoes (metabolic *Culex* and *kdr Aedes*) can be sprayed at 0.66 ounce per acre or higher. The highest label rate of 1-ounce/acre might be needed only under rare occasions to control highly resistant mosquitoes like triple *kdr* mutated *Ae aegypti*.

ReMoa Diluent™

The ReMoa Tri formulation is so unique that diluting it with regular mineral oil (which is a common diluent in the industry) will reduce the efficacy of the product. Mineral oil and other petroleum-based diluents alter the polarity of the ReMoa Tri formulation which reduces the penetration of active ingredients into the cuticle. If dilution is necessary to increase volume at low application rates, then use VBC's recommended ReMoa Diluent to maintain efficacy in the field.

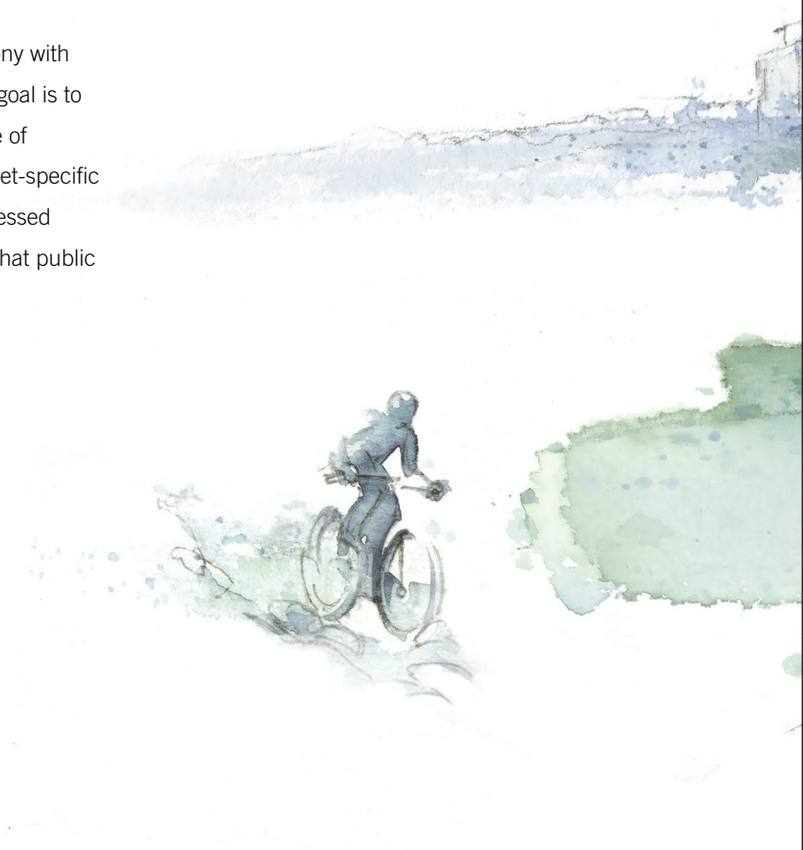
Resistance Monitoring: Bottle Bioassays

ReMoa Tri has a novel combination of three active ingredients with three different modes of action. Traditional bottle bioassays use technical grade active ingredients mixed in acetone to coat the bottles. However, acetone as a carrier reduces the efficacy of the ReMoa Tri combination. Since each active ingredient has a different mode of action, isolating individual actives for measuring resistance will underestimate the combined efficacy of all the actives on resistance development. Because of the uniqueness of the ReMoa Tri formulation, bottle bioassays will underestimate the mortality and development of resistance to the combined actives. Research at two different academic institutions and at the Centers for Disease Control and Prevention are in progress to determine the appropriate method for estimating resistance profile for ReMoa Tri.

Art & Science
of Public Health

At Valent BioSciences, we believe it is always better to work in harmony with nature rather than against it. As a global leader in public health, our goal is to help realize human potential by bringing together the art and science of sustainable insect control with the most comprehensive range of target-specific biorational solutions. In everything we do, you will find that idea expressed through artistic representations of our products and the adversaries that public health professionals around the world face every day.

ARTWORK BY NARDA LEBO



To learn more about ReMoa Tri™
call **800.323.9597** or
scan the **QR code**

VALENT BIOSCIENCES

Valent BioSciences is an ISO 9001 Certified Company

valentbiosciences.com/publichealth

Valent BioSciences
1910 Innovation Way, Suite 100
Libertyville, Illinois 60048

FPO
FPO
FPO FPO
FPO FPO FPO