Insecticide Mode of Action

Technical Training Manual

BASF

The Chemical Company

contents

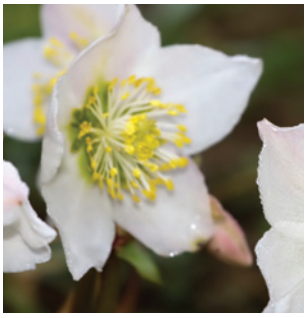
Inside this pocket is the complete Insecticide Resistance Action Committee (IRAC) Classification chart in poster format as a companion to this book.

Introduction

Insecticides are essential tools for preventing or minimizing insect damage to, and significantly increasing the quality and quantity of crops, as well as for improving the quality of life for humans, domestic animals and livestock. There are currently more than 20 different mechanisms, or modes of action, by which various commercial insecticides control insects by disrupting specific vital biological processes, but not all of these can be used against any particular pest insect. Despite the best efforts of the entire crop protection industry, a new insecticide mode of action comes to market only every 5 or 10 years, the last being in 2007.

Nevertheless, mode of action diversity is the most important tool we have for ensuring our sustained ability to control insect pests. Repeated application of insecticides with the same mode of action contributes to resistance by killing the susceptible insects and leaving those with resistance to that entire class of insecticides. By rotating pest control chemicals that work through different modes of action, insecticide resistance can be forestalled or avoided altogether.

Insects are animals, with similarities to and differences from other animals. Indeed, all living things share a common set of biological processes that make life possible, and the more closely related two organisms are, the more vital processes they have in common. Ideally, insecticides would specifically target vital processes unique to pest species, but that is seldom possible. While insects are close relatives of mites and ticks which are also often pests, they are also kin to lobsters, crabs and shrimp, which are not. These relationships have led, on the one hand, to important acaricides for controlling mites and ticks, but on the other, to collateral toxicity of insecticides to non-target soil-dwelling and aquatic arthropods, as well as to bees and other beneficial insects. While some insecticides target processes unique to insects and closely-related arthropods, such as the biosynthesis of chitin, a tough, semitransparent polysaccharide that is the main component of the insect's exoskeleton, most gain species selectivity by other means. Key insecticide target groups include: neuromuscular poisons, respiratory poisons, gut disruptors and insect growth regulators.



The Evolution of Insect Control

As many of us learned in the movie *Jurassic Park*, vertebrates have been plagued by insect pests for hundreds of millions of years. Humans, like other mammals, were always hosts to lice, flies, predatory bugs and mosquitoes. Immediate physical reactions such as grooming, swatting or squashing and mud baths were probably the only means of combating aggressive insect behavior until the development of civilization. While just about every conceivable approach has been tried since then, including magic spells, prayer and, as recently as 1866, even ecclesiastical trials and excommunication of the pests, not until the advent of synthetic insecticides in the early 1900s did growers have an effective and consistent means to address the damage caused by insects. Chemical insecticides have unquestionably been the most successful tools for protecting our crops, structures and livestock from insect pests, and controlling the transmission of many insect-borne diseases.

The earliest insecticides were natural substances derived from minerals or toxic plants. The first known example was the control of insects and mites with ground sulfur compounds by the Sumerians as early as 2500 BC.

Sulfur and various mineral-derived compounds containing aluminum antimony, arsenic, lead, mercury or phosphorus were used throughout history, with some of these still in use today. The most toxic of these compounds have been phased out due to health and/or environmental concerns, or restricted to applications where an acceptable environmental impact can be achieved.

One of the earliest known uses of a poisonous plant to control insects was of hellebore by the ancient Romans. Not entirely safe, it was also used as a rat poison. Much more relevant to the history of insecticides is the pyrethrum daisy, the powdered dried flowers of which have been used in China since 100 CE to control lice. It was traded along the silk road and was brought to Europe by Marco Polo in the 13th century. Pyrethrum is still used in organic farming and in household insecticides, as are its purified active constituents, which are known as pyrethrins. One of the largest groups of modern synthetic insecticides, the pyrethroids, is modeled on the pyrethrins.

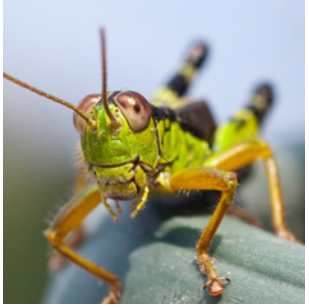
from top to bottom:

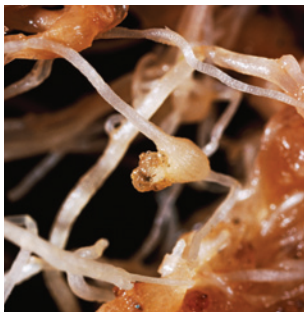
Primates spend several hours a day removing pests. The hellebore plant contains toxic alkaloids that are the source of the plant's toxicity. Pyrethrum daisies are drying in the sun. A soldier is wearing a mosquito net treated with alpha-cypermethrin, a widely used synthetic product modeled on pyrethrins.

Since the 1940s, mineral and botanical insecticides have been largely replaced by successive generations of synthetic organic insecticides and microbially-derived products with improved properties. There are now approximately 275 insecticide active ingredients registered globally, and the value gained from their proper use is significant. A 2009 study estimated that U.S. farmers gained a net value of approximately $21.7 billion each year from the use of insecticides, a return of $19 for every $1 spent. Without insecticides, it is estimated that yields of most crops would decline by 40% to 50%. As the world's population is estimated to increase by over 1 billion people by 2020, the capacity of producers to meet the increasing need for quality food and fiber will be stretched to the limit. (Source: Crop Life Foundation.) Many factors will affect producers' ability to meet this increased demand, including weather variability, climate change, government policy and shifting dietary demands. The adoption of new technologies by developing countries, the availability of and efficient use of land, water and crop production tools, as well as the continued availability of and sustainability of effective pest control technologies, may enable producers to meet the growing near-term needs of a growing population.

In 2011, the global insecticide/acaricide/nematicide market was $14 billion, with $11.6 billion in crop uses (foliar, soil and seed treatments) and $2.4 billion in non-crop applications. (Source: Phillips McDougall-AgriService.) The crop segment (e.g., maize, cotton, fruit, vegetables, rice, soybeans, etc.) is divided into the following pest sub-segments: chewing insects, piercing & sucking insects, mites and nematodes. These four classes of pests can cause significant crop damage and often occur concurrently on the same crop and in the same season.

With the high demand for cost-effective technologies comes the responsibility of product stewardship regarding environmental awareness, insect resistance management, non-target impacts, dietary residues and consumer and applicator exposure. With increasing pressure on crop production and quality, growers will rely more heavily on multiple insecticide treatments to control an increasingly complex diversity of pests.





from top to bottom:

Four pest types that threaten our capacity to produce food and fiber are: chewing insects like grasshoppers, ants, termites, beetles and caterpillars; piercing and sucking insects like aphids, whiteflies, hoppers, and scales; mites; and nematodes.

Insecticide Mode of Action

above:
Nicotine, a natural insecticide produced by tobacco plants, mimics the chemical messenger acetylcholine in the nervous systems of both insects and humans – two quite different organisms. This illustrates the challenge of developing insecticides that affect harmful insects but not beneficial ones.

Vital processes in organisms are carried out largely by proteins – macromolecules composed of chains of amino acids that occur in many different forms and are the workhorses of the body. The best known of the tens of thousands of different proteins in the human body are structural ones that occur in large quantities, such as keratin, which forms our hair and nails, and collagen, which forms cartilage and the scaffold upon which most tissues are built. Some insects and other arthropods produce a specialized structural protein called silk.

However, most proteins in the body are not structural, but are intricate molecular machines that occur in minute quantities – sometimes just a few molecules per cell – and perform various essential functions. Many of these non-structural proteins are: 1) enzymes that catalyze biochemical reactions, 2) receptors that transduce signals, or 3) channels and other types of transport proteins, which help substances cross cell membranes. Because of their complexity and the key functions they carry out, enzymes, receptors and channel proteins are often affected by drugs, toxins or insecticides.

Like drugs, modern insecticides act specifically – often targeting a single protein. The challenge of controlling pests with insecticides is that the more similar two organisms are, the more similar their proteins are as well. This in turn increases the challenge to develop low risk and selective products. For example, the natural insecticide nicotine mimics the action of the endogenous (substance that originates within an organism, tissue or cell) chemical messenger acetylcholine (ACh) in the insect nervous system, and has the same action in humans. Nicotine, though natural, is much more toxic to humans than any commercial insecticide. The nicotine contained in 30 - 40 cigarettes can be lethal to an adult when administered as a single dose.

What is a Target Site?

Insecticide target protein molecules are many times larger than the insecticides that act on them, and can have more than one site where small molecules like insecticides can bind. While nicotine and many other toxicants and drugs exert their effects on the body by preempting the action of an endogenous substance at a site on a protein that is already specialized for binding a small molecule, other toxicants and drugs act on sites for which there is no known endogenous ligand, but where there happens to be a pocket in which a small molecule can tightly bind and disrupt the function of the protein. The location on or within a particular protein where the toxicant binds and exerts its toxic action is known as the target site, and the interactions of the toxicant with that site define the toxicant's mode of action.

Mode of Action Classification

Mode of action (MoA) is the most fundamental property of an insecticide – more so even than chemical structure itself, as compounds of widely different chemical structure can bind at the same target site and have exactly the same mode of action. Furthermore,

heritable changes that hamper action at the target site can confer resistance to entire classes of insecticides. The Insecticide Resistance Action Committee (IRAC) is a specialist technical group of the industry association CropLife, organized to provide a coordinated industry response to prevent or delay the development of resistance in insect and mite pests. IRAC classifies insecticides into groups with a common mode of action and then into chemical subgroups within those groups.

While consolidating the 50 or so chemical insecticide classes into mode of action groups is very useful, further grouping the 26 recognized mode of action groups into four categories provides a broader understanding of this relationship. The four categories are: neuromuscular toxins, which attack the nervous system or muscles; insect growth regulators (IGRs), which affect growth and development; respiratory poisons, also called metabolic poisons, which affect energy metabolism; and gut disruptors, which destroy the integrity of the gut lining. In addition to these four categories, there is a group of compounds that are thought to be non-specific multi-site inhibitors, which interact with one or more specific target sites, as well as a group of compounds that are thought to act specifically, but whose targets are currently unknown. The insert in the front pocket of this manual illustrates the complete IRAC classification grouped into the four categories mentioned above.

Some of the important properties of insecticides, such as speed of action, spectrum of control and environmental safety, are characteristic of their mode of action. Neuromuscular disruptors and respiratory disruptors directly affect the coordination and energy state, respectively, so they are usually rapid and broad in spectrum of activity, controlling a wide variety of pests. While this sometimes affects non-targeted, beneficial insects, many of the newer neuromuscular disruptors and even some respiration disruptors, acting at novel target sites, are shown to have low toxicity, and only affect target pests. Insect growth regulators, on the other hand, are generally low risk to non-target organisms, but they are usually slow-acting and limited in spectrum. Furthermore, they are usually only effective at certain stages of the insect's life cycle, such as during molting. It is not surprising, then, that neuromuscular toxins lead the insecticide market by a very large margin, making up more than 90% of the dollar value of the global insecticide market.

Types of Insecticide Target Proteins

Classification of insecticide targets by type of protein is also informative, and is shown by the color coding in the complete IRAC Classification of Insecticide Modes of Action included as an insert to this manual. Neuromuscular disruptors act mostly on ion channels (shown in light green), except for the octopamine agonists, which act on a G-protein-coupled receptor (in light blue), and the acetylcholinesterase inhibitors, which inhibit an enzyme (light orange). The target sites of the chitin synthesis inhibitors and some of the other insect growth regulators (dark green) are not yet known, while the mimics of the juvenile hormones and ecdysone receptor agonists act on nuclear receptors (shown in dark blue) involved in the regulation of gene expression during development. ACCase inhibitors (light orange) are classified as insect growth regulators because they are primarily active on

immature insects. They inhibit an enzyme involved in the biosynthesis of fatty acids, which are essential components of cell membranes.

On the other hand, most respiratory poisons are enzyme inhibitors except for uncouplers, which are ionophores (in red), which means that there is no protein target, and the insecticide molecules themselves carry ions across membranes.

Some insecticide target proteins are very complex, containing more than one target site where agents bind to disrupt function. Among the most complex of these are the ion channels, which are also the most important insecticide targets. Inorganic ions and the ion channels that facilitate their movement across membranes, are the basis of bioelectricity, which drives many vital processes. Electrical current requires the flow of charged particles, which, in the body, are sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-) ions. Positive ions are atoms with a deficit of electrons, whereas negative ions have an excess of electrons, making them negatively charged. Being charged, like ions repel each other and easily permeate polar media like water, but they cannot enter nonpolar areas like cell membranes, except via polar channels traversing those membranes that are formed by ion channel proteins. These transmembrane proteins occur in many varieties that are specific for particular ions, and literally form channels or pores through which ions can tunnel across the membrane.

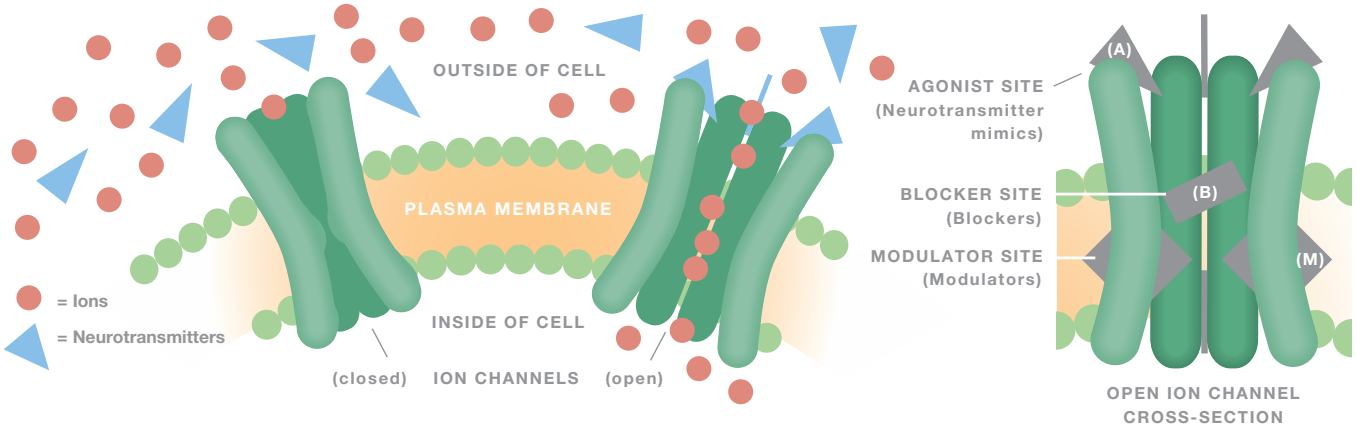
A simple ion channel could be formed from a relatively small and simple molecule, but the complexity and much of the sensitivity comes from the mechanisms that open and close the channel to allow ion flow only under certain conditions. Opening and closing of the pore, known as gating, depends on a signal such as a neurotransmitter substance, or a physical stimulus such as heat, pressure or electrical potential. Flux of the ions across the membrane can generate an electrical signal, and, in the case of Ca^{2+}, the ion itself also can interact with intracellular proteins to cause many effects.

Insecticides can affect ion channels in various ways. Channel blockers enter and become trapped in the pore, blocking ion flow through the channel. Agonists bind to and mimic the action of the neurotransmitter at the neurotransmitter binding site, while antagonists also bind to the neurotransmitter site but hinder activation of the ion channel. Lastly, modulators are compounds that bind to a modulatory site and modify the normal function of the channel. Some modulators activate the channel, others keep it open much longer than normal and still others prevent it from opening at all.

All three of these binding sites are present within the cys-loop ligand-gated ion channels, which are target proteins of five of the eleven groups of insecticides that act on the neuromuscular system. These channels are composed of five subunits, which are similar if not completely identical, and fit together like staves to form a barrel around the pore.

About half of each subunit is embedded in the membrane and half protrudes from the surface of the cell. In the resting state, the staves form a tight barrel and the pore is too small to pass ions, so the channel is closed. Channel blocker insecticides can bind in the pore either when it is open, closed, or both, depending on the compound. Ions cannot pass through the pore when a blocker is bound. The neurotransmitter binds to the agonist sites, which are situated between adjacent subunits in the extracellular region. Since there are five subunits, there can be up to five agonist sites per channel. Like the agonist sites, the modulator sites of cys-loop channels are also situated between subunits, but within the membrane, far removed from the extracellular agonist site. One can imagine that binding of the agonist or modulator, or both, moves the staves of the barrel apart and opens the channel. Antagonists that bind to the agonist site while the channel pore is closed are called competitive antagonists because they compete with agonists for the binding sites and prohibit opening of the channel. Compounds that bind to the modulatory site in the closed state are called allosteric antagonists, and they can prevent opening of the channel by agonists. The modulatory site on the cys-loop ligand-gated ion channels is known as the macrocyclic lactone binding site and is the target site of avermectins on glutamate-gated chloride channels, and spinosyns on nicotinic acetylcholine receptors. A macrocyclic lactone binding site also exists on GABA receptors and is the target of some novel experimental insecticides that may soon be commercialized.

below, left:
Ion channels are activated by neurotransmitters or physical stimuli to conduct ions across the cell membrane.



ADME – Absorption, Distribution, Metabolism and Excretion

In order for an insecticide to act at its target site, it must enter the insect through one or more absorption routes, including absorption through the cuticle, orally through the consumption of treated foliage or sap, or inhalation through the spiracles as a vapor. Once absorbed into the body, the active ingredient then distributes throughout the body to reach the target sites, which may only occur deep within certain tissues. At the same time, natural defense mechanisms of the insect are acting to break down and excrete the insecticide molecules. These processes, which together with mode of action determine the biological effect of the insecticide, are absorption, distribution, metabolism and excretion, respectively.

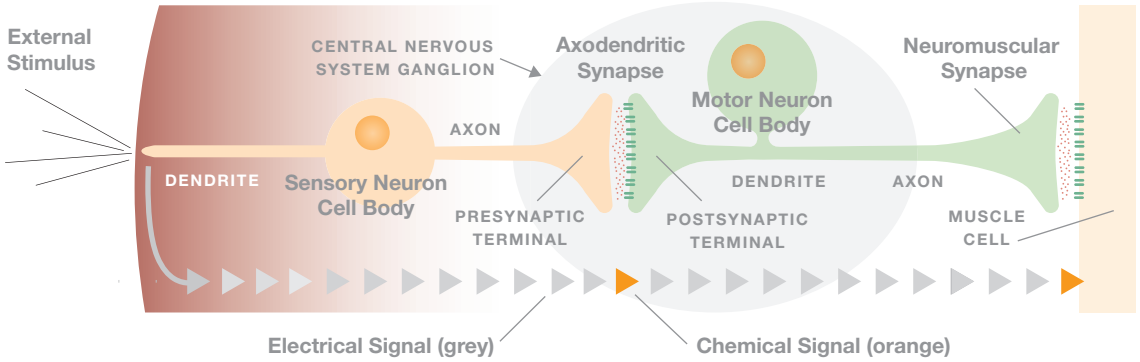
above:
Insecticides can have various effects on ion channels: agonists (A) bind at the neurotransmitter site and open the channel, antagonists (A) occupy the neurotransmitter site and prevent activation, blockers (B) occlude the channel pore, while modulators (M) act at other sites to modify channel function.

The Insect Neuromuscular System

Elements of the Insect Neuromuscular System

Most highly effective insecticides act on the nervous system – the control center of the body, or on the muscles, which transduce the activity of the nervous system into behavior. The complex network of nerve cells, which are also called neurons, perceives and acts upon external cues from light, sound, touch, smell and taste sensors, as well as internal input from sources such as hormones, body temperature, hunger and limb position sensors, to produce the controlled orchestration of muscular contractions that results in the behavior that allows the insect to grow and prosper in its environment. It's not surprising that disrupting this fine-tuned control system can rapidly affect insect behavior and halt feeding, which is why neuromuscular disruptors are by far the most–used insecticides.

The Monosynaptic Reflex Arc of an Insect



right:

A monosynaptic reflex loop illustrates how sensory input is translated into motor activity through neurons, which carry electrical signals, and synapses, which translate those into chemical signals and relay them between cells.

While the neuromuscular system is complex, composed of many circuits that control different body parts and behaviors, it is assembled from a much smaller variety of well-understood modular components. To understand the action of insecticides, we need to understand the functions of these components and the effects of insecticides on them.

To illustrate the essential components of the nervous system involved in insecticide mode of action, we consider the simplest type of neural circuit, the monosynaptic reflex arc shown above, as would be involved, for example, in the well-known knee-jerk reflex. Insects have analogous reflexes. Starting on the left side of the diagram, a sensory neuron receives an external stimulus, such as the tap of the physician's hammer on a patient's knee or the bending of a sensory hair on an insect's leg, which generates an electrical signal that travels down the dendrite or input side of the cell, past the sensory neuron cell body and then along the axon to its terminus in the synapse: the junction with the next cell. At the synapse, the electrical signal is converted into a chemical signal that is transmitted across the synaptic space to the postsynaptic cell, by a neurotransmitter substance that is emitted from the presynaptic terminal to activate receptors on the postsynaptic cell, which in the case of a monosynaptic reflex arc is a motor neuron. The signal in the motor neuron travels to the

neuromuscular synapse, where it triggers release of a chemical neurotransmitter, which activates the muscle.

Bioelectricity and Axonal Conduction

Dendrites and axons conduct signals electrically, but by different mechanisms. Like wires, both of these structures are tubular, constructed of a conductor – the cytoplasm, ensheathed by an insulator – the cell membrane. Wires are passive conductors: charged particles are forced into one end and flow out the other, minus a few that have escaped through the insulation. Cytoplasm, however, is a poor conductor and membranes are poor insulators, making dendrites and axons poor passive conductors. Fortunately, dendrites are short enough that passive conduction suffices, but passive conduction is inadequate in axons, which must carry signals over much longer distances. Instead, the axon conducts signals actively as all-or-none impulses called action potentials, without decrement, by regenerating the impulse at each point along its length.

Axonal conduction can be understood through the actions of three transmembrane proteins. The first of these is present in all animal cells and is not directly involved in the action potential, but rather in establishing the transmembrane potential and ion gradients that drive the action potential as well as many cellular processes. This protein resides in the cell membrane and uses energy from adenosine triphosphate (ATP) to pump sodium ions out of the cell and potassium ions into the cell.

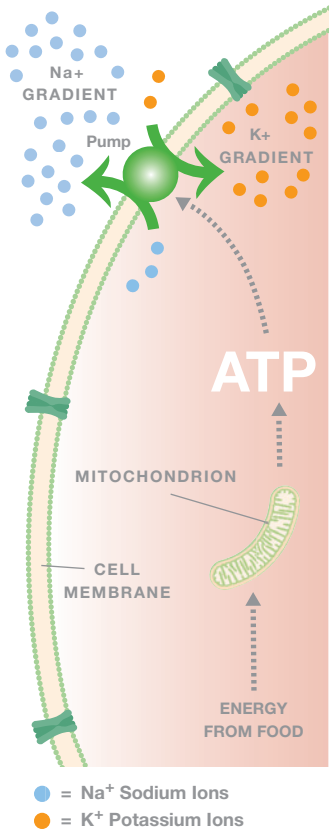
ATP is the energy currency of cells: energy obtained from foodstuffs is harnessed by subcellular organelles called mitochondria to form a high energy phosphate bond in the ATP molecule, and most cellular processes are driven by the stored energy released when that bond is broken. Insecticides that disrupt the production of ATP will be discussed in another section.

For each ATP molecule it consumes, the sodium-potassium pump expels three sodium ions from the cell but brings in only two potassium ions, leading to a net expulsion from the cell of one positive charge for each pump cycle, eventually making the interior of the cell negative by 50 to 100 mV. Another consequence of pump activity is that the potassium ion concentration becomes 10 or more times higher inside of the cell than out, and the sodium ion concentration becomes much higher on the outside.

The sodium-potassium pump is one of the most important proteins in the body, consuming about one third of all energy expended by an animal and about two-thirds of all energy expended in the nervous system. It is easy to see how building up an electrical potential can be useful as a form of energy storage, but the real repository of the energy expended by the pump is the transmembrane gradients of sodium and potassium ions.

below:

The origin of bioelectricity in animals; energy-rich molecules derived from food are processed by the mitochondria within cells to produce ATP. By pumping Na^+ ions out of the cell and K^+ ions in, the Na^+, K^+ pump establishes transmembrane potential and ion gradients that serve as bioelectric batteries to supply energy to many processes, including action potential conduction.



Ion concentration gradients have the capacity to do work because ions have a strong tendency to move from areas of high concentration to areas of low concentration, even against electrical potential. The reason for this is the simple, yet extremely subtle second law of thermodynamics: systems tend to move from a state of order to a state of disorder. The pump has established highly-ordered, high-energy gradients for sodium and potassium ions by separating those ions across the cell membrane. All that is needed to harness this order are transmembrane ion channels specific for sodium and potassium ions.

Both the sodium and potassium channels have gates that sense and respond to the transmembrane electrical potential. These ion channels are shown in green in the illustrations on the facing page. The Na^+ and K^+ concentration gradients are depicted by the number of ions on either side of the membrane. At the negative resting potential established by the Na^+, K^+ pump, the sodium channel is closed but poised to open in response to positive signals. The flow of positively-charged sodium ions into the cell through the open channel drives the interior of the cell positive. The sodium channels that are still closed sense this and they also open, so there is positive feedback, which takes over and soon all of the sodium channels are open and the cell interior becomes highly positive, as shown in the central part of the illustration at right, labeled “Active”. The transmembrane potential is shown in the corresponding line chart below the illustrations. This depolarization can occur in less than one millisecond. At the peak of positivity, two processes kick in to terminate the action potential: the sodium channels become temporarily inactivated, halting the sodium influx, and voltage-dependent potassium channels open, allowing K^+ ions to flow out of the cell and restore the negativity.

After the action potential is over, the sodium channels remain inactivated and the potassium channels remain open, making the conduction of another action potential impossible. This refractory period, which lasts about one hundredth of a second, ensures that the action potential travels only forward, self-propagating along the axon membrane. The open K^+ channels driving repolarization are shown in the right side of illustration A, labeled “Refractory”, on the next page.

To understand how the action potential moves, it helps to think of these diagrams as a snapshot in time, depicting three regions of the membrane in different stages of the sequence, as an action potential travels from right to left. In the resting area on the left, where the action potential is approaching, the inside of the axon is negative and the potential is -75 mV. In the active region just to the right (orange), the Na^+ permeability is high, which has resulted in Na^+ influx and charge reversal. This has driven the membrane potential positive. The action potential keeps moving toward the left because the excess positive charge on the inside flows into the adjacent resting section, causing depolarization and activation of the sodium permeability in this area.

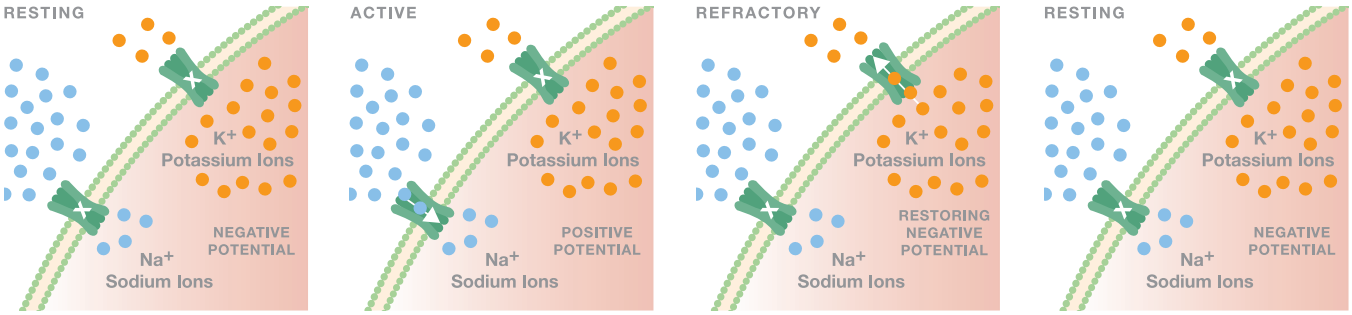
To the right of the orange active section is the blue refractory section, from where the action potential has just come.

Here, two processes are occurring to restore the membrane to its resting state. First, the Na^+ channels that generated the action potential have become inactivated so that no more Na^+ flows in, and second, K^+ channels have opened to allow K^+ ions to flow out of the cell and restore the internal negativity. In addition to restoring the resting conditions, these processes also ensure that the action potential does not travel backward. The region of membrane on the far right has fully recovered and is ready for the next action potential.

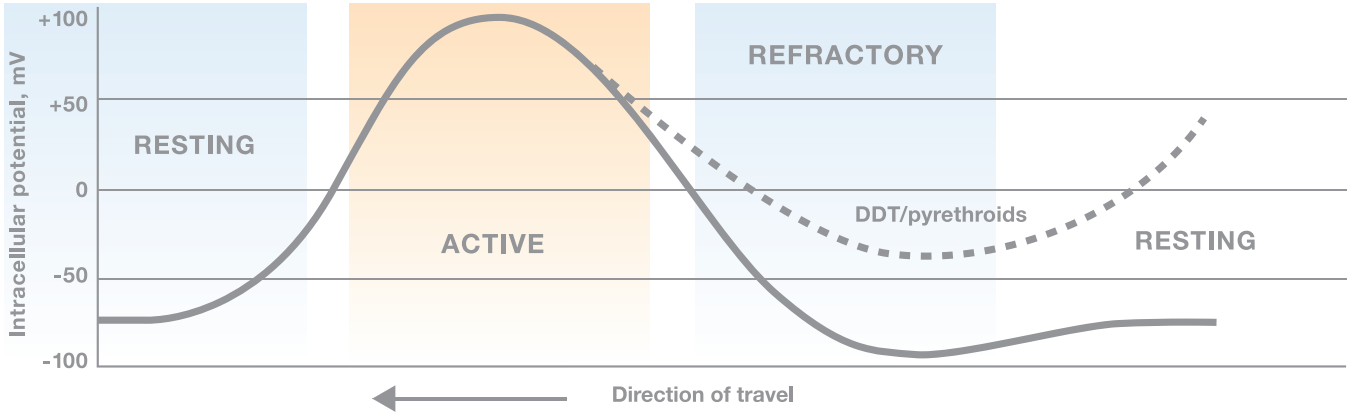
Of the three proteins involved in action potential conduction – the sodium-potassium pump, the sodium channel and the potassium channel – only the sodium channel is a target of insecticides.

Pyrethroids and DDT slow the closing of sodium channels after an action potential, causing the cell to become re-excited. Indoxacarb and metaflumizone block the sodium channels, which prevents action potential generation.

A. Intracellular Action Potential States



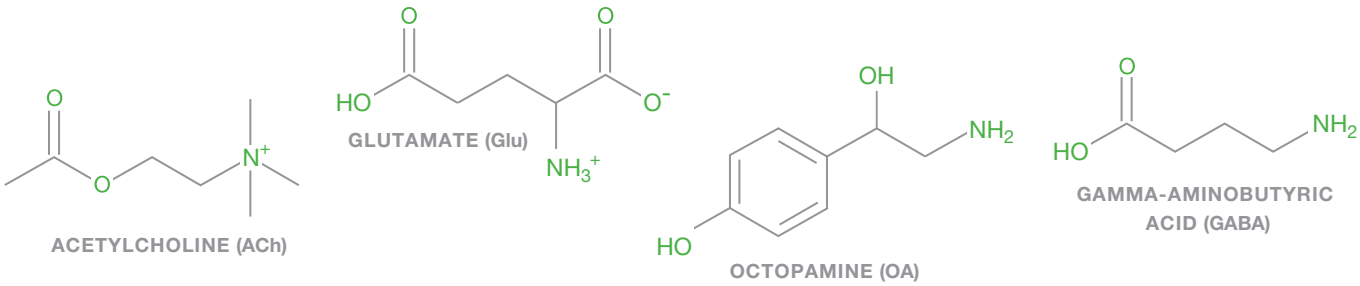
B. Intracellular Action Potential Chronology



Synaptic Transmission – Cholinergic Synapses

Although the two cells forming a synapse are separated by a gap of only 30 millionths of a millimeter, or about one thousandth the diameter of a human hair, the action potential cannot traverse it, and instead triggers the release of a chemical neurotransmitter from the presynaptic terminal to transmit the signal to the postsynaptic cell. Many different neurotransmitters are known, but only four are widely used in the insect neuromuscular system. Acetylcholine (ACh) is the major, if not the only, fast excitatory neurotransmitter in the insect central nervous system (CNS), and synapses that use it, known as cholinergic synapses, can transmit an excitatory signal from the presynaptic cell to the postsynaptic cell. The chemical structure of acetylcholine is shown below.

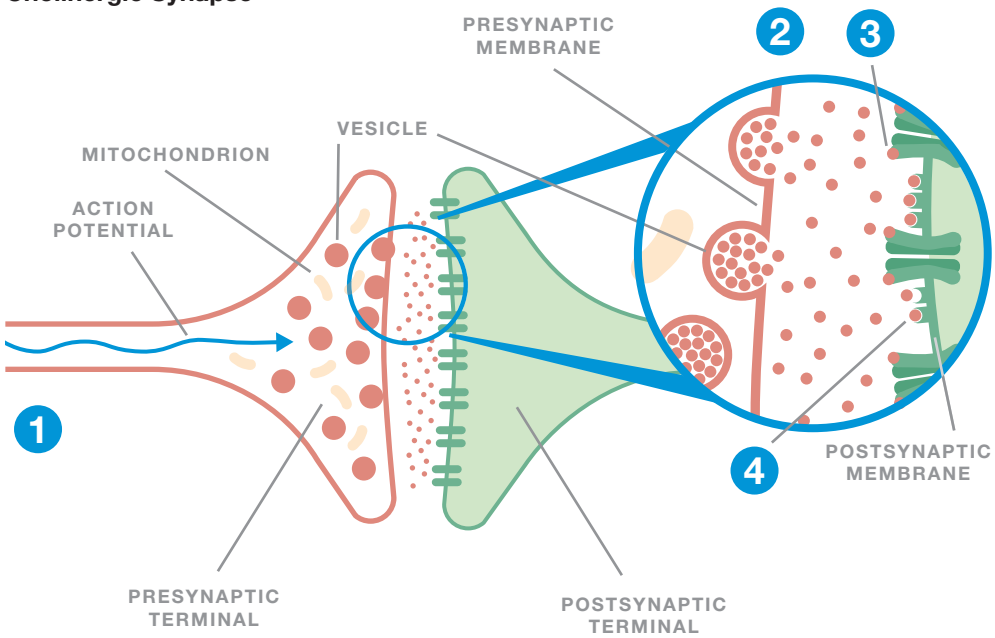
Molecular structures of the four major neurotransmitters of the insect neuromuscular system.



With at least four distinct target sites, cholinergic synapses are by far the most important in terms of insecticide action, but GABA, glutamate and octopamine synapses are also important.

Neurotransmission at a cholinergic synapse:
1) A nerve signal (action potential) flows down the nerve axon and reaches the presynaptic terminal.
2) Once excited by the nerve signal, synaptic vesicles fuse with the presynaptic membrane and liberate neurotransmitter molecules.
3) Neurotransmitter molecules bind to and activate receptors on the postsynaptic membrane and a new impulse is generated.
4) To end the signal, acetylcholinesterase enzymes degrade the neurotransmitter.

Cholinergic Synapse



The presynaptic terminal is loaded with membrane-bound vesicles packed with ACh. When an action potential arrives down the axon, the resulting depolarization triggers the fusion of some of these vesicles with the presynaptic membrane, resulting in the release of their contents into the synaptic cleft.

Once free in the cleft, the individual molecules of ACh diffuse across to the postsynaptic membrane, which is studded with ACh receptors, so called because their purpose is to receive the ACh signal transmitted by the presynaptic terminal. There are several different types of ACh receptors, but those on the postsynaptic membranes in insects are thought to be all of the nicotinic type, so called because they are sensitive to the ACh-mimicking action of nicotine. Activation of the nicotinic receptor by the binding of two ACh molecules gates a hydrophilic channel in its center that permits the passage of Na^+ ions into the postsynaptic cell, driving the intracellular potential positive. If enough receptors are activated, an action potential can be generated in the postsynaptic cell.

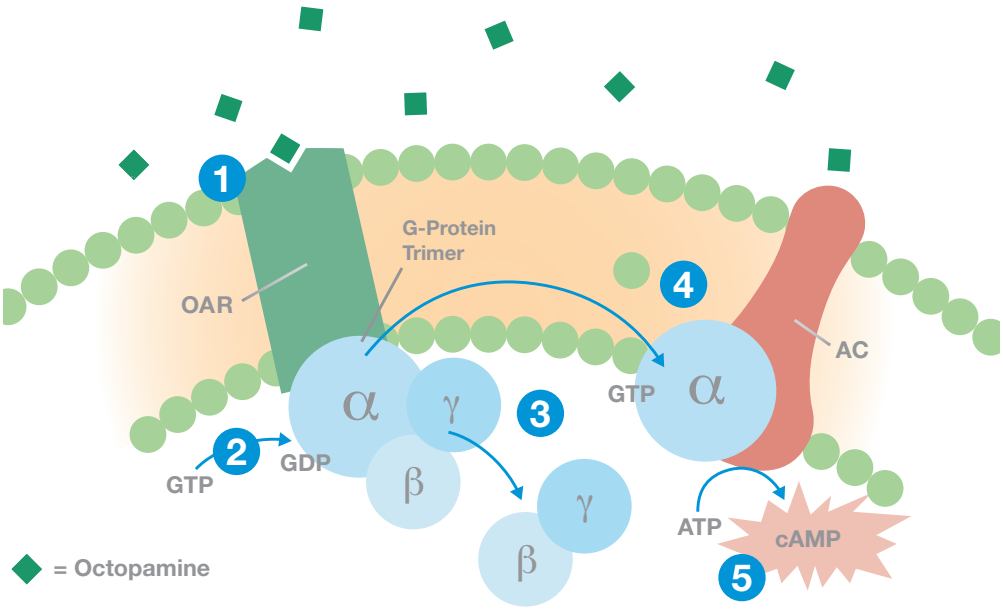
It is important that ACh be quickly removed from the synaptic cleft after signal transmission, so that the signal has a finite duration, and this is accomplished by a high concentration of the acetylcholinesterase enzyme (AChE) in the cleft. AChE cleaves ACh into its acetic acid and choline components, which no longer stimulate the receptors and are transported back into the presynaptic cell where they are recombined into ACh and repackaged into vesicles.

In general, synapses using any other neurotransmitter function by the same principles described for nicotinic synapses, but have transmitter-specific synthesis, reception, degradation and reuptake mechanisms. ACh is the only neurotransmitter that is degraded by an enzyme in the synaptic cleft; all others are taken back up intact into the presynaptic terminal by specific transporters. In addition to the nicotinic receptor, the acetylcholinesterase enzyme is also an important insecticide target site.

Excitatory, Inhibitory and Modulatory Neurotransmission

ACh is the major fast excitatory neurotransmitter in the insect CNS, but glutamate is the fast excitatory neurotransmitter that insect motor neurons release onto muscle cells to elicit contractions. Not all neurotransmission is excitatory, however. Inhibition is also extremely important in the nervous system. It is well established in insects that gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the CNS as well as at neuromuscular synapses. GABA activates receptors that gate chloride channels, and the influx of negatively charged chloride ions has an inhibitory effect on the postsynaptic cell, which counteracts the effect of excitatory input. Inhibitory glutamate-gated chloride channels (GluCls) are also widespread on insect muscle and nerve cells, and while they have not yet been demonstrated to participate in inhibitory neurotransmission, that is considered probable.

right:
Octopamine receptors: 1) octopamine binds to the receptor (OAR); 2) GTP replaces GDP; 3) G-protein dissociates into GTP-α and β γ; 4) GTP-α binds to adenylate cyclase; and 5) cAMP is produced from ATP.

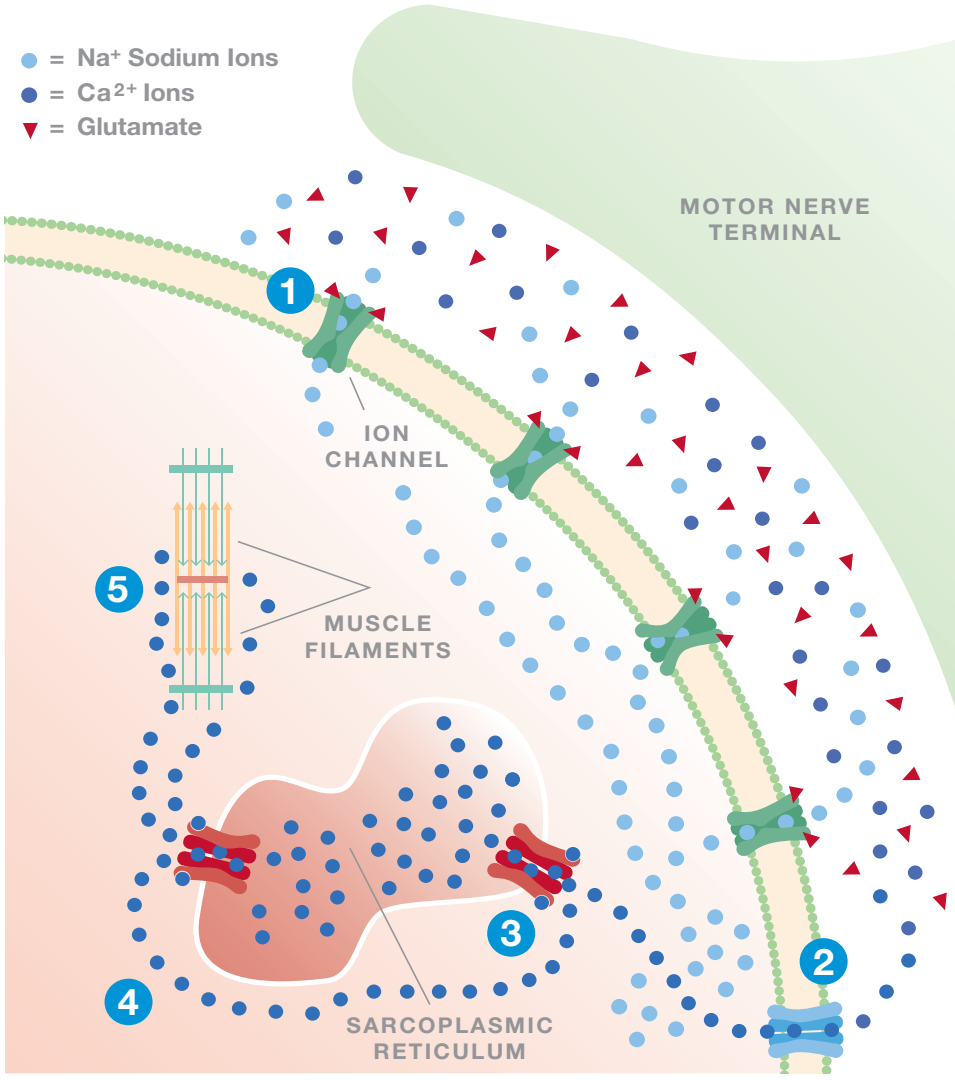


In addition to excitatory and inhibitory neurotransmitters, there are also modulatory neurotransmitters; octopamine is the major modulatory neurotransmitter in insects, playing a role similar to that of adrenaline in mammals in having a plethora of effects that increase the general level of arousal. Octopaminergic neurons release octopamine onto neurons in the CNS and onto many peripheral tissues, where, instead of opening ion channels, octopamine receptors activate biochemical signaling pathways that modulate the function of the postsynaptic cells. Octopamine receptors belong to the class of G-protein-coupled receptors, or GPCRs. While GPCRs are extremely important as the targets of 40% of all prescription pharmaceuticals, there is currently only one insecticide that acts on the octopamine receptor, which is the only known GPCR insecticide target.

Neuromuscular Transmission and Excitation-Contraction Coupling

The last process required for understanding insecticide mode of action on nerve and muscle is muscle excitation-contraction coupling. Muscle contraction is ultimately brought about by Ca^{2+}-sensitive contractile proteins in muscle cells that use energy from ATP to cause cell shortening in the presence of cytoplasmic Ca^{2+} ions. The cytoplasmic free Ca^{2+} ion concentration in resting muscle cells is maintained at a very low level by ATP-driven pumps in the plasma membrane that pump Ca^{2+} ions out of the cell, and by similar pumps in the membrane that encloses an extensive intracellular storage organelle called the sarcoplasmic reticulum (SR), where large amounts of free Ca^{2+} ions are stored. The SR membrane has a high density of calcium-activated calcium channels called ryanodine receptors (RyRs), which release SR Ca^{2+} ions into the cytoplasm when activated by cytoplasmic Ca^{2+} ions, increasing the cytoplasmic Ca^{2+} ion concentration even further. This inherent positive feedback can produce the rapid rise in cytoplasmic Ca^{2+} ion concentration needed to activate the contractile proteins in the muscle cell. Diamide insecticides chlorantraniliprole, cyantraniliprole and flubendiamide activate RyRs, inducing muscle contractions.

The mechanism of excitation-contraction coupling in muscle is shown below. The command for the muscle to contract is transmitted to the muscle cell from motor neurons. Release of the neurotransmitter glutamate onto the muscle membrane from motor nerve terminals leads to activation of excitatory glutamate receptors, which allow sodium ions to flow into the cell and cause a small depolarization. This depolarization triggers the activation of L-type voltage-dependent calcium channels in the plasma membrane, which allow enough Ca^{2+} ions into the cell to activate ryanodine receptors in the sarcoplasmic reticulum membrane, leading to the release of massive amounts of free Ca^{2+} ions into the cytoplasm from SR stores. The rise in cytoplasmic Ca^{2+} ion concentration initiates shortening of muscle contractile filaments, causing the muscle cell to contract, and also activates Ca^{2+} pump proteins that pump Ca^{2+} ions back into the SR and into the extracellular spaces, terminating the contraction and restoring the resting state. It should be noted that the excitatory glutamate receptors in the postsynaptic muscle membrane are not cys-loop receptors and are not targets of any insecticides, in contrast to the GluCls, which also occur on the muscle membrane, and are targets of avermectins and milbemycins.



left:
Ryanodine receptors in muscle excitation-contraction coupling: 1) glutamate released from the motor nerve terminal activates receptors in the cell membrane; 2) Influx of Na^{+} ions through glutamate receptors depolarizes the sarcolemma, activating L-type voltage-dependent Ca^{2+} channels; 3) Ca^{2+} ions entering through L-type Ca^{2+} channels bind to and activate ryanodine receptors (RyRs) in the SR membrane; 4) released Ca^{2+} ions activate more RyRs, amplifying Ca^{2+} release; 5) Ca^{2+} ions bind to contractile filaments in the cytoplasm, initiating shortening; the contraction is ended by proteins that pump Ca^{2+} ions from the cytoplasm back into the SR and extracellular spaces.

Neuromuscular Disruptor Insecticides

IRAC Group 1: Acetylcholinesterase (AChE) Inhibitors

Acetylcholinesterase (AChE), a critical enzyme in the function of the insect central nervous system, is the target of inhibition by organophosphate (OP) and carbamate insecticides. Many competing products in these groups, from various companies, dominated the insecticide market from the 1950s to the 1980s. Although their use has been in slow decline, due largely to regulatory action but also to resistance, OPs and carbamates still accounted for 18.1 and 8.3 %, respectively, of the global agricultural insecticide market in 2010, allowing AChE to keep its place as the most important insecticide target site. Organophosphates and carbamates are broad-spectrum insecticides with a large variety of crop and non-crop uses. The group includes several of the most important soil-applied and foliar insecticides for row crops and vegetables. Some members of these classes also control nematodes and mites.

IRAC Group 1A: Carbamates

Scottish missionaries to the kingdom of Calabar in Southern Nigeria in the 1840s regularly observed poisonings by seeds of the local plant *Physostigma venenosum*, which were fed to suspected witches as a trial by ordeal. If the accused vomited after chewing and swallowing 20 to 30 Calabar beans, he survived and was exonerated. The carbamate physostigmine (eserine), isolated from the Calabar bean in 1864, soon found use as a drug that is still in use today to treat myasthenia gravis, glaucoma, Alzheimer's disease and delayed gastric emptying.

The insecticidal activity of carbamates was first discovered in 1947 at the Geigy Company in Switzerland, but it wasn't until 1956 that carbaryl, the first successful carbamate insecticide, was introduced by Union Carbide. Since then, BASF, DuPont, FMC Corporation, Syngenta, Dow AgroSciences, Bayer and other companies have developed and commercialized their own proprietary carbamate insecticides, and this group still accounts for 8.3% of the insecticide market, although use is declining as older products are phased out.

Carbamates are mostly broad spectrum insecticides used on cotton, fruit, vegetables, row and fodder crops. Carbaryl, with a broad spectrum and low mammalian toxicity, is sold under the trade name Sevin®. Some carbamates are systemic and can be applied by soil application or seed treatment.

Examples of Carbamate Insecticides

- Common name carbaryl – trade name Sevin®
- Common name carbosulfan – trade name Marshal®
- Common name methomyl – trade name Lannate®
- Common name aldicarb – trade name Temik®

- Common name thiodicarb – trade name Larvin®
- Common name oxamyl – trade name Vydate®
- Common name triazamate – trade name Aztec®
- Common name carbofuran - trade name Furadan®

IRAC Group 1B: Organophosphates

Organophosphates were discovered in the early 1930s at the Bayer division of the German chemical conglomerate I.G. Farben, and are still one of the largest families of insecticides. Though their use is declining due to regulatory action, organophosphates are still widely used for their broad spectrum of activity, flexibility in use, and good residual characteristics.

Notable members of the group include Perfekthion® insecticide (dimethoate) and Abate® mosquito larvicide (temephos), by BASF. Perfekthion insecticide is used for the control of aphids and certain other pests in wheat, rye, triticale, sugar beet and other beet crops, seed crops and ornamental plant production. Abate mosquito larvicide, important in the war against malaria, controls mosquitoes that vector human diseases. When applied to standing water where mosquitoes breed, Abate mosquito larvicide kills the larvae, interrupting the life-cycle of the protozoal malaria parasites.

Integrated campaigns including the use of Abate mosquito larvicide are responsible for significantly reducing the occurrence of guinea worm disease (dracunculiasis) worldwide. When the World Health Organization began its effort to eradicate this parasite in 1986, there were 3.5 million new cases each year across 21 countries in Africa and Asia. From January to September 2013, there were only 129 new cases in four African countries, down 75% from the same period in 2012, and dracunculiasis is on track to be the first parasitic disease and only the second disease, after smallpox, to be completely eradicated worldwide.

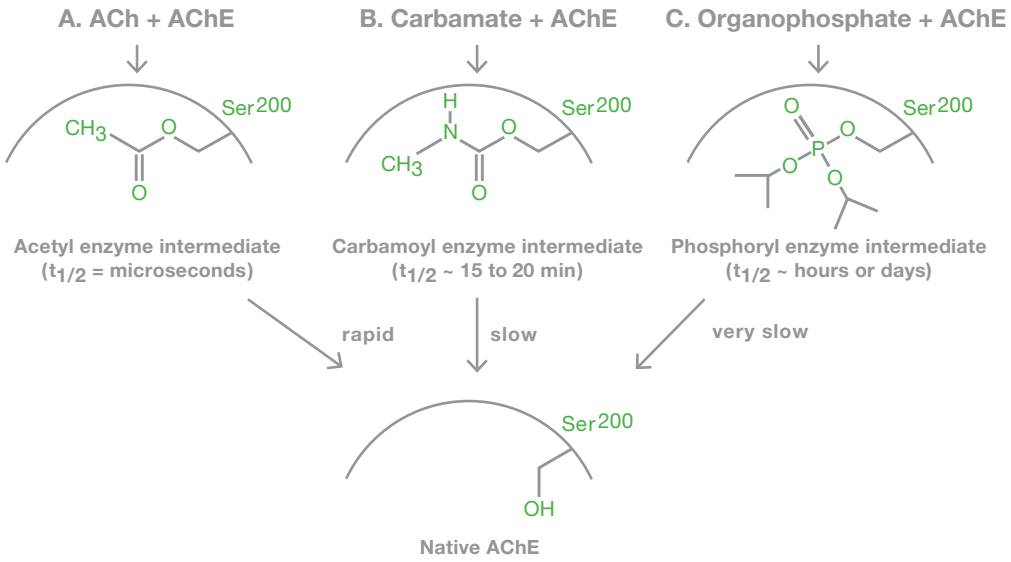
Examples of Organophosphate Insecticides

- Common name terbufos – trade name Counter®
- Common name dimethoate – trade name Perfekthion® insecticide
- Common name chlorpyrifos – trade names Lorsban®, Dursban®
- Common name methamidophos – trade names Monitor®
- Common name acephate – trade name Orthene®
- Common name profenofos – trade name Curacron®
- Common name chlorethoxyfos – trade name Fortress®
- Common name phorate – trade name Thimet®
- Common name malathion – trade name Cythion® insecticide (discontinued)
- Common name temephos – trade name Abate® mosquito larvicide

Mode of Action and Resistance: Acetylcholinesterase inhibitors bind to and inhibit the enzyme that's normally responsible for breaking down ACh after it has carried its message across the synapse. This allows the ACh to continue stimulating the postsynaptic neuron, leading to overstimulation of the nervous system and eventual death of the insect.

AChE is one of the fastest enzymes known – each molecule being able to degrade 25,000 ACh molecules per second. AChE is a type of hydrolytic enzyme known as a serine esterase, so called because of the presence of the amino acid serine (Ser^{200}) in the active site, whose hydroxyl side chain becomes esterified (ester group added to molecule) by the substrate during catalysis. The acetyl enzyme intermediate forms rapidly, and releases the acetate group with a half-life of microseconds. Carbamates and organophospates are suicide inhibitors of AChE. They enter the active site of the enzyme and react with the catalytic serine residue, but the carbamoylated and phosphorylated enzymes are much more stable than the acetylated form, so the enzyme is inhibited. Resistance to AChE inhibitors is often due to enhanced metabolism, but modified AChE also often plays a role in many cases.

Mode of Action of AChE Inhibitors



right:
Mode of Action of AChE Inhibitors:
A) Acetylation of the catalytic serine by ACh leads to an acetyl enzyme intermediate that releases the acetate group within a few micro-seconds.
B) Carbamoylation of the catalytic serine by a carbamate insecticide inhibits the enzyme for 15-20 minutes before releasing the carbamoyl group.
C) Phosphorylation of the serine residue by an OP insecticide leads to longer-lasting inhibition, on the order of hours or days.

Environmental and Toxicological Considerations: Carbamates and organophosphates have varying levels of toxicity to non-target organisms, including humans. As a group, these are among the most toxic insecticides to man. Some are toxic to birds and fish and their uses have accordingly been restricted by regulatory agencies.

Symptoms of acute poisoning by organophosphates and carbamates can develop within minutes to hours, depending on the route of exposure. Early symptoms include headache, nausea, dizziness, pupillary constriction and hypersecretion (sweating, salivation, watery eyes, and runny nose). The primary cause of death in organophosphate poisoning is respiratory failure.

Many early symptoms can be easily confused with other illnesses like heat stress, over-fatigue and lack of sleep. Although similar in the symptoms they elicit, the treatment for carbamate versus organophosphate acute overexposure can vary. Always seek medical attention if the above symptoms are exhibited after exposure, as a life-threatening condition may exist.

Carbamates and organophosphates break down readily in the environment and are not considered persistent; nor do they biomagnify (increase in animal tissues through the food chain). Some have very high water solubility and have the potential to leach into groundwater.

IRAC Group 2: GABA-Gated Chloride Channel Antagonists

IRAC Group 2A: Cyclodiene Organochlorines

Cyclodienes were introduced in the 1940s. Their stability in soil and the environment, broad spectrum of activity, high level of performance and relatively low cost gained the group extensive use globally. However, due to widespread insect resistance, persistence, long-range transport and bio-magnification in wildlife food chains, the use of cyclodienes - once a mainstay of the insecticide market – has seen a period of rapid decline. Endosulfan, the sole member of this class in wide use until recently, is banned in over 80 countries but still used extensively in India, China and a few other countries. Under a global ban that went into effect in mid-2012, cyclodienes should be phased out over five years.

Agricultural uses of lindane were banned under the Stockholm Convention in 2009, and it is now only allowed as a topical treatment for lice and scabies.

Examples of Cyclodiene Organochlorides

- Common name endosulfan – trade name Thiodan® – discontinued in many areas of the world
- Common name gamma-HCH or lindane – discontinued for crop protection in many areas of the world

IRAC Group 2B: Phenylpyrazoles

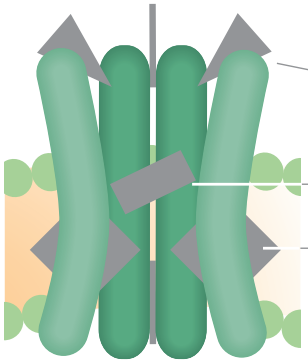
Phenylpyrazoles are a family of GABA-gated chloride channel antagonists discovered in 1987 by Rhone-Poulenc. Fipronil was the first phenylpyrazole, registered as Regent® insecticide by Rhone-Poulenc in 1993 and acquired by BASF in 2003.

Fipronil has proven to offer low dose, highly effective insect control against a broad range of pests. Currently, fipronil is registered in more than 70 countries for the control of pests on more than 100 crops. Key agricultural uses include sugar cane, rice and maize. Highly effective in non-agricultural areas, fipronil has become the world's leading termiticide and is a key component in urban pest control programs against cockroaches and ants.

Examples of Phenylpyrazole Insecticides

- Common name fipronil – trade names (crop) Regent® insecticide, Standak® seed treatment, Cosmos® insecticide – trade names (non-crop) Termidor® termiticide/insecticide, Impede® turf and ornamental insecticide
- Common name ethiprole – trade names Curbix®, Kirappu®

Mode of Action and Resistance: As mentioned in the section "Excitatory, Inhibitory and Modulatory Neurotransmission," gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter used to transmit signals that inhibit the activity of postsynaptic cells. A certain amount of inhibition in the nervous system is essential for normal function, and blocking of the GABA-gated chloride channels by cyclodienes and phenylpyrazoles leads to overstimulation and convulsions.



OPEN ION CHANNEL CROSS-SECTION

Blockers interfere with inhibitory neurotransmission by occluding the chloride channel pore. In addition to GABA receptors, insects and some other invertebrates also have glutamate-gated chloride channels that may play a role in inhibitory neurotransmission. Unlike other GABA-gated chloride channel antagonists, which are specific for GABA-gated chloride channels, fipronil also potently blocks two types of glutamate-gated chloride channels. This unique multi-target mode of action may reduce the potential for resistance development.

Resistance to chloride channel antagonists is often target-based. An alanine residue located near the intracellular mouth of the pore in transmembrane segment two is important for the binding of all GABA-gated chloride channel antagonists. Mutation of this residue to serine or glycine is associated with high levels of resistance to cyclodienes in many insects, but only marginally affects the effectiveness of fipronil. However, mutation of this residue to asparagine in some insects has been found to confer high levels of resistance to fipronil.

Environmental and Toxicological Considerations: Fipronil has low toxicity to mammals and waterfowl, but is highly toxic to fish and songbirds. It is also highly toxic to some aquatic invertebrates and honeybees. Low application rates combined with formulation innovations and improved application methods are intended to reduce the risks associated with this compound's use. In addition, a product stewardship program has been implemented to reinforce proper use and monitor mis-use.

IRAC Group 3: Sodium Channel Modulators

IRAC Group 3A: Pyrethroids

Pyrethrum is the powdered dried flower head of the pyrethrum daisy, a species of chrysanthemum that has been used as an insecticide since the 1st century CE in China and still enjoys worldwide sales of Euro 350 million. Pyrethrins are the insecticidal compounds that occur naturally in this material. Synthetic analogs of pyrethrins, called pyrethroids, were pioneered by chemist Michael Elliot at Rothamsted Experimental Station in the United Kingdom, culminating in the discovery of deltamethrin and cypermethrin in 1977.

Pyrethroids have been specifically designed to be more environmentally stable than Pyrethrins, whose activity is measured in hours. They provide long-lasting control and improved mammalian safety relative to other products in use at the time they were developed. These compounds are generally effective against caterpillars, beetles, certain aphids and mites in crops, and are used for mosquito, termite and cockroach control in non-crop segments. In addition, certain members of the class are used to control ecto-parasites on pets and humans. Key applications include foliar sprays in vegetable crops, oilseed rape and cotton, as well as soil and foliar uses in maize.

Alpha-cypermethrin, a third generation pyrethroid, was introduced to the market in 1983, and is now one of the top-selling insecticides globally. Alpha-cypermethrin formulations developed by BASF are registered in 40 countries around the world and used on more than 90 crops, controlling a broad spectrum of crop pests. BASF's alpha-cypermethrin formulations incorporated into Interceptor® mosquito long-lasting insecticidal nets also play a key role in preventative applications against vectors of tropical diseases like malaria and dengue fever.

Examples of Pyrethroid and Pyrethrin Insecticides

- Common name alpha-cypermethrin – trade names Fastac® insecticide, Fendona® insecticide, Mageos® insecticide, Interceptor®, Carifend®
- Common name esfenvalerate – trade name Asana XL®
- Common name cyfluthrin - trade name Baythroid®
- Common name permethrin – trade names Ambush®, Pounce®
- Common name bifenthrin – trade names Capture®, Brigade®
- Common name deltamethrin – trade name Decis®
- Common name tefluthrin – trade name Force®
- Common name zeta-cypermethrin – trade names Fury®, Mustang Max®
- Common name lambda-cyhalothrin – trade names Karate®, Warrior®
- Common name fluvalinate – trade name Mavrik®
- Common name etofenprox – trade name Trebon®

IRAC Group 3B: DDT, Methoxychlor

First synthesized by an Austrian student in 1873, DDT, one of the best known insecticides, was rediscovered as a toxicant in 1940 by chemist Paul Mueller at the Geigy Chemical Company in Basel. Introduced in 1942, DDT was the most widely used insecticide for 20 years. Its enormous value in combating malaria and typhus in World War II and thereafter, earned Mueller the 1948 Nobel Prize for Physiology or Medicine.

DDT was used on a large scale as a crop insecticide because of its low mammalian toxicity. It proved to be highly persistent, however, and more than one billion pounds accumulated in the environment by 1968, disrupting ecological food chains. Environmental concerns led the EPA to ban DDT in 1972, and have virtually eliminated all uses globally, although it has recently been reintroduced in Africa for the control of mosquito disease vectors by interior wall and ceiling treatments in dwellings. Another member of this group, methoxychlor, was introduced as a replacement for DDT, but has been banned in the United States and Europe since 2003 due to its potential for bioaccumulation and endocrine disruption.

Examples of DDT and Methoxychlor

- Common name DDT – no registered trade names
- Common name methoxychlor – no registered trade names

Mode of Action and Resistance: Sodium channel modulators are neurotoxins that act on the action potential sodium channel. They slow the closing and inactivation of the channel, causing it to remain open longer than normal, which has the effect of prolonging the action potential, as shown by the dashed trace in the lower part of the figure on page 11. When the action potential is not promptly terminated, it can re-excite the same area of membrane, leading to repetitive firing.

Because nerve axons occur throughout the insect's body, even near the surface of the cuticle in sensory organs and motor nerve terminals, pyrethroids and DDT cause symptoms as soon as they enter the body and are considered extremely fast-acting, causing immediate "knockdown".

Pyrethroid and DDT resistance is widespread and can be metabolic or target-site-based. A number of cytochrome P450s that are overexpressed in pyrethroid-resistant insects have been identified. Metabolic resistance confers resistance to certain pyrethroids, whereas target-based resistance extends to all pyrethroids and DDT, and is known as knockdown resistance *(kdr)*. In contrast to the cys-loop ligand-gated ion channels, in which the channel is formed from five similar or identical subunits, the voltage-dependent sodium channel is formed from four similar domains of a single polypeptide chain. These domains act like pseudosubunits, each forming a stave of the channel barrel. Interestingly, mutations in at least three of the domains have been found to confer *kdr* resistance, but domain II is

the most common location. Five different amino acid residues in domain II are mutated in various *kdr* insects, defining a binding pocket for pyrethroid and DDT molecules. There may also be corresponding binding pockets in other domains.

Environmental and Toxicological Considerations: Pyrethroids have low mammalian toxicity, and although highly toxic to fish and aquatic organisms, they have a low bioaccumulation potential. Skin sensitization affects some people using both pyrethrins and synthetic pyrethroids. The sensitization is caused by the modulatory effect on sodium channels of sensory nerve endings in the skin. It affects sensitive areas of the body, such as the face, causing a tingling, burning or numbing feeling that usually lasts less than 24 hours. Applicators who have an allergic reaction to these insecticides must either increase the amount of personal protective equipment worn during handling, or stop working with this class of insecticides. Pyrethroid exposure can also cause nausea and paralysis. There is no antidote to acute pyrethroid poisoning, so symptoms are treated individually as they occur.

Pyrethroids and DDT are unusual among biologically active compounds in having a strong negative temperature dependence of activity at their target site, which increases their activity at low temperature. This contributes to their high mammalian safety.

In the environment, synthetic pyrethroids are rapidly degraded in soil and plants. The major degradation mechanisms are catalyzed by UV light, water and oxygen. Pyrethroids do not biomagnify – they have low water solubility and are strongly adsorbed to soil particles, which results in low soil mobility and minimizes the potential for leaching. (Note: adsorption is the adhesion of molecules to a surface, unlike absorption, which happens when a fluid permeates or is dissolved by a liquid or solid).

DDT is extremely persistent in the environment and can accumulate through the food chain. While the toxicity of DDT to non-target invertebrates probably stems from sodium channel effects, effects on vertebrates may be due to endocrine disruption. DDE, a metabolite of DDT is notorious for causing eggshell thinning in birds that led to severe declines in bird populations in the 50s and 60s, especially of large predatory species such as the bald eagle and California condor. Like DDT, methoxychlor is also an endocrine disruptor.

IRAC Group 4: Nicotinic Acetylcholine Receptor (nAChR) Agonists

IRAC Group 4A: Neonicotinoids

Neonicotinoids provide excellent acute and residual control of sucking insects, including aphids, leafhoppers, planthoppers and whiteflies, as well as certain chewing insects including Colorado potato beetle, rice water weevil and codling moth. In addition, two neonicotinoids, thiacloprid and acetamiprid, have proven to be effective in the control of many Lepidoptera pests. Imidacloprid, commercialized in 1991, is the most widely used crop insecticide worldwide and is also registered for many non-crop uses, particularly as

a spot-on flea treatment, turf treatment for white grubs and as a termiticide. The extremely potent aphid antifeedant effect of the group reduces the insect-vectored transmission of certain viruses. + neonicotinoid insecticides have been commercialized.

Possessing high water solubility and robust plant systemicity, neonicotinoids can be applied by foliar spray, soil treatment, seed treatment, trunk injection or painting onto plant tissue. The application rates for neonicotinoids are low compared to most insecticide groups.

Examples of Neonicotinoids

- Common name imidacloprid – trade names Admire®, Gaucho®, Merit®, Provado®
- Common name acetamiprid – trade names Assail®, Intruder®
- Common name clothianidin – trade name Poncho®
- Common name dinotefuran – trade names Safari®, Starkle™, Venom®
- Common name nitenpyram – trade name Bestguard®
- Common name thiacloprid – trade name Calypso®
- Common name thiamethoxam – trade names Cruiser®, Actara®

IRAC Group 4B: Nicotine

Nicotine is a natural insecticide, made by plants (e.g., tobacco) for defense against insects. Nicotine-based insecticides have been banned by the EPA since 2001 because of their high acute toxicity.

IRAC Group 4C: Sulfoxaflor

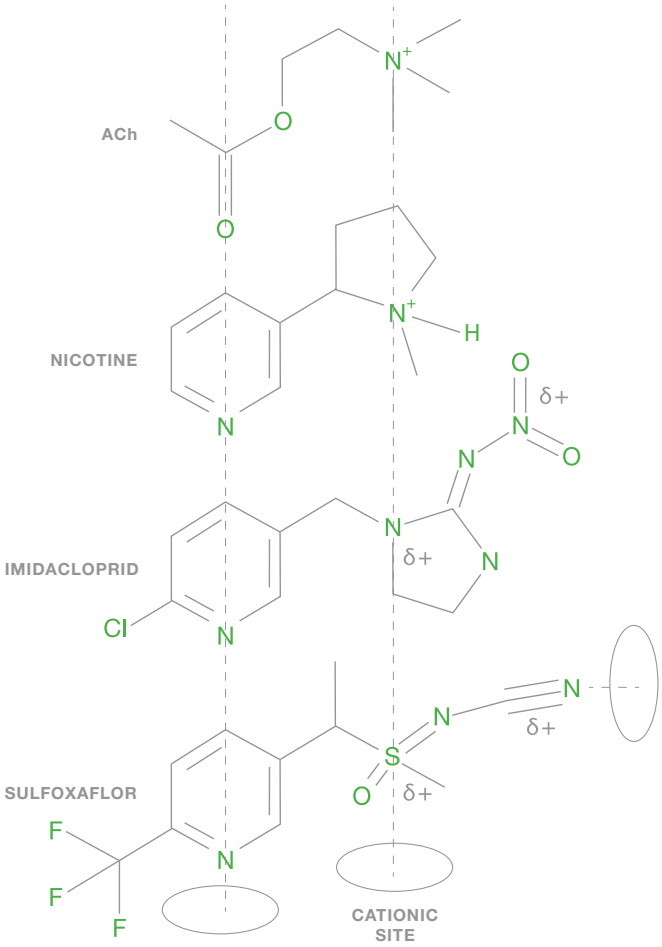
Sulfoxaflor, introduced by Dow AgroSciences in 2011, is a novel sulfoximine nAChR agonist offering excellent broad spectrum activity against key sap-feeding pests, excellent residual activity and control of many imidacloprid-resistant insects.

Example of Sulfoxaflor

- Common name sulfoxaflor – trade names Transform®, Closer®

Mode of Action and Resistance: Group 4 insecticides act as agonists of acetylcholine receptors, meaning that they mimic the action of the neurotransmitter acetylcholine (ACh). The similarity between these chemicals is shown by the alignment of their structures in the figure on page 25 with key areas on the receptor that interact with certain parts of the molecules (ellipses). The cationic site is the best understood, containing a tryptophan residue that attracts the charged nitrogen atom in ACh and nicotine. The corresponding nitrogen atom in imidacloprid and the sulfur atom of sulfoxaflor carry a partial positive charge that also binds to this tryptophan residue in the insect nAChR but does not bind very well to mammalian nAChRs. The insect receptors also contain groups shown by the ellipse on the right, that bind the cyano group of sulfoxaflor and the nitro group of imidacloprid, contributing further to the insect selectivity of these molecules. A third area on the receptor, shown by the ellipse on the lower left, binds the carbonyl group of ACh and the pyridine group of the other molecules.

The popular view is that neonicotinoids and indeed nicotine itself, continually stimulate the receptors and in so doing cause nerve overstimulation, but it is becoming clear that the action is more complicated. Most receptors do not remain activated indefinitely when an agonist is bound, but instead become desensitized. Desensitization involves a conformational transition of the nicotinic acetylcholine receptor (nAChR)-insecticide complex to a very stable desensitized state that binds the insecticide 500-fold more strongly than the activated state. Desensitized nAChR-neonicotinoid complexes no longer conduct ions, and are essentially inhibited. While most receptors undergo desensitization, some insects have been found to also possess non-desensitizing receptors, which, lacking a high-affinity desensitized state, are relatively insensitive to neonicotinoids.



Several of the prime insect pests targeted by group 4 insecticides have shown a high potential for resistance development, primarily due to enhanced metabolic degradation. The first case of field-evolved target site resistance has recently arisen in populations of the green peach aphid, *Myzus persicae,* in Spain and France, resulting from a point mutation in the ACh binding site of one of its six nAChR subunit genes. This same resistance mutation has also been found in Asian cotton aphid populations, and confers high levels of resistance to sulfoxaflor as well as to neonicotinoids.

Environmental and Toxicological Considerations: In contrast to nicotine itself, neonicotinoids and sulfoxaflor are highly selective for insect over mammalian nicotinic acetylcholine receptors (nAChRs), giving these classes of insecticides low mammalian toxicity and excellent environmental profile. Nevertheless, these insecticides are highly toxic to bees. Labels for this class of insecticides therefore prohibit application to flowering plants likely to be visited by honeybees. In contrast to neonicotinoids and sulfoxaflor, nicotine is highly toxic to humans. Signs and symptoms of nicotine over-exposure in humans includes nausea, vomiting, headache and diarrhea, and in the most serious cases, respiratory failure and reduced consciousness.

Moderately persistent in the environment, neonicotinoids do not biomagnify (biological magnification). They are highly water soluble, leading to label restrictions of some uses in areas prone to leaching into groundwater. In contrast, sulfoxaflor is rapidly degraded in soil and water.

IRAC Group 5: Nicotinic Acetylcholine Receptor (nAChR) Allosteric Modulators – Spinosyns

Spinosyns are microbial insecticides with a unique mode of action, that control some of the most stubborn vegetable pests, including the Colorado potato beetle, diamondback moth, European maize borer, hornworms, thrips and leafminers. Spinosyns are unique tetracyclic macrolides derived from a species of actinobacteria (gram-positive bacteria) discovered in soil.

The microbially produced natural product, spinosad, so named because it consists primarily of the natural spinosyns A and D in a ratio of 85 to 15, respectively, was commercialized by Dow AgroSciences in 1995 as a family of products, including Tracer®, Success™ and Spintor®, under the family trade name Naturalyte®. Its main usage is on fruit, vegetable, cotton, maize and vine pests, and because it is a natural product, it is approved for organic farming. In 2007, Dow introduced spinetoram, a semisynthetic spinosyn derivative with a broader spectrum of pest coverage and higher potency than spinosad.

Examples of Spinosyns

- Common name spinosad – trade names Spintor®, Success™, Tracer®
- Common name spinetoram – trade names Delegate®, Radiant®

Mode of Action and Resistance: While neonicotinoids selectively desensitize those nAChRs that have the capacity to undergo desensitization, which are known as nAChD receptors, spinosyns are highly selective allosteric modulators acting at the macrocyclic lactone site (page 7) of non-desensitizing nAChRs, known as nAChN receptors, which are found in certain insects. Thus, not only do spinosyns and neonicotinoids act at distinct target proteins, but they act at different types of sites on these proteins: while neonicotinoids mimic the action of ACh at its binding site, spinosyns bind to a site that modulates the receptor remotely (or allosterically) with respect to the ACh binding site. Nicotinic acetylcholine receptors are kept activated by spinosad, leading to nervous system hyperexcitation and contractive paralysis.

Because of the unique mode of action, most resistant insects are not cross-resistant to spinosyns. Resistance due to enhanced metabolic degradation is known, but most cases of resistance are target site-based, associated with loss-of-function mutations in the α6 nicotinic receptor subunit. While such mutations can occur somewhat frequently, they also appear to be accompanied by a fitness cost so that this type of target site resistance to spinosyns is effectively managed by IRAC-recommended rotation schedules.

A point mutation in the macrocyclic lactone binding site (page 7) of the α6 nAChR subunit of Western flower thrips has recently helped localize the action of spinosyns to this site.

Environmental and Toxicological Considerations: This group is considered integrated pest management (IPM) friendly, with no known significant adverse effects on beneficial arthropods such as ladybird beetles, lacewings and spiders. Although there is high intrinsic toxicity when applied to or ingested by worker honeybees, there is low acute toxicity after residues have dried on plant foliage. Extremely high doses of spinosyns are required to elicit a response in mammals and non-target organisms. Spinosyn labels carry the lowest human hazard rating assigned by the EPA.

Spinosyns are non-volatile materials that bind moderately to strongly to soils. On plant surfaces, spinosyns readily degrade and have a half-life of a few days. Spinosyns are stable in water, but their use in crops poses minimal risk to aquatic organisms when label directions are followed. Always refer to and follow local label guidelines.

IRAC Group 6: Chloride Channel Activators – Avermectins and Milbemycins

Avermectins and milbemycins are closely related, naturally occurring macrocyclic lactones generated by soil-dwelling actinobacteria. All are insecticidal, acaricidal and nematicidal, but different products within this group have various advantages in terms of spectrum and animal safety.

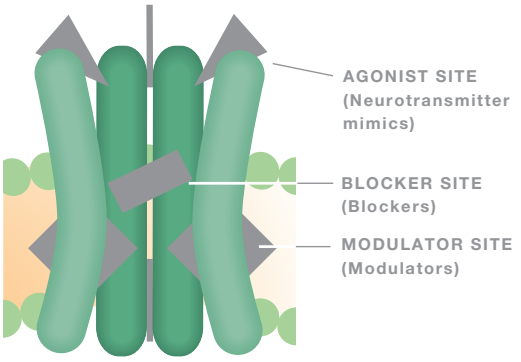
Abamectin, the leading product in this group, was introduced in 1985. Derived from the fermentation of *Streptomyces avermitilis*, abamectin controls pests like leaf miners and mites and is used mainly in vegetable and fruit crops. Major markets include the U.S., Brazil, Mexico, Italy, Egypt, France, Spain, Indonesia and Argentina. Emamectin benzoate was introduced in 1998 for use on vegetable crops and was later expanded to cotton for bollworm control. The main markets are Japan, Australia, South Korea, USA, Taiwan and Mexico. Milbemectin, a mixture of natural endotoxins, acts as a contact miticide on all mite life stages and significantly reduces the fecundity of adult females.

Examples of Chloride Channel Activators

- Common name abamectin – trade names Agri-Mek®, Zephyr®
- Common name emamectin benzoate – trade name Proclaim®
- Common name milbemectin – trade names Milbeknock™, Ultiflora™

Mode of Action and Resistance: Avermectins and milbemycins activate glutamate-gated chloride channels (GluCls), which are widespread on insect muscle and nerve cells. They are positive allosteric modulators acting at the macrocyclic lactone binding site on these channels. While GluCls have not yet been shown to participate in inhibitory neurotransmission, that is considered likely. Activation of GluCls is inhibitory, leading to flaccid paralysis.

Multiple mechanisms of abamectin resistance have been described in the twospotted spider mite, *Tetranychus urticae,* including enhanced metabolism by oxidases, glutathione S-transferases and esterases, as well as target site resistance. Target site resistance has been shown to be due to a mutation of glycine to glutamate at position 323 in GluCl of *Tetranychus urticae*, which corresponds to known ivermectin resistance mutations in nematode parasites, and is in the modulatory macrocyclic lactone binding site (see figure below left).



OPEN ION CHANNEL CROSS-SECTION

Environmental and Toxicological Considerations: Avermectins and milbemycins are potentially highly toxic to fish, mammals and aquatic organisms, but due to low dermal penetration and specifically formulated low assay products, workers can apply these products safely when label directions are followed. High toxicity is also exhibited when bees are exposed to direct treatment or to residues. These products should not be applied or allowed to drift onto blooming plants or other non-target areas.

These compounds are rapidly absorbed by plants. Remaining surface residues are rapidly degraded by UV light, which reduces their bioavailability to bees. There is no bioaccumulation potential, since chloride channel activators bind tightly to the soil and do not leach.

IRAC Group 9 : Selective Homopteran Feeding Blockers

Selective homopteran feeding blockers control homopterous insects, a group that includes aphids, cicadas, whiteflies and leafhoppers. These insecticides are used on a variety of crops, including vegetables, potatoes, rice, stone fruits and ornamentals, causing rapid cessation of feeding in homopterans and some other insects. Considered a major rotation partner for neonicotinoids, selective homopteran feeding blockers work through direct contact with the pest, systemically within the plant, or locally systemic: penetrating leaf tissues and forming a reservoir of active ingredient within the leaf. Because they are considered of low toxicity to beneficial insects, selective homopteran feeding blockers fit well into integrated pest management systems.

IRAC Group 9B: Pymetrozine

Pymetrozine was introduced in 1999 by Novartis (now Syngenta).

Example of Pymetrozine

- Common name pymetrozine – trade names Chess®, Plenum®, Fulfill®

IRAC Group 9C: Flonicamid

Flonicamid was developed and introduced on a global basis in the late 1990s by ISK Biosciences Corporation and its parent company, Ishihara Sangyo Kaisha but is distributed in the Americas and Europe by FMC.

Example of Flonicamid

- Common name flonicamid – trade names Aria®, Beleaf®, Carbine®, Teppeki®

Mode of Action and Resistance: The antifeedant effect of Group 9 insecticides is due to action of the compounds on chordotonal organs, proprioceptive sensory organs present throughout the insect's body that are important in hearing, gravity perception and fine motor coordination. The molecular target has not yet been identified.

Although their modes of action are different, neonicotinoid-resistant insects are often cross-resistant to pymetrozine, apparently due to cytochrome P450-dependent monooxygenases that can metabolize both types of compounds. Resistance to flonicamid has not been reported, and there is also no known target site resistance to Group 9 insecticides.

Environmental and Toxicological Considerations: Selective feeding blockers have a very favorable toxicity profile and are generally considered of low toxicity to bees and other beneficial insects.

IRAC Group 14: Nicotinic Acetylcholine Receptor Channel Blockers

These compounds are known as thiocarbamate insecticides or nereistoxin analogs, because they are all pro-insecticides that are bioactivated to the same active form – nereistoxin. Cartap is used for controlling microlepidoptera or micromoths, such as the diamondback moth *(Plutella xylostella)*, and the leaf-mining moth *(Tuta absoluta).*

The diamondback moth is one of the most important pests of cruciferous crops in the world and will usually only feed on plants that produce glucosinolates, a class of organic compounds that contain sulfur and nitrogen and are derived from glucose and an amino acid. It has a short life cycle (14 days), is highly fertile and capable of migrating long distances. The tomato leafminer (*Tuta absoluta)* is a serious pest of tomato crops in Europe and South America. It also attacks plants from the nightshade family, including potato, eggplant and tobacco. In favorable conditions, eight to ten generations can occur in a single year.

Examples of Nicotinic Acetylcholine Receptor Channel Blockers

- Common name bensultap – trade name Bancol®
- Common name cartap – trade name Padan®
- Common name thiocyclam – trade name Evisect®
- Common name thiosultap

Mode of Action and Resistance: Thiocarbamate insecticides are metabolically bioactivated to nereistoxin, which is a blocker of nAChR-gated ion channels. This action is clearly different from neonicotinoids. Resistance to cartap has been observed in both the Diamondback moth *(Plutella xylostella)* and *Tuta absoluta*. In the latter, resistance appears to be due to enhanced degradation by cytochrome P450-dependent monooxygenases.

Environmental and Toxicological Considerations: Thiocarbamate insecticides are slightly toxic to mammals, fish and birds, and slightly to moderately toxic to bees.

IRAC Group 19: Octopamine Receptor Agonists

Amitraz is an insecticide/acaricide introduced in 1975 that is primarily used to control ticks, lice and mange mites on cattle, swine and dogs. It is also used to control psyllids, whiteflies and mites on certain crops.

Example of Octopamine Receptor Agonists

- Common name amitraz – trade names Mitaban®, Preventic®

Mode of Action and Resistance: Octopamine receptor agonists mimic the action of the neurotransmitter octopamine in insects. Octopamine is the “insect adrenaline”, modulating the function of the central nervous system and enhancing the level of excitability of many tissues in the body. Activation of octopamine receptors is coupled by a GTP-binding protein to the production of the intracellular messenger cyclic-AMP (page 14), elevation of which triggers many excitatory effects related to fight-or-flight, and too much of which results in tremors and convulsions, as well as suppression of feeding and reproduction at lower doses. Amitraz is the only octopamine receptor agonist in current use.

Resistance to Amitraz appears to be mainly metabolic. No target site resistance is known.

Environmental and Toxicological Considerations: Amitraz has low toxicity to mammals, but is suspected to be a carcinogen. It is practically non-toxic to bees, but may adversely affect avian reproduction. It is highly toxic to many aquatic vertebrates and invertebrates, but poses a low risk because of its rapid environmental dissipation.

IRAC Group 22: Voltage Dependent Sodium Channel Blockers

IRAC Group 22A: Indoxacarb

Introduced by DuPont in 2000, indoxacarb is a prodrug (precursor of a drug), which is bioactivated by amidases and esterases in insects but catabolized (broken down metabolically) in mammals. Indoxacarb controls most Lepidoptera, as well as certain members of other insect orders, including Coleoptera, Hemiptera, Diptera, Orthoptera and Hymenoptera.

Example of Indoxacarb

- Common name indoxacarb – trade names Avaunt®, Provaunt®, Steward®

IRAC Group 22B: Metaflumizone

Metaflumizone, a new class of chemistry co-developed by BASF and Nihon Nohyaku Co., does not require bioactivation. It controls most Lepidoptera, as well as certain members of other insect orders, including Coleoptera, Hemiptera, Diptera, Orthoptera and Hymenoptera. Metaflumizone effectively controls pests in a variety of crop markets, including cotton, potatoes, fruits and leafy vegetables. It is also used for the control of ants and other nuisance pests.

Example of Metaflumizone

- Common name metaflumizone – trade names Alverde® insecticide, Siesta™ fire ant bait

Mode of Action and Resistance: Sodium channel blocking insecticides bind in and obstruct the sodium channel pore, blocking nerve action potentials and paralyzing insects. Paralysis by sodium channel blockers has been called relaxed paralysis, to distinguish it from the tetanic paralysis caused by many other insecticides.

Many cases of resistance to indoxacarb have been reported, all of which appear to be due to enhanced metabolism. So far, resistance to metaflumizone has not been reported, and metaflumizone controls most indoxacarb-resistant insects.

Environmental and Toxicological Considerations: Indoxacarb was classified by the EPA as a reduced-risk pesticide. It is practically non-toxic to bees and poses little risk to humans and other non-target organisms when used according to the label. Metaflumizone is generally considered non-toxic to mammals and birds, and slightly toxic to fish and bees. It has low impact on key beneficial insects. Although highly toxic to some aquatic invertebrates, it is strongly adsorbed to soil, is rapidly degraded in the environment, and is not expected to leach.

IRAC Group 28: Ryanodine Receptor Modulators - Diamides

The insecticides in IRAC Group 28 represent a relatively new mode of action. Flubendiamide was co-developed by Nihon Nohyaku Company and Bayer CropScience and introduced in 2007, while chlorantraniliprole was launched by DuPont in 2008. Both products have broad-spectrum larvidical activity on Lepidoptera pests and can be used on a wide range of crops. They provide effective control of pest populations resistant to other insecticidal products. Cyantraniliprole was introduced by DuPont in 2012 as a second generation ryanodine receptor modulator offering cross-spectrum foliar and systemic activity against chewing and sucking insects.

Examples of Diamide Insecticides

- Common name flubendiamide – trade names Belt®, Phoenix®
- Common name chlorantraniliprole – trade names Altacor®, Coragen®
- Common name cyantraniliprole – trade names Exirel®, Benevia®

Mode of Action and Resistance: IRAC group 28 insecticides activate ryanodine receptors, which are calcium-activated calcium channels in the sarcoplasmic reticulum of muscle cells whose function is to amplify a small trigger calcium signal to produce the massive calcium release from intracellular stores that is needed for muscle contraction (page 14-15). Ryanodine receptors are also found in neurons of the central nervous system where it may be involved in Ca^{2+}-signaling. Direct activation of ryanodine receptors by these insecticides causes sustained muscle contractions leading to rapid feeding cessation, regurgitation, lethargy and tetany (sustained contraction of muscles).

Resistance to diamides appeared soon after commercialization in diamondback moth *(Plutella xylostella)* populations in several Asian countries, due to a point mutation in the proposed diamide binding site of the ryanodine receptor. Diamide resistance has also been reported in *Liriomyza* in Florida, and both the Beet armyworm *(Spodoptera exigua)* and rice borer *(Scirophaga)* in Indonesia.

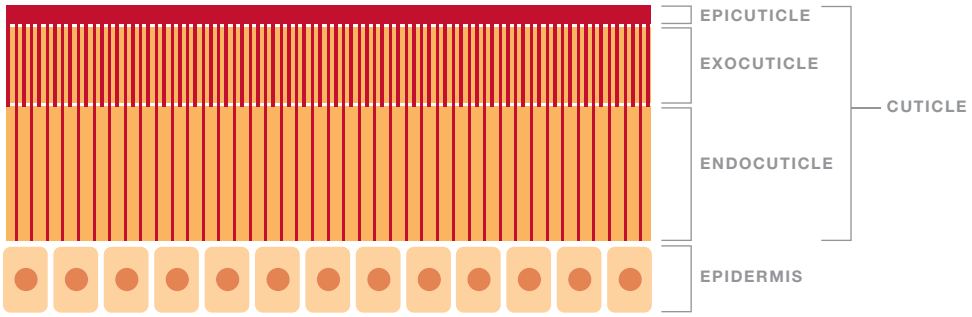
Environmental and Toxicological Considerations: Chlorantraniliprole is practically non-toxic to terrestrial and aquatic vertebrates. Flubendiamide has been shown to have low toxicity to mammals, but poses no risk when used according to label directions. Diamides have exhibited low toxicity to most beneficial insects, including bees. Cyantraniliprole has high acute toxicity to bees if applied during flight, but the dried residue has minimum impact on bees. While there is toxicity to some aquatic invertebrates, the potential for bio-accumulation is minimal when used according to label directions.

Insect Growth and Development

The Insect Integument

The insect's integument, or skin, is also its exoskeleton, serving as the attachment point for muscles, as well as a protective shield for internal organs.

The insect integument consists of a single layer of epidermal cells attached to a basement membrane, or thin layer of tissue, from which arises the cuticle. The cuticle consists of an outer thin, waxy, and water-resistant epicuticle, which is important for water retention, and the much thicker inner procuticle, which consists of two layers built from interwoven fibers of protein and the long-chain polysaccharide chitin, a polymer of N-acetylglucosamine. The procuticle's inner endocuticle layer is tough but flexible, and is predominant in caterpillars, while its outer exocuticular layer is often made harder by cross-linking of the fibers and the addition of minerals, as in beetles.

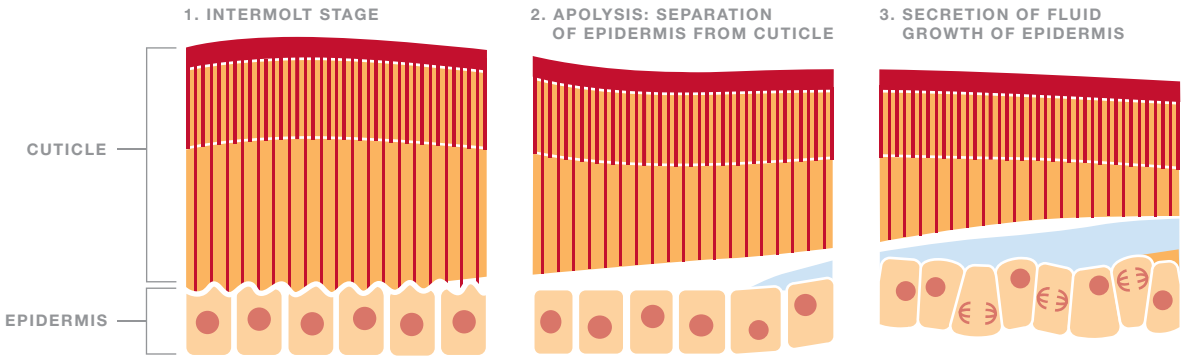


left:
Diagram of the multi layered structure of the insect cuticle. The shaded areas represent the single cuticle layers: non-chitinous epicuticle; procuticle consisting of exocuticle and endocuticle, with chitin fibers oriented parallel to the surface. An epidermal cell layer underlies the endocuticle.

Insect growth progresses through stages: eggs hatch into an immature stage, and the insect may pass through several more immature stages before emerging as an adult. As the exoskeleton cannot expand, it must be shed and replaced with a larger one at each molt to the next stage.

Insect Molting Process

Molting, also known as ecdysis, can be likened to putting on an overcoat under a sweater and then removing the sweater. The epidermis first separates from the cuticle and then expands by cell division as it secretes an inactive digestive fluid into the void. The epidermis then secretes a new cuticle and activates enzymes in the digestive fluid so the old cuticle can be partially digested and re-absorbed through pores in the new cuticle. Finally, the insect breaks open the old cuticle along pre-formed fault lines and wriggles out. The insect may swallow air to expand the new cuticle before it hardens, which can take a few hours, and it sometimes eats the old cuticle to recover the nutrients.

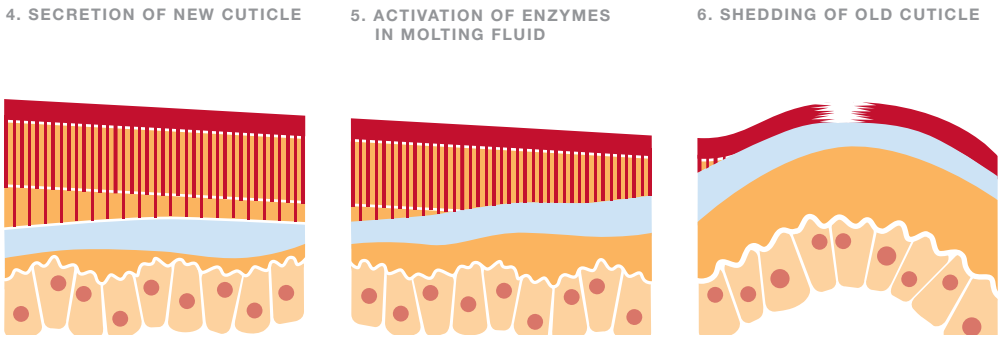


A molt can involve a change not only in size, but also of form, to a more mature life stage. A molt to a more mature form is called a metamorphosis, while a molt to a new immature stage is called an immature molt. Insects may go through more than one immature molt and even more than one metamorphosis, such as larva to pupa and pupa to adult, but the molt to the adult stage is the final one.

The major developmental hormone in insects, the steroid hormone ecdysone, plays a central role in all molting and metamorphosis. Ecdysone is produced and released by the prothoracic gland in the thorax, under control of the nervous system. When the brain has determined that the time is right, two pairs of neurosecretory neurons secrete a neuropeptide into the hemolymph that acts on the prothoracic gland to stimulate release of ecdysone. The hormone enters cells of the epidermis and other tissues where it binds to the ecdysone receptor, EcR. This hormone-receptor complex moves into the nucleus where it complexes with another DNA-binding protein called USP, forming a tertiary complex that binds to specific short DNA sequences, activating genes required for molting.

While a pulse of ecdysone induces molting, the nature of the molt depends on juvenile hormone (JH), so named because its presence during the ecdysone peak prevents metamorphosis to a more mature stage. Juvenile hormone is produced by a pair of glands behind the brain called the *corpora allata*, under the control of two neurohormones released by the brain. JH production stops during metamorphosis and circulating JH is degraded by a pair of enzymes. It reappears in the adult, where it regulates female reproductive maturation.

Juvenile hormone enters the nucleus and binds to its receptor called Met, a DNA binding protein that pairs with itself and other DNA binding proteins to form dimers (a molecule composed of two simpler molecules). The dimers bind to short DNA segments, called juvenile hormone response elements, in order to switch on genes. The juvenile hormone receptor is called Met because it was identified in methoprene resistance. The anti-metamorphic effect of juvenile hormone is due to the switching on of a single gene that represses the expression of the genes needed for adult development.



below:
A cicada molting.

There are seven groups of insect growth regulators in the IRAC classification. Both the juvenile hormone and ecdysone mimics bind to and activate the receptors for these hormones, while four groups of chitin synthesis inhibitors hinder the formation of new cuticle during the molt by unknown mechanisms. The newest group of insect growth regulators does not affect molting and metamorphosis directly, but affects development by inhibiting the biosynthesis of fats, which are required for growth.

Growth and Development Disruptor Insecticides

IRAC Group 7: Juvenile Hormone Mimics

Juvenile hormone (JH) mimics do not control adult insects or early larval stages, but because of their antimetamorphic effect, they prevent transformation to the adult stage. They also have an ovicidal effect, which is very important for many uses. They are used as mosquito larvicides, for controlling fleas on domestic animals, and as an additive to cattle feed to prevent fly breeding in the dung. Pyriproxyfen is also used to control whiteflies in cotton.

Examples of Juvenile Hormone Mimics

IRAC Group 7A: Juvenile Hormone Analogues

- Common name hydropene – trade name Gentrol®
- Common name kinoprene – trade name Enstar® II
- Common name methoprene – trade names Altosid®, Precor®

IRAC Group 7B: Fenoxycarb

- Common name fenoxycarb – trade names Insegar®, Logic®

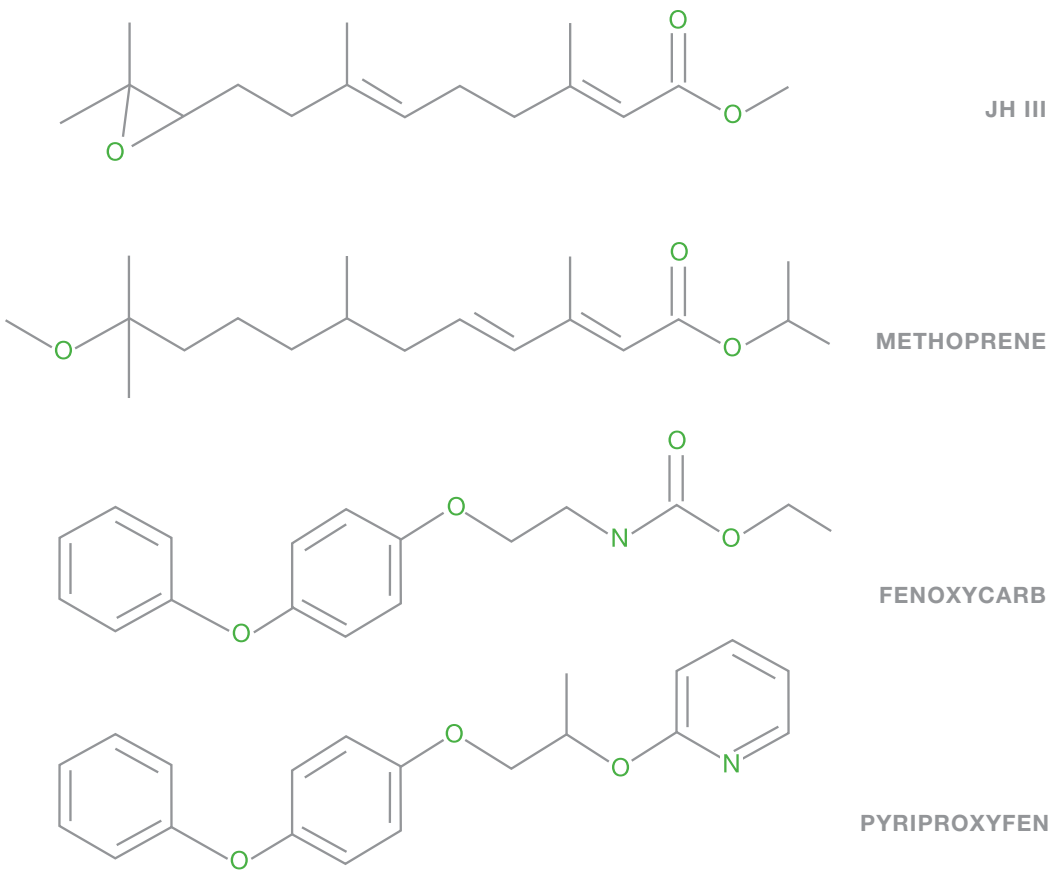
IRAC Group 7C: Pyriproxyfen

- Common name pyriproxyfen – trade names Cyclio®, Nylar®

Mode of Action and Resistance: The figure on page 37 shows the chemical structure of the most common form of JH, JH III, along with examples of the three classes of insecticides that mimic it. Clearly, methoprene is a very close chemical analog, and in fact all of the JH mimics work by binding to and mimicking the action of JH at its receptor. Activation of JH receptors during molting inhibits the expression of genes needed to form larval or adult structures. In immature molts this has little or no effect, but it can severely disrupt metamorphosis, leading to incomplete molting, or in some cases, a successful molt to a supernumerary immature stage.

Although target site resistance to JH mimics was generated in the laboratory in fruit flies, resistance in the field appears to be largely metabolic, due to enhanced metabolism by P450 monooxygenases and glutathione S-transferases (GSTs).

Environmental and Toxicological Considerations: JH mimics are relatively non-toxic to mammals and most other organisms, but have moderate to high acute toxicity to estuarine invertebrates. These insecticides vary in their environmental persistence, but most have extremely low water solubility and are not considered to have a potential to leach.



IRAC Group 10: Mite Growth Inhibitors

These compounds interfere with mite development and are active against eggs, larvae and nymphs, but not adults.

IRAC Group 10A: Clofentezine, Hexythiazox and Diflovidazin

Clofentezine, hexythiazox and diflovidazin are acaricides. Clofentezine is used for the control of spider mites on a wide range of crops, including fruits and nuts. It acts primarily as an ovicide, but has some activity against early motile stages of mites.

Hexythiazox is used to control mites on a variety of crops, including alfalfa, mint, carrot, clover seeds, caneberries, Christmas trees, citrus, cotton, potatoes, nuts, strawberries and other fruits. Hexythiazox has ovicidal, larvicidal and nymphicidal activities and can be applied at any stage of plant growth from budding to fruiting. It has very long residual activity of up to four months. Many countries recommend one application per year on registered crops to minimize the emergence of resistant mites.

Examples of Clofentezine and Hexythiazox

- Common name clofentezine – trade name Apollo®
- Common name hexythiazox – trade names Onager,® Savey®

IRAC Group 10B: Etoxazole:

Etoxazole controls spider mites in a variety of crops, including citrus, strawberries, nuts, vegetables, ornamentals, cotton and pome fruits. It kills mite eggs and nymphs and prevents adults from laying viable eggs. Etoxazole controls mites resistant to hexythiazox and clofentezine.

Example of Etoxazole

- Common name etoxazole – trade name Zeal®

Mode of Action and Resistance: Clofentezine and Diflovidazin are close analogs and are grouped with Hexythiazox because they commonly exhibit cross-resistance, despite being structurally distinct. The target site of these compounds is considered unknown, but recent investigations of resistance mechanisms indicate that these compounds may be chitin synthase inhibitors.

Etoxazole has been shown to inhibit chitin biosynthesis in whole larvae and isolated integuments of *Spodoptera frugiperda* and to cause similar symptoms to triflumuron. Furthermore, a mutation in the chitin synthase gene confers resistance to etoxazole, clofentezine and hexythiazox.

Environmental and Toxicological Considerations: Both clofentezine and hexythiazox exhibit low acute mammalian toxicity, have low solubility, degrade rapidly and have very low soil mobility once applied. They have low toxicity to terrestrial arthropods, including predatory mites, making them useful in integrated pest management programs. Both products are toxic to certain aquatic organisms. Hexythiazox is highly toxic to Daphnia on a chronic basis.

Etoxazole is relatively non-toxic to mammals, birds and honeybees, moderately toxic to fish, and extremely toxic to oysters and freshwater invertebrates. Terrestrial degradation studies indicate that etoxazole breaks down readily in soil and does not accumulate.

IRAC Group 15: Inhibitors of Chitin Biosynthesis, Type 0, Lepidopteran (Benzoylureas)

Chitin is an essential structural component of insect cuticle. Group 15 insecticides interfere with the biosynthesis of chitin in Lepidoptera and some other orders. When an insect cannot make chitin, it dies during the molt.

Insecticides in this group are used to control certain major pests in a variety of crops, including boll weevils and Lepidoptera (moths and butterflies) in cotton and Lepidoptera and Coleoptera (beetles) in citrus, rice, tea, soybeans, vegetables and ornamentals. These insecticides generally enter the insect orally through the consumption of treated foliage or insecticide baits.

BASF's Cascade® insecticide and Tenopa® insecticide, members of IRAC Group 15, contain the active ingredient flufenoxuron, available in Asia for use in fruit and vegetable crops. BASF's Nomolt® insecticide with the active ingredient teflubenzuron is sold in South America and Europe for use in soybean, maize, cotton, fruits and vegetables. Nomax® insecticide containing the same a.i., is used solely in Brazil for soybean pest control.

Examples of Inhibitors of Type 0, Lepidopteran

- Common name chlorfluazuron – trade names Atabron®, Ishipron®
- Common name fluazuron – trade name Acatak®
- Common name flucycloxuron
- Common name diflubenzuron – trade name Dimilin®
- Common name flufenoxuron – trade names Cascade® insecticide, Tenopa® insecticide
- Common name hexaflumuron – trade name Shatter®
- Common name lufenuron – trade name Match®
- Common name novaluron – trade names Diamond®, Rimon®
- Common name noviflumuron – no registered trade names
- Common name teflubenzuron – trade names Nomolt® insecticide, Nomax® insecticide
- Common name triflumuron – trade names Alsystin®, Baycidal®, Starycide®

Mode of Action and Resistance: Chitin biosynthesis inhibitors interfere with the formation of chitin during molting, resulting in a weak, soft exoskeleton and deformed appendages and sexual organs. The molecular target of the chitin biosynthesis inhibitors is not known.

Environmental and Toxicological Considerations: Chitin biosynthesis inhibitors have low toxicity to mammals, but in the environment, particularly aquatic ecosystems, they can be very toxic to non-target insects and other arthropods. Most have extremely low water solubility and are not considered to have potential to leach through the soil. Some chitin synthesis inhibitors can persist in the environment and are active at very low levels.

IRAC Group 16: Inhibitors of Chitin Biosynthesis, Type 1, Homopteran

The mode of action and environmental considerations of IRAC Group 16 chitin biosynthesis inhibitors resemble those of Group 15, but in both cases the target is not known. The major difference between the two groups is that IRAC Group 15 acts on Lepidoptera and Coleoptera orders, while IRAC Group 16 affects Homoptera, an order of insects that includes cicadas, aphids and other insects with sucking mouthparts.

The sole member of this group, buprofezin, is used to control a variety of sucking insects, including mealybugs, planthoppers, scales and whiteflies. Buprofezin is registered for use on a wide range of crops and is an effective tool for insecticide resistance management.

Example of Inhibitors of Chitin Biosynthesis, Type 1, Homopteran

- Common name buprofezin – trade name Applaud®

Mode of Action and Resistance: Chitin biosynthesis inhibitors interfere with the formation of chitin during molting, resulting in a weak, soft exoskeleton and deformed appendages and sexual organs. The molecular target of the chitin biosynthesis inhibitors is not known.

Environmental and Toxicological Considerations: Chitin biosynthesis inhibitors have low toxicity to mammals, but in the environment, particularly the aquatic environment, they can be very toxic to insects and other arthropods. Most have extremely low water solubility and are not considered to have a potential to leach through the soil. Some chitin synthesis inhibitors can persist in the environment and are active at very low levels.

IRAC Group 17: Molting Disruptor, Dipteran

The only member of IRAC Group 17 is cyromazine, which disrupts the growth and development of larval life stages of the order Diptera, including mosquitoes, gnats, onion maggots, fruit flies and midges, all of which have a single pair of wings and are classified as true flies. Cyromazine is used as a foliar spray to control leaf miners and other fly larvae in vegetables, mushrooms, ornamentals and as a feed-through product in animal health. It is broadly systemic and moves within plants by translaminar and acropetal action.

Example of Molting Disruptor, Dipteran

- Common name cyromazine – trade name Trigard®

Mode of Action and Resistance: Cyromazine disrupts growth and development of dipteran larvae by an unknown mechanism. It appears to affect the hormonal control of molting, but the mechanism of action is unknown. While resistance to cyromazine has been reported in house flies, leafminers and blow flies, it does not appear to be target site-based.

Environmental and Toxicological Considerations: Cyromazine is practically non-toxic to mammals, birds and most non-target organisms. Against aquatic Diptera larvae, it has low acute toxicity, but is highly toxic on a chronic basis, as expected from its mode of action. It has not been shown to leach or accumulate in soil and has low potential for bioaccumulation.

IRAC Group 18: Ecdysone Receptor Agonists (Diacylhydrazines)

Diacylhydrazines are the only group of ecdysone receptor agonist insecticides, which induce premature molting in insects by mimicking the action of the molting hormone ecdysone. Activity is limited to Lepidoptera and Coleoptera, and because the compounds do not penetrate the cuticle well, they must be ingested. Ecdysone receptor agonists provide rapid control in comparison to most insect growth regulators, causing feeding cessation within 3 to 14 hours.

Diacylhydrazines were discovered and commercialized by the Rohm and Haas Company, which was subsequently acquired by the Dow Chemical Company. Tebufenozide, introduced in 1995, acts specifically on Lepidoptera, as does methoxyfenozide, introduced in 1998, which provides broader spectrum control at 3 to 4 times lower use rates than tebufenozide. Halofenozide, introduced in 1998, controls soil-dwelling Coleoptera and Lepidoptera larvae in turf. Chromafenozide, jointly discovered and commercialized by the Nippon Kayaku and Sankyo companies, is registered for control of Lepidoptera pests on vegetables, fruits, vines, tea, rice and ornamentals in Japan.

Examples of Diacylhydrazines

- Common name chromafenozide – trade name Matric®
- Common name halofenozide – trade name Mach 2®
- Common name methoxyfenozide – trade names Intrepid®, Runner®
- Common name tebufenozide – trade names Confirm®, Mimic®

Mode of Action and Resistance: Diacylhydrazine insecticides bind in the ecdysone binding site of the ecdysone receptor-usp dimer, causing it to activate ecdysone-responsive genes that are normally activated during molting and metamorphosis. One of the earliest symptoms, occurring within 3 to 14 hours, is feeding cessation, a normal effect of ecdysone that allows insects to clear food from the gut in preparation for molting. Separation of the old cuticle from the epidermis and synthesis of the new cuticle begins during this time also. The continued activation of ecdysone receptors, in contrast to the brief activation by the pulse of ecdysone in a normal molt, does not allow the proper timing of gene activation. This results in an improperly formed cuticle and mouth parts that are soft and mushy and unable to break the insect out of the old cuticle. The selectivity of diacylhydrazines for Lepidoptera is due in large part to the high selectivity for lepidopteran ecdysone receptors.

Resistance to diacylhydrazines has occurred in several lepidopteran species, due to enhanced oxidative metabolism. Target site resistance has not been reported.

Environmental and Toxicological Considerations: Diacylhydrazines have an excellent environmental profile, are essentially non-toxic to mammals and other vertebrates, and have exhibited low toxicity towards most non-target invertebrates, including beneficial insects and bees.

IRAC Group 23: Inhibitors of Acetyl CoA Carboxylase (Tetronic and Tetramic Acid Derivatives)

Tetronic and tetramic acid derivative insecticides were discovered and commercialized by Bayer CropScience. Spirodiclofen, the first product in this group, was introduced in 2003 as a selective acaricide on citrus, grapevines and pome fruits. Spiromesifen, introduced in 2005, controls whiteflies in addition to mites, and spirotetramat, introduced in 2008, is systemic and controls aphids, scales, mealybugs, psylla, phylloxera and thrips, as well as mites and whiteflies. It is used on cotton, maize, ornamentals, potatoes, leafy greens, sweet potatoes and fruit crops. Group 23 insecticides act primarily by ingestion against immature stages, and also reduce the fecundity (fertility) of adult stages.

Examples of Inhibitors of Lipid Synthesis

- Common name spirodiclofen – trade name Envidor®
- Common name spiromesifen – trade name Oberon®
- Common name spirotetramat – trade name Movento®

Mode of Action: Group 23 insecticides inhibit acetyl coenzyme A carboxylase, the enzyme that catalyzes the first step in fatty acid biosynthesis. Important components of cell membranes, fatty acids are needed for growth and development. Exposure to group 23 insecticides inhibits lipid biosynthesis, stopping the development of immature insects, including whitefly pupae, which are not controlled by many other insecticides. Adults are not acutely affected, but their fecundity is decreased. Target site resistance to spirotetramat has been identified in the greenhouse whitefly, due to a mutation in the ACCase gene.

Environmental and Toxicological Considerations: Tetronic and tetramic acids are not acutely toxic to mammals and other vertebrates, and pose minimal risk. Reproductive effects on mammals have been observed in laboratory studies, but are not a concern. Only moderate effects of spitotetramat have been found on beneficial arthropods, which make the product suitable in integrated pest management (IPM). Early in 2010, spirotetramat's registration was withdrawn by the U.S. EPA due to a procedural issue raised by environmental groups. Later in 2010, its use was reinstated in the USA. The product is controversial with environmental groups and commercial beekeepers who claim that it is potentially toxic to honeybee populations.

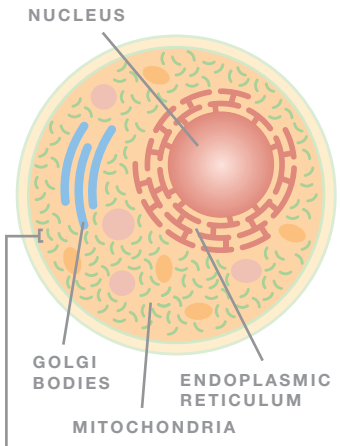
Cellular Respiration in Insects

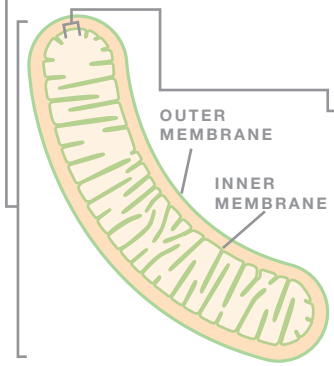
While avoiding calories is of great concern for many of us, insects are considered pests because they take calories from us. The calorie is a measure of food energy, which insects obtain from the same nutrients that we do, including carbohydrates, fats, proteins and organic acids. Gut enzymes break large nutrients like carbohydrates and proteins into their smaller building blocks – sugars and amino acids, respectively, and these smaller molecules are carried by the hemolymph (blood) to all cells of the body. These nutrients are taken up by the cells and some are processed by intracellular enzymes and then by mitochondria, to convert their energy to a standard form that all cellular processes can use.

As mentioned earlier in the discussion of bioelectricity, this "energy currency of the cell" is the adenosine triphosphate (ATP) molecule, which stores the energy captured from nutrients in the high-energy chemical bond between the last two phosphate groups of its triphosphate chain. Attaching a phosphate group to a molecule is a chemical reaction known as phosphorylation. ATP is created in the mitochondria by phosphorylation of adenosine diphosphate (ADP), and it donates energy to other molecules by phosphorylating them with its terminal phosphate group, aided by specific enzymes.

All animal and plant cells contain mitochondria, which evolved from bacteria that once infected cells and came to live symbiotically within them. Mitochondria are about the size of bacteria, which means if we were their size, a human or insect cell would seem as large as a football field. Muscle cells and other cells that require a lot of energy can contain thousands of these tiny power plants, which, like conventional power plants, burn or combust fuel to capture its chemical energy in a usable form. Burning, or combustion, is the sequence of energy-liberating chemical reactions between a fuel and an oxidizer, which in the case of both conventional and cellular power plants is oxygen. A coal power plant combusts coal with oxygen, releasing heat to drive electricity-generating steam turbines, while cellular power plants combust a variety of nutrients with oxygen to produce high-energy phosphate bonds in ATP. Coal is almost pure carbon, and the waste product of its combustion with oxygen is carbon dioxide, or CO_2. Combustion of nutrients with oxygen in mitochondria also produces CO_2 waste gas from the carbon in the nutrients, while water is produced by combining the hydrogen from nutrients with oxygen.
The process carried out by mitochondria is called cellular respiration because it is the cellular cognate of the inhalation and exhalation carried out by our lungs that we normally think of as respiration. In fact, most of the oxygen that we inhale is consumed by cellular respiration in mitochondria, and most of the CO_2 that we exhale is the waste produced by the mitochondria. You could say that every breath we take is for our mitochondria.

Typical Cell

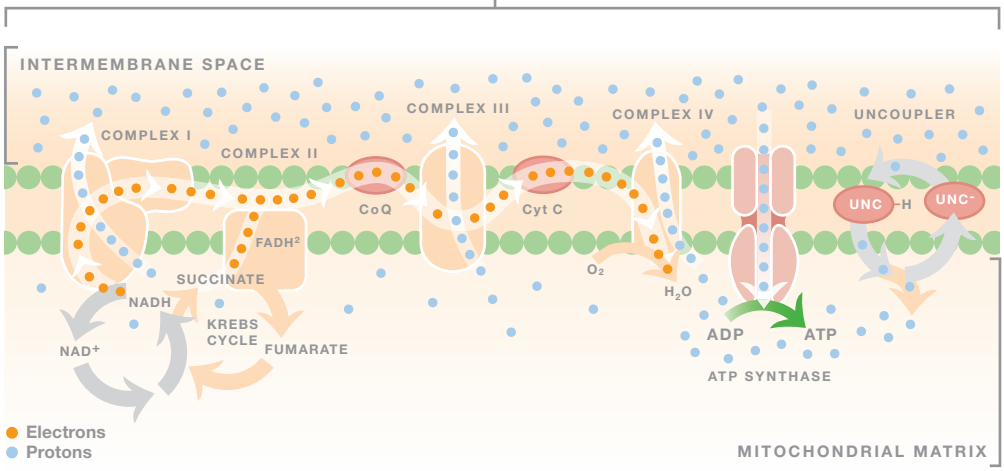




Typical Mitochondrion

The mitochondrion is bounded by an outer membrane and divided by a highly invaginated (folded inward) inner membrane that encloses and separates the inner matrix compartment from the outer compartment known as the intermembrane space. Certain intermediates derived from the metabolism of nutrient molecules in the cell penetrate into the mitochondrial matrix, with the help of specific transport proteins. In the matrix, these intermediates are fully oxidized by a biochemical pathway known as the Krebs cycle to produce energy in the form of ATP and two other energy-rich molecules, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$).

Electrons in molecules exist at specific energy levels, and their transfer between molecules transfers this energy as well. Four giant protein complexes embedded in the inner mitochondrial membrane, together with the small molecules coenzyme Q (CoQ) and cytochrome C (CytC), form the electron transport chain, as shown in the figure below. High-energy electrons from NADH enter the electron transport chain via complex I while those from $FADH_2$ enter via Complex II. After passing through this chain, they are accepted by oxygen to form water in a reaction catalyzed by complex IV.



Excess energy derived from the electron transfers carried out by complexes I, III and IV is used to pump ions across the inner mitochondrial membrane, creating an ion gradient. As discussed earlier in connection with the Na^+, K^+ pump, an ion gradient has potential energy, or the capacity to do work, because of the entropy-driven tendency of the ions to move from an ordered (high concentration) to a disordered (low concentration) state. The ions that are pumped by the electron transport complexes are protons, the nuclei of hydrogen atoms, which are always present in aqueous solutions as a result of the dissociation of water molecules. Protons give water its acidity. In fact, the widely known measure of acidity, pH, is actually a measure of proton concentration.

ATP synthase, also known as F_0F_1-ATPase, is the enzyme in the inner mitochondrial membrane that uses the energy of the proton gradient across it to power the phosphorylation of ADP to form ATP. It is a rotary enzyme composed of two motors that are connected by a common shaft to exchange rotational energy with one another. During ATP synthesis, the movement of protons from the intermembrane space to the matrix, through the membrane-embedded F_0 motor, generates torque, causing the F_0 motor to rotate. Rotation is transmitted by the shaft to the F_1 motor in the matrix, where it drives the phosphorylation of ADP to form ATP.

The entire process described in the preceding three paragraphs is known as oxidative phosphorylation. The energy released by oxidation of nutrients in the electron transport chain is coupled by a proton gradient to the phosphorylation carried out by ATP synthase. All six groups of respiration disruptor insecticides act on the inner mitochondrial membrane of mitochondria, either by inhibiting ATP synthase or one of the electron transport complexes, or by uncoupling oxidation from phosphorylation by making the membrane leaky to protons so that it cannot support a proton gradient. While all six of these groups starve cells of energy by preventing ATP synthesis, they do so in different ways, offering a richness of insecticide target sites.

Respiration Disruptor Insecticides

IRAC Group 12: Inhibitors of Mitochondrial ATP Synthase

IRAC Group 12 insecticide /acaricides starve cells of energy by inhibiting ATP synthase.

IRAC Group 12A: Diafenthiuron

A broad spectrum acaricide and insecticide, diafenthiuron is registered for use on cotton, soybeans, vegetables, fruit and ornamentals. It controls all post-hatch stages of mites, whiteflies and aphids. Its ability to control all sucking pests of cotton as well as mites, with no known toxicity to beneficial insects, is unique and very valuable in cotton integrated pest management (IPM) programs.

Example of Diafenthiuron

- Common name diafenthiuron – trade name Polo®

Mode of Action and Resistance: Diafenthiuron is a proinsecticide, which means that it must be converted to another compound – the drug – in order to be toxic. Activation of diafenthiuron to its carbodiimide drug occurs on the leaf surface, catalyzed by light, or in the insect, catalyzed by P450 monooxygenases. Diafenthiuron carbodiimide binds to the glutamate residue in the transmembrane F_0 subunit of ATP synthase where protons from the intermembrane space dock to begin their journey across the inner mitochondrial membrane. Binding of diafenthiuron carbodiimide to this site blocks proton transport and ATP synthesis. Diafenthiuron was launched in 1991 and resistance has not yet been reported.

Environmental and Toxicological Considerations: Diafenthiuron has low toxicity to birds, mammals and beneficial insects, and is only slightly toxic to predatory mites. It is toxic to fish, but presents little hazard because it is rapidly degraded.

IRAC Group 12B: Organotins

Organotin miticides are unusual among modern insecticide/acaricides in containing a metal atom. They control all motile stages of phytophagous mites, with long residual activity. Azocyclotin is an acaricide for use in citrus, fruits, grapes, vegetables, hops, cotton and ornamentals. Cyhexatin controls plant-feeding mites that are resistant to other acaricides and is used in a variety of crops, including almonds, walnuts, hops, ornamentals and fruit. Fenbutatin-oxide is an acaricide used on apples, citrus, pears, and ornamentals.

Examples of Organotins

- Common name azocyclotin – trade names Peropal®, Caligur®
- Common name cyhexatin – trade name Plictran®
- Common name fenbutatin-oxide – trade names Vendex®, Torque®

Mode of Action and Resistance: Organotins inhibit ATP synthase. Like diafenthiuron, they act at the proton binding site in the F_0 subunit. Cases of resistance to fenbutatin-oxide, cyhexatin and azocyclotin have all been reported, but target site resistance has not been found.

Environmental and Toxicological Considerations: Organotin miticides are generally shown to have no toxicity to beneficial arthropods and birds, but are highly toxic to aquatic organisms, including invertebrates, fish and algae. In mammals, they have low acute toxicity but are highly toxic on a chronic basis and are severe eye irritants.

IRAC Group 12C: Propargite

Propargite is a contact acaricide that controls motile stages of phytophagous (or plant feeding) mites on almonds, beans, carrot seeds, Christmas trees, conifers, clover seeds, maize, cotton, fruit, hops, mint, ornamentals, potatoes, peanuts, sorghum, sugar beets and walnuts. It's also effective for postharvest use in sweet cherries and citrus.

Example of Propargite

- Common name propargite – trade names Comite®, Omite®

Mode of Action and Resistance: Propargite inhibits ATP synthase. While resistance has been reported, target site resistance has not been found.

Environmental and Toxicological Considerations: Propargite is not shown to have toxicity to birds and beneficial arthropods when used as directed, but is highly toxic to fish and some aquatic invertebrates. It causes eye and skin irritation in humans.

IRAC Group 12D: Tetradifon

Tetradifon is an acaricide that gives long residual control of eggs and immature stages of phytophagous mites on top-fruit, vegetables, ornamentals, hops, cotton, nuts, tea, sugarcane and forestry. It is a non-systemic compound with activity on eggs (ovidical) and immature life stages.

Example of Tetradifon

- Common name tetradifon – trade name Tedion®

Mode of Action and Resistance: Tetradifon inhibits ATP synthase in the F_0 subunit. While resistance is known, target site resistance has not been described.

Environmental and Toxicological Considerations: Tetradifon is not phytotoxic to most crops and is considered non-toxic to non-target organisms, including beneficial arthropods.

IRAC Group 13: Uncouplers of Oxidative Phosphorylation via Disruption of the Proton Gradient

The three insecticides that act as uncouplers of oxidative phosphorylation represent three different chemical families.

Pyrroles, discovered in the late 1980s at American Cyanamid, now BASF, are exemplified by chlorfenapyr, a broad spectrum insecticide/miticide currently registered in more than 30 countries for crop and specialty uses. A foliar insecticide in the crop segment, chlorfenapyr is highly effective against Lepidoptera and Coleoptera larvae as well as mites and thrips.

It is also excellent for use in urban pest control against ants, cockroaches, bed bugs and termites in soil and wood.

Chlorfenapyr is active against larvae and adults of many insect and mite pests, and is used in a wide range of crops, including vegetables, tree fruits, vines and ornamentals. Its contact activity and lack of repellency make it an excellent product against many non-crop pests, including termites, ants, cockroaches and bed bugs.

Chlorfenapyr is a pro-insecticide, requiring bioactivation by oxidative metabolism within the insect. Target pests acquire the compound primarily through consumption of treated residues or contact with treated surfaces. Chlorfenapyr has excellent translaminar movement, which means that when applied to the top of a leaf, it will cross to the bottom surface where many pests feed.

DNOC (4,6-dinitro-o-cresol, also known as 2-methyl-4,6-dinitrophenol) is an insecticide/acaricide used as a dormant spray for the control of insects, mites and disease on top fruit. It is also used as a herbicide.The compound is highly toxic and its registrations and uses are extremely limited. DNOC was banned in the U.S. in 1991 and in the E.U. in 1999.

Sulfluramid is a pro-insecticide that is bioactivated by oxidative metabolism in the insect. It is used in bait stations for ants, termites, cockroaches and wasps, but its uses are being phased out in the U.S. by 2016 because of human toxicity.

Examples of Uncouplers of Oxidative Phosphorylation

- Common name chlorfenapyr – trade names Alert® insecticide, Pirate® insecticide (discontinued), Phantom® insecticide, Stealth® insecticide, Rampage®, Secure® insecticide, Mythic® termiticide/insecticide
- Common name DNOC
- Common name sulfluramid – trade name Firstline®

Mode of Action and Resistance: Uncouplers are the only insecticides that do not act on a protein target. Most are weak acids that can accept a proton in the proton-rich intermembrane space, transport it across the inner mitochondrial membrane, deposit it in the matrix and return across the membrane to pick up another proton and repeat the cycle. The result of this proton shuttling is that the energy stored in the proton gradient is dissipated as heat, without being used for ATP synthesis. In the absence of a proton gradient, ATP synthase runs in reverse, quickly hydrolyzing the available ATP to futilely pump protons back into the intermembrane space. ATP is quickly depleted, leading to rapid paralysis and death.

Any lipophilic weak acid could pick up protons in the intermembrane space and transport them into the matrix, but in order to act as an uncoupler, the deprotonated form of the molecule, which has a negative charge, must diffuse back across the inner mitochondrial

membrane to pick up another proton in the intermembrane space, thus sustaining a cycle of proton transport. Most charged molecules cannot traverse the nonpolar interior of the membrane. In a polar medium, such as water, a charged atom in a molecule attracts polar water molecules that shield its charge, thus reducing repulsive forces between like-charged molecules. This shielding would not occur inside the non-polar lipid membrane, so there would be strong repulsion between charged molecules. Uncoupler molecules are able to shield the charge internally within the molecule, by delocalizing it over many atoms. This greatly reduces the electrostatic repulsion between like molecules in a nonpolar medium. The active form of chlorfenapyr is very good at delocalizing the negative charge over a system of double bonds, and is one of the most potent uncouplers known.

Without a target site, uncouplers are not subject to target site resistance. Laboratory tests have shown no indication of cross-resistance with other insecticides.

Environmental and Toxicological Considerations: Chlorfenapyr is highly toxic to birds, but the results of extensive avian field studies and a probabilistic risk analysis indicate that the use of chlorfenapyr in agriculture presents a low risk to avian species. Greenhouse and non-agricultural uses of chlorfenapyr do not pose significant risk to birds, due to its low water solubility and immobility in soils, which precludes it from leaching into water supplies. Chlorfenapyr is strongly adsorbed by various soil types and degrades in the soil gradually over time.

IRAC Group 20: Mitochondrial Complex III Electron Transport Inhibitors

The three insecticides that act as mitochondrial complex III electron transport inhibitors represent three different chemical families.

IRAC Group 20A - Hydramethylnon

Hydramethylnon, discovered by American Cyanamid, now BASF, is an insecticide used in granule and gel bait formulations to control ants and cockroaches in pastures, rangeland, ornamentals and indoor areas. One of the leading products in this category, Amdro® Pro, is marketed by BASF for controlling ants.

Example of Hydramethylnon

- Common name hydramethylnon – trade names Amdro® Pro, Siege® cockroach gel bait

IRAC Group 20B - Acequinocyl

Acequinocyl is used for control of all stages, including eggs, of phytophagous mites in fruit crops, including apple, cherry, citrus, melon, peach and pear, as well as ornamentals and vegetables.

Example of Acequinocyl

- Common name acequinocyl – trade names Kanemite®, Shuttle®

IRAC Group 20C - Fluacrypyrim

Fluacrypyrim is a strobilurin acaricide introduced in 2002 for use on fruit crops. Its route of exposure is through ingestion of treated residues or by contact with treated surfaces. It exhibits rapid activity on all mite life stages, with residual control lasting up to one month.

Example of Fluacrypyrim

- Common name fluacrypyrim – trade name Titaron® is discontinued

Mode of Action and Resistance: Hydramethylnon inhibits the electron transport chain at complex III by an unknown mechanism. True resistance has not been documented. Presumed cases of resistance have so far been traced to acquired aversion to bait components.

Acequinocyl is a pro-insecticide, whose deacetylated drug binds to the Q_0 center (ubiquinol oxidation site) in the cytochrome b subunit of mitochondrial electron transport complex III, where fluacrypyrim also binds. Mutations of two different amino acids in the binding site are known to confer resistance to acequinocyl and fluacrypyrim in mites.

Environmental and Toxicological Considerations: Hydramethylnon is non-toxic or slightly toxic to mammals, birds and bees. It is moderately toxic to fish and freshwater invertebrates, but presents minimal risk because of its low water solubility and rapid degradation in water and light. Acequinocyl is practically non-toxic to mammals, birds, fish, bees, beneficial insects and mites. It is highly toxic to aquatic invertebrates, requiring adequate risk reduction measures for field use. Fluacrypyrim is considered of low risk of toxicity to mammals, birds, fish, bees and most non-target insects.

IRAC Group 21: Mitochondrial Complex I Electron Transport Inhibitors

IRAC Group 21A: METI Acaricides and Insecticides

Tebufenpyrad, one of the compounds in this group, is the active ingredient in Masai® insecticide/acaricide, developed by BASF. Effective as an acaricide, this product controls spider mites and citrus red mites on cotton, citrus, fruit and vegetable crops and ornamentals.

Other Group 21A insecticide/acaricides are used to control leafhoppers, mites and whiteflies in fruit crops, vegetables, ornamentals, nuts and cotton. They are generally fast-acting and offer long-lasting control of all life stages of susceptible insects and mites.

Examples of Mitochondrial Complex I Electron Transport Inhibitors

- Common name fenazaquin – trade name Magister®
- Common name fenpyroximate – trade names Fujimite®, Danitron®
- Common name pyrimidifen – trade name Miteclean®
- Common name pyridaben – trade names Nexter®, Pyramite® miticide/insecticide, Sanmite®
- Common name tebufenpyrad – trade name Masai® insecticide/acaricide
- Common name tolfenpyrad – trade name Hachi-Hachi®

IRAC Group 21B: Rotenone

Rotenone (Group 21B) is effective on aphids, beetles, moths, spider mites and thrips in fruit and vegetable crops, as well as on fire ants and mosquito larvae in pond water. It is also used to control fish populations in the field of water management and to eliminate invasive fish species.

Example of Rotenone

- Common name rotenone – trade name Prentox®

Mode of Action and Resistance: Group 21 insecticide/acaricides inhibit mitochondrial electron transport complex I, leading to rapid paralysis and death. Resistance to METI acaricides has developed in several mite species, but surprisingly has not yet been found to be target-site-based. Resistance is metabolic, but often extends to other members of this group, because of chemical similarities.

Environmental and Toxicological Considerations: Group 21 insecticides are generally non-toxic to slightly toxic to mammals and birds, but tend to be highly toxic to fish and aquatic invertebrates. Some, such as fenazaquin, are also toxic to predatory mites.

IRAC Group 24: Mitochondrial Complex IV Electron Transport Inhibitors.

IRAC Group 24A: Phosphine

Phosphine is a colorless, odorless, flammable toxic gas with the chemical formula PH_3. Pellets of aluminum phosphide, calcium phosphide or zinc phosphide release phosphine gas upon contact with atmospheric moisture or rodents' stomach acid. These pellets also contain agents to reduce the potential for ignition or explosion of the released phosphine. Phosphines are fumigants used as rodenticides and to control a broad spectrum of insect pests of grains and non-food/non-feed plant and animal products, such as animal hides, leather products, feathers, wood chips, bamboo, paper and dried plants and flowers in sealed containers or structures. There are no homeowner or agricultural row crop uses for these products.

Because the previously popular fumigant methyl bromide has been phased out in some countries under the Montreal Protocol, phosphine is the only widely used, cost-effective, rapidly acting fumigant that does not leave residues on the stored product.

Examples of Phosphine

- Common name phosphine – trade name - Profume®
- Common name phosphides

IRAC Group 24B - Cyanide

Cyanide is produced by certain bacteria, fungi and algae, and is found in a number of plants. Cyanide is also found in small amounts in certain seeds and fruit stones. In plants, cyanide is usually bound to sugar molecules in the form of cyanogenic glycosides that defend the plant against herbivores. Highly toxic to humans and animals, cyanide has been banned for use as a pesticide.

Example of Cyanide-Derived Chemistry

- Common name sodium cyanide, calcium cyanide and potassium cyanide – no registered trade names

Mode of Action and Resistance: Phosphine gas and cyanide are considered to inhibit mitochondrial electron transport complex IV, the last complex in the electron transport chain, which uses electrons from cytochrome C to reduce molecular oxygen to water. Pests developing high levels of resistance toward phosphine have become common in Asia, Australia and Brazil. High level resistance is also likely to occur in other regions, but may not have been as closely monitored. Phosphine resistance has recently been found to be due to any of several mutations that cluster around the catalytic center in the enzyme dihydrolipoamide dehydrogenase, which is a component of four major multienzyme complexes in mitochondria, not including complex IV, suggesting that Electron Transfer Complex IV might not be the target of phosphine.

Environmental and Toxicological Considerations: Phosphines are Restricted Use Pesticides – RUP; Category I, due to high acute oral toxicity of the pellets and inhalation toxicity of phosphine gas.

IRAC Group 25: Mitochondrial Complex II Electron Transport Inhibitors

This group includes the selective acaricides cyenopyrafen, which controls mites on ornamentals, top fruits, tea, vegetables, and non-bearing fruit trees, as well as cyflumetofen, a new acaricide developed by Otsuka and offered by BASF in certain areas of the world. Expected to launch in 2014, cyflumetofen will be used to protect a variety of crops, including tree nuts, pome fruits, grapes, vegetables and citrus.

Examples of Mitochondrial Complex II Electron Transport Inhibitors

- Common name cyenopyrafen – trade name Starmite®
- Common name cyflumetofen – trade names Danisaraba®, Nealta® miticide, Sultan™ miticide

Mode of Action and Resistance: Cyenopyrafen and cyflumetofen are pro-insecticides that are metabolized to corresponding enol products that inhibit mitochondrial electron transport complex II, leading to rapid paralysis and death due to cellular energy starvation. Resistance has not yet been reported.

Environmental and Toxicological Considerations: Cyenopyrafen and cyflumetofen have been shown to have very low toxicity to mammals, birds, bees and beneficial insects, including predatory mites. They have some toxicity to fish and aquatic invertebrates, but pose minimal risk because they are rapidly degraded in water and soil.

Microbial Gut Disruptor Insecticides

Microbial insecticides are microscopic organisms that infect and incapacitate or kill insects, and can include viruses, bacteria, fungi, protozoa and nematodes, or the toxins produced by them. Microbial insecticides kill insects by various mechanisms. Because the activity of microbial organisms is so specific, each application may control only a portion of the pest complex present in a field.

IRAC Group 11: Microbial Disruptors of Insect Midgut Membranes – Includes Transgenic Crops Expressing *Bacillus thuringiensis* Toxins

The most important microbial insecticide, *Bacillus thuringiensis* or Bt, is a gram-positive, rod-shaped, spore-forming bacterium that takes its name from the German state of Thuringia where it was isolated in 1911 as a bacterial disease of flour moth caterpillars. Like most pathogenic organisms, Bt is active only against a very narrow range of host species. A strain of Bt had already been isolated in Japan in 1901 as the cause of a silk worm malady, and more than 100 other strains active against specific target insects have since been found, the most important of which are *kurstaki,* used on Lepidoptera; *israelensis* against Diptera; and *san diego* and *tenebrionis* against Coleoptera. *Bacillus sphaericus* and *B. firmus* are related species with activity against mosquito larvae and certain nematodes, respectively.

Bt is transmitted by spores: dormant bacterial cells that are long-lived and resistant to heat, desiccation and radiation. Spores contain genetic material, cytoplasm and all other materials needed to sustain life, but are in a form of suspended animation. Bt spores are packaged with crystals that help them infect the next host insect. After ingestion by a suitable insect, the crystals dissolve, liberating protein toxins that destroy the gut lining, facilitating infection of host tissues by the actively replicating bacterial cells. Towards the end of the infection, as the decaying body of the dead host becomes less hospitable, the bacteria re-enter the sporulation phase to begin the process of transmission to the next host.

BT can be grown in a fermentation broth to produce spores and crystals that can be formulated into sprays, which have been used on crops since 1938. Because of their high specificity, Bt sprays are considered very safe and are approved for organic farming. However, Bt sprays have low capacity to reach cryptic insects, and heat, desiccation or exposure to ultraviolet radiation can reduce their effectiveness. Therefore, proper storage conditions, timing and application procedures are important, and multiple sprays may be needed to provide adequate control.

Advances in genetic engineering technology made it possible to move the toxin genes from bacteria into crop plant germ lines. Beginning in 1996, cotton, maize, potato and

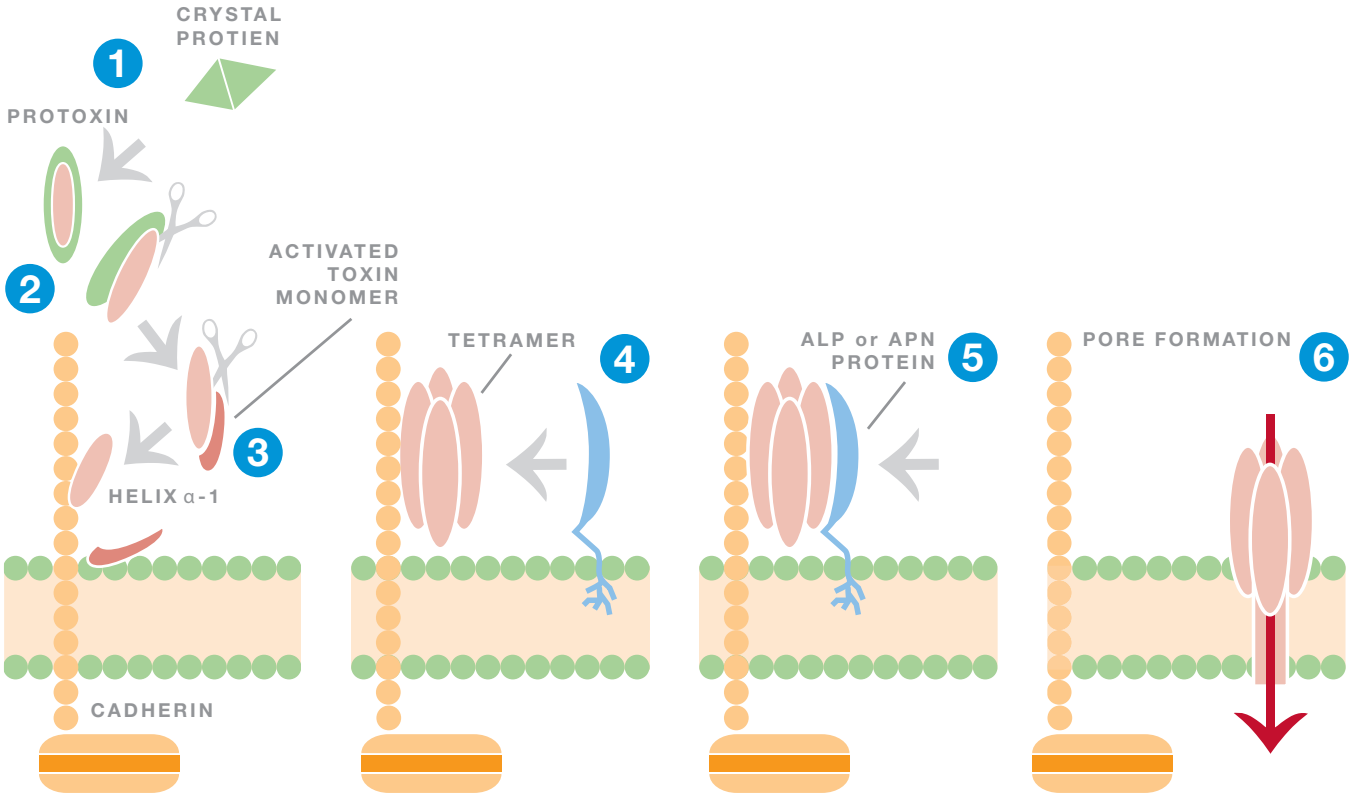
soybean varieties genetically modified to produce insecticidal levels of Bt toxin proteins were introduced worldwide, significantly reducing the use of insecticides against lepidopteran and coleopteran pests on these crops. In 2011, an estimated 65% of corn and 75% of cotton hectares in the U.S. were planted with varieties expressing Bt toxins.

Examples of Bt Insecticides

- Common name *B.t. israelensis* – trade names Sentry®, Gnatrol®
- Common name *Bacillus sphaericus*
- Common name *B.t. aizawai* – trade name XenTari®
- Common name *B.t. kurstaki* – trade names Javelin®, Turex®, DiPel®
- Common name *B.t. tenebrionis* – trade names M-Trak®, Novodor® (both discontinued)

Mode of Action and Resistance: When Bt crystals are ingested by an insect, they dissolve under the alkaline conditions of the gut to liberate one or more protein toxins. Of those, only the crystal, or Cry toxins, have been engineered into crops. More than 500 Cry proteins in 67 classes were identified in the various known Bt strains. Cry toxins liberated from Bt crystals are actually protoxins, requiring two successive transformations in the host before they become active. The first step is the cleavage by gut protein-digesting enzymes to yield an activated toxin monomer. The activated monomer passes through the peritrophic membrane and binds to a specific cadherin on the brush border membrane of epithelial cells. Cadherins are proteins on the surface of cells that serve a variety of functions, including binding cells together into tissue. Binding to a cadherin triggers removal of the toxin fragment's N-terminal end, known as helix α-1. With helix α-1 gone, toxin monomers are able to self-assemble into tetramers, which bind to secondary receptors on the epithelial surface. These receptors can be either aminopeptidase N or alkaline phosphatase, both of which are abundant. After binding, the toxin tetramer partially inserts into the membrane to make pores that lyse the cells and destroy the integrity of the gut. This gut destruction by the action of the toxin is sufficient to kill the insect by fluid loss and septicemia. In bacterially infected hosts, the actively replicating bacteria can also invade and replicate in the host tissues. Although a few days may elapse before the insect dies, it stops feeding soon after ingesting Bt.

From the beginning, it was realized that widespread use of Bt crops would speed resistance development in target pests if countermeasures were not taken, and the best practical strategy was the high dose refuge strategy. In this strategy, a high dose of Bt is expressed, so that survivors are rare. Furthermore, a significant number of non-Bt trait plants are required in the vicinity of fields sown with Bt trait varieties. These must not be treated with insecticides, in order to ensure the availability of an excess of susceptible insects to mate with the rare Bt survivors. Bt resistance genes are recessive, so offspring of a union between susceptible and resistant individuals would be susceptible and unable



to survive on the Bt crop. This strategy has been recommended for all Bt crops, and when implemented has proven effective in delaying resistance. Where it has not been used, resistance has indeed developed.

Several different resistance mechanisms affecting the various steps of toxin action have been observed in the lab, but only modified cadherins no longer recognized by the toxin active fragments have been observed as a resistance mechanism in the field. Resistance due to modified cadherins has occurred in several lepidopteran species, but is unstable and reverts in the absence of selection.

Environmental and Toxicological Considerations: The toxins produced by entomopathogenic *Bacillus* are highly selective for a narrow range of target insects and non-toxic to other organisms. Therefore, there are minimal risks associated with exposure to microbial products. If inhaled or rubbed on the skin, the spores and the dust or liquids used as carriers of the products may cause allergic reactions.

The major environmental concerns with "Bt crops" and their wide-spread use, are effects on non-target insects and potential transgene flow to native plant species. As of 2013, extensive studies have failed to identify any such negative impacts of Bt crops, and significant reduction of chemical insecticide use has in fact been realized. (Source: Informa Healthcare USA, Inc.)

left:
Mode of action of Cry toxins; (1) Crystal solubilization; (2) protoxin proteolytic activation; (3) activated toxin monomer binding to cadherin and cleavage of helix α-1; (4) pre-pore tetrameric structure formation; (5) tetramer binding to APN or ALP; and (6) pore formation.

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Non-Specific Multi-Site Insecticides

IRAC Group 8: Miscellaneous Non-Specific (Multi-Site) Inhibitors

In contrast to the insecticides previously discussed, most of which interact selectively with only one specific protein, Group 8 insecticides are reactive compounds that chemically modify proteins in a specific way that can affect multiple targets. This makes them less selective, but also makes them almost immune to target site-based resistance, compared to more specific compounds.

IRAC Group 8A: Alkyl Halides

Methyl bromide, which represents alkyl halide compounds, has been used as a structural fumigant as well as a pre-plant soil fumigant to control pests across a wide range of agricultural and commercial sectors. Methyl bromide is an odorless, colorless gas that can be produced either in the laboratory or biologically by bacteria, fungi and seaweed.

Example of Alkyl Halides

- Common name methyl bromide

Mode of Action and Resistance: Methyl bromide is a reactive chemical that reacts and donates its methyl group to sulfur-containing amino acids in proteins, thereby disrupting the function of many proteins. Because there is no single target site, target site resistance is unlikely.

Environmental and Toxicological Considerations: Brief exposure to high concentrations and prolonged inhalation of lower concentrations can be toxic to humans. It can cause respiratory distress, cardiac arrest and central nervous system effects. Signs of exposure include nausea, abdominal pain, weakness, confusion, pulmonary edema and seizures. Persistent neurological deficits are frequently present after moderate to severe poisoning. Because methyl bromide was determined to be a significant ozone-depleting substance, its general use was phased out under the Montreal Protocol in 2005 in industrialized countries, and will be eliminated in developing countries by 2015. Selected uses, including quarantine applications, will remain and are exempt from the phase-out regulation as long as effective alternatives are not available.

IRAC Group 8B: Chloropicrin

Chloropicrin was first synthesized in 1848 and was patented as an insecticide in 1908. It was used as a toxic tear gas in World War I for its severely irritating, lachrymatory and toxic effects. It moves rapidly in soil and is used as a soil fumigant for the control of insects, nematodes and fungi in a wide range of agricultural and non-agricultural crops, including fruits, vegetables and ornamental plants. It is often used in combination with other fumigants (1,3-Dichloropropene; Iodomethane; Methyl Bromide) for greater potency and spectrum of activity, and as a warning agent for these otherwise odorless fumigants.

Example of Chloropicrin

- Common name chloropicrin – trade name Metapicrin®

Mode of Action and Resistance: Chloropicrin is highly reactive and reacts with sulfur-containing amino acids in multiple proteins and other biomolecules.

Environmental and Toxicological Considerations: Chloropicrin is a strong lachrymator (tear gas) and is severely irritating to eyes, skin and mucosal membranes of the respiratory and gastrointestinal tracts, causing nausea, vomiting, difficulty breathing and respiratory tract inflammation. Because of its high volatility, the main route of human exposure to chloropicrin is inhalation. Damage to the respiratory tract can lead to pulmonary edema and death. Chloropicrin can be absorbed systemically through inhalation, ingestion and the skin. It is severely irritating to the lungs, eyes and skin, causing potentially fatal tissue damage and edema at higher levels. In the atmosphere, it is rapidly degraded and does not deplete the ozone layer.

IRAC Group 8C: Sulfuryl Fluoride

With methyl bromide being phased out in both developed and non-industrial nations, the use of sulfuryl fluoride as a replacement has increased rapidly. This odorless, colorless gas is used as a fumigant to kill insects post-harvest on products like grains, fruit and nuts, and to control drywood termites in structures. It is also being evaluated as a soil fumigant

Example of Sulfuryl Fluoride

- Common name sulfuryl fluoride – trade names Vikane®, Profume®

Mode of Action and Resistance: As a fumigant, sulfuryl fluoride penetrates materials and insect bodies rapidly. Containment is important, to ensure that it is present in the insect's body long enough to be broken down to release toxic fluoride ions, which are known to inhibit several enzymes. The insecticidal mechanism of fluoride ions is not well understood, but is thought to involve inhibition of one or more key enzymes. Its protective action against dental caries is due to the formation of a complex with magnesium and phosphate that inhibits the enzyme enolase, which is important for sugar utilization by bacteria. Resistance to sulfuryl fluoride has not yet been reported.

Environmental and Toxicological Considerations: Sulfuryl fluoride is a colorless odorless gas that is highly toxic to humans if inhaled. Symptoms may include weakness, nausea, vomiting, hypotension, metabolic acidosis, hypocalcemia, cardiac dysrhythmia and pulmonary edema, which can be fatal if not treated. Fumigation of food with sulfuryl fluoride leaves a significant residue of fluoride ions behind. In January 2011, the US EPA proposed significant restrictions on the use of this compound in the food industry on the grounds of fluoride's negative effects on children, but a final decision is still pending. Sulfuryl fluoride has been classified as a greenhouse gas, but it is not ozone-depleting.

IRAC Group 8D: Borates (Sodium Borate, Sodium Tetraborate Decahydrate)

Boric acid and borate salts have been registered since 1948 as nonselective herbicides, fungicides and insecticides. Many formulations are available, including liquids, soluble and emulsifiable concentrates, granules, powders, dusts, pellets, tablets, solids, pastes, baits and crystalline rods, The major insecticide uses are as a wood preservative and for control of cockroaches and other crawling pests, where it is safe for household and kitchen use.

Examples of Borates

- Common name sodium borate – trade name Niban®
- Common name boric acid – trade names CB Borid®, CB Drax®, MotherEarth®, Perma-Dust®, Eaton's® Answer®, BorActin™, InTice™
- Common name borax (sodium tetraborate, NaB_4O_7) – trade name Solubor®
- Common name disodium octaborate ($Na_2B_8O_{13}$)
- Common name sodium metaborate ($NaBO_2$) – trade name Tim-bor®

Mode of Action and Resistance: All borate salts dissolve in the body to yield boric acid. There is no information available on the mode of action of boric acid and borates. The symptomology in insects includes reduced appetite, weight loss, desiccation and mortality. Boric acid powder is said to abrade the exoskeleton of crawling insects, leading to desiccation, but this appears to be supposition and is probably an oversimplification. Resistance to boric acid and borates has not been reported.

Environmental and Toxicological Considerations: Boric acid and borates occur naturally and are re-released into the environment by many human activities including the use of borate salt laundry products, coal burning, power generation, chemical manufacturing, copper smelters, rockets, mining operations and industries using boron compounds in the manufacture of glass, fiberglass, porcelain enamel, ceramic glazes, metal alloys and fire retardants. These products are non-mutagenic, non-carcinogenic, and relatively non-toxic to mammals, but have been fatal to humans and livestock when accidentally consumed in large amounts.

IRAC Group 8E: Tartar Emetic

Tartar emetic, antimonyl potassium tartrate, is used as the toxic agent in ant poisons and for the control of thrips. It is only used today in South Africa and Zimbabwe.

Example of Tartar Emetic

- Common name tartar emetic – trade names Tartox®, Brennotox®

Mode of Action and Resistance: The mode of action is unknown. Resistance in thrips has been reported, but the mechanism has not been determined.

Environmental and Toxicological Considerations: Tartar emetic is highly irritating to skin, eyes and mucus membranes.

Insecticides of Unknown Mode of Action

IRAC Group UN: Unknown or Uncertain Mode of Action

The compounds in this group have an unknown or uncertain mode of action because the target protein responsible for the biological activity is unknown or uncharacterized.

Azadirachtin

Azadirachtin is the principal insecticidal component of extracts of seeds of the neem tree, a large, fast-growing mahogany of tropical and subtropical regions of India, Pakistan, Bangladesh and Southeast Asia. Neem seeds were traditionally ground to a powder and mixed with water to control insects on crops. Azadirachtin is used to kill locusts, aphids, beetles, borers, bugs, caterpillars, flies, leafhoppers, leafminers, mealybugs, mole crickets, nematodes, psyllids, sawflies, scales, thrips, weevils, whiteflies and fruit flies.

Example of Azadirachtin

- Common name azadirachtin – trade names Ecozin®, Azatrol®

Mode of Action and Resistance: Azadirachtin acts on insect gustatory receptors to inhibit feeding, and also interferes with development by inhibiting synthesis of the neuropeptide that triggers ecdysone release. The molecular targets mediating these effects are not known. Resistance to azadirachtin has not been reported.

Environmental and Toxicological Considerations: Azadirachtin is practically non-toxic to mammals, birds and plants. It is moderately toxic to aquatic invertebrates, but exposure is negligible due to low application rates and rapid degradation. Since it is only active by ingestion of treated foliage, exposure of non-target insects and honeybees is minimal.

Bifenazate

Bifenazate is a nonsystemic contact acaricide with long residual action, used to control all stages of phytophagous mites on citrus, tree fruits, vines, hops, nuts, vegetables, ornamentals, cotton and maize.

Example of Bifenazate

- Common name bifenazate – trade names Acramite®, Enviromite®, Floramite®

Mode of Action and Resistance: The mode of action of bifenazate has recently been determined to be inhibition of mitochondrial electron transport complex III by binding in the Q_0 center (ubiquinol oxidation site) in the cytochrome b subunit. Mutations of two different amino acids in the binding site confer resistance to bifenazate and also to the group 20 miticide acequinocyl. Bifenazate will be reclassified in IRAC group 20D.

Environmental and Toxicological Considerations: Bifenazate is practically non-toxic to mammals, moderately toxic to birds and highly toxic to fish and aquatic invertebrates. It is moderately toxic to bees, but non-toxic to predatory mites and beneficial insects.

Benzoximate

Benzoximate is an acaricide used to control all stages of spider mites on pome fruit, stone fruit, citrus fruit, vines and ornamentals. It was commercialized in Japan in 1971 but discontinued there in 1998 and is currently registered only in Italy, South Korea, South Africa and Switzerland.

Example of Benzoximate

- Common name benzoximate

Mode of Action and Resistance: The mode of action of benzoximate is not known. Resistance has been reported in citrus red mite, European red mite and twospotted spider mite.

Environmental and Toxicological Considerations: Benzoximate is relatively non-toxic to mammals and fish, but is moderately toxic to predatory mites.

Cryolite

Cryolite is an uncommon mineral salt of sodium, fluoride and aluminum that was first discovered on the west coast of Greenland. The supply was depleted by 1987, and synthetic cryolite is now produced from the common mineral fluorite. Cryolite is used at very high application rates of 5-30 kg/ha to control Lepidoptera and Coleoptera on certain fruits, vegetables and citrus. 92% of total cryolite applied in the U.S. is used on grapes in California.

Example of Cryolite

- Common name cryolite – trade name Kryocide®

Mode of Action and Resistance: Cryolite is thought to act through the release of fluoride ions. The insecticidal mechanism of fluoride ions is not well understood, but is thought to involve inhibition of one or more key enzymes. Its protective action against dental caries is due to the formation of a complex with magnesium and phosphate that inhibits the enzyme enolase, which is important for sugar utilization by bacteria. The only known case of resistance to cryolite was in walnut husk fly, reported in 1943.

Environmental and Toxicological Considerations: Cryolite does not contaminate ground or surface water and is considered low risk to non-target organisms. However, like sulfuryl fluoride, it is currently under review by the US EPA because of the possibility of negative effects of fluoride on the neurodevelopment of children.

Chinomethionat

Chinomethionat is a quinoxaline fungicide and acaricide introduced in 1968 to control powdery mildew and spider mites on fruits, ornamentals, cucurbits, cotton, coffee, tea, tobacco, walnuts, vegetables and glasshouse crops. It is nonsystemic, with contact activity only.

Example of Chinomethionat

- Common name chinomethionat (oxythioquinox in Australia) - trade name: Morestan®

Mode of Action and Resistance: Chinomethionat reacts with sulfur-containing amino acids in proteins, thereby disrupting the function of many enzymes and other proteins. Because there is no single target site, target site resistance is unlikely. Chinomethionat resistance has not been reported.

Environmental and Toxicological Considerations: Chinomethionat has been shown to have low toxicity to mammals, birds and bees, but is highly toxic to fish and some aquatic invertebrates.

Dicofol

Dicofol is a selective miticide in use since 1957 for control of many phytophagous mites on a wide range of crops, including fruits, vines, ornamentals and field crops. It is nonsystemic, with contact action.

Example of Dicofol

- Common name dicofol – trade name Kelthane®

Mode of Action and Resistance: Although dicofol is a close structural analog of DDT, and is even produced in some insects as a metabolite of DDT, its effects on sodium channels are weak, and the mode of action is considered to be undetermined. Mites with target site resistance to pyrethroids are not cross-resistant to dicofol, and dicofol resistance has so far been found to be due to enhanced metabolism.

Environmental and Toxicological Considerations: Dicofol has moderate toxicity to mammals and birds, but is highly toxic to aquatic organisms, including fish and invertebrates. Dicofol is moderately persistent, and has moderate bioaccumulation potential in fish, with a half-life of several weeks. Labeling and other risk mitigation measures must be followed to avoid contamination of surface water from spray drift and runoff.

Pyridalyl

Pyridalyl was introduced by Sumitomo in 2004 for the control of Lepidoptera and thrips. It is highly selective for these orders, a preferred characteristic in integrated pest management programs. It appears to have a new mode of action and is active against insects resistant to other compounds.

Example of Pyridalyl

- Common name pyridalyl – trade name Pleo®

Mode of Action and Resistance: Pyridalyl is selectively cytotoxic to cells of target species, by an unknown mechanism.

Environmental and Toxicological Considerations: Pyridalyl has low toxicity to non-target organisms. However, because it has a high potential for bioaccumulation and is persistent in soil, sediment and water, and may accumulate with repeated use, it is only approved in the U.S. for greenhouse use.

Pyrifluquinazon

Pyrifluquinazon was introduced in Japan in 2007 to control aphids, whiteflies, thrips, mealybugs and scale insects on vegetables and ornamentals. It received U.S. registration for greenhouse use on ornamentals in 2013.

Example of Pyrifluquinazon

- Common name pyrifluquinazon – trade name Colt®

Mode of Action and Resistance: Pyrifluquinazon modifies insect behavior, rapidly stopping feeding such that insects starve to death. It may have the same mode of action as pymetrozine, but this has not yet been determined. Resistance to pyrifluquinazon has not yet been reported.

Environmental and Toxicological Considerations: U.S. registration of pyrifluquinazon restricts it to greenhouse use on ornamentals because of its persistence and high toxicity to freshwater invertebrates.

Product Safety

Today and in the future, farmers need to produce more food and fiber from less cultivated land. This requires a careful and thoughtful balance of agricultural technologies, including crop protection, plant biotechnology and other more traditional farming practices, to sustain yields. Crop protection products protect against pests, weeds, and diseases, not only during cultivation, but also during storage, where crops are highly susceptible to damage. Without crop protection products, food losses would be significant. The challenge is to do all this while minimizing the impact on the environment – the ultimate resource for future generations of farmers and consumers.

BASF is committed to protect human health and the environment through the development of innovative products, technologies and services, to promote and support the safe and responsible use of our crop protection technologies, and to support the inclusion of crop protection products in sustainable agriculture worldwide.

As we work to bring new products to market, we place a huge emphasis on consumer safety, conforming to rigorous internal and external guidelines. Pesticides are some of the most researched and regulated products on earth. Each product takes eight to ten years to develop. Before registration is granted, more than 800 specific tests of a product's environment and health impact must be conducted. During the registration process, a label is created. The label contains directions for proper use of the material in addition to safety restrictions. This allows new products to be introduced in a safe, predictable manner, while providing consistent and clear guidance to national and international food safety authorities, farmers, distributors, and retailers.

It goes without saying that we meet all relevant laws, regulations and international agreements by acting in accordance with the principles of Responsible Care®, the FAO (United Nations' Food and Agriculture Organization) international code of conduct on the distribution and use of pesticides and our own high internal standards. However, as a good corporate citizen, we want to go the extra mile to help farmers use our products in a responsible way, with due care for both human health and the environment.

BASF works closely with customers and suppliers to help them adopt and further develop consistent global standards, and to promote best practice. By ensuring the appropriate use of crop protections products, we train farmers to grow more food and avoid crop losses.

Product stewardship is at the very heart of our contribution to sustainable agriculture. This begins at the research and development phase of a product, continues through distribution and use, storage, and ultimately, safe disposal of any waste. This lifecycle approach to product management ensures responsible and ethical management of our crop protection and biotechnology products, and protects the health of farmers and consumers, as well as the environment.

above:
The first documented case of insecticide resistance was of San Jose scale to lime sulfur, reported in 1914 in Washington state. In 1947, resistance to DDT was confirmed in houseflies after only four years of use. Since then, resistance to each new insecticide class has appeared in a number of key pest species within 2 to 20 years after its introduction.

Insecticide Resistance

What is Insecticide Resistance? Resistance is an inherited change in susceptibility of a pest population to an insecticide, which is generally reflected in the product's failure to achieve the expected level of control or efficacy as defined by the labeled use rate, frequency of application, length of control or economic threshold. Control or efficacy can be defined in terms of insect population reduction, yield or quality protection, or improvement of plant vigor or health, where collectively these benefits result in a financial return on investment.

Resistance arises because of artificial selection of insect populations with an insecticide. As a result of continued applications (exposure) over time, initially very rare naturally occurring traits that confer resistance are favored and thereby selected. The surviving insect population becomes increasingly difficult to control at the labeled rate and application interval, as individuals with these traits selectively thrive and proliferate. This in turn leads to more frequent applications of the insecticide in order to achieve the same level of control. Both the intensity of the resistance and the frequency of insecticide-resistant individuals in the population increase as more frequent, less effective treatments are applied. Eventually, no control is provided and users switch to another pesticide if one is available. This phenomenon of sequential insecticide applications, treatment failures and resistance selection has been described as the 'pesticide treadmill', and the sequence is familiar.

The Arthropod Pesticide Resistance Database *(http://www.pesticideresistance.com/)* is a comprehensive list of confirmed cases of resistant arthropod species. As of this writing, insecticide/miticide resistance has been confirmed in 553 pest species.

Examples of insects that tend to readily develop resistance to insecticides

Insect	Insecticides Resisted
Green peach aphid	Neonicotinoids, carbamates, organophosphates, pyrethroids, cyclodienes
Diamondback moth	Many insecticides
Colorado potato beetle	Many insecticides
House fly	Many insecticides
Maize earworm	Pyrethroids, cyclodienes, carbamates, organophosphates
Bed bugs	Pyrethroids, cyclodienes, organophosphates
Western root worm	Cyclodienes, carbamates, organophosphates, Bt toxins
Indianmeal moth	Organophosphates, cyclodienes, Bt toxins, pyrethrins
Anopheles mosquitoes	Many insecticides
Tobacco budworm	Many insecticides
Greenhouse whitefly	Many insecticides

Resistance to insecticides reduces the effectiveness of insect control, resulting in lowered agricultural productivity and higher human health risks. At the same time, however, resistance stimulates investment in research leading to the development of new compounds to replace older chemicals.

The speed of resistance development depends on several factors, including how fast the insects reproduce, measured in generations per crop season, the migration and host range of the pest, the availability of nearby susceptible populations, the persistence and specificity (site of action) of the crop protection product, and the rate, timing and number of applications made to a specific population. Resistance increases more rapidly in situations such as greenhouses, where insects or mites reproduce quickly, there is little or no immigration of susceptible individuals and the user may spray more frequently.

Insecticide Resistance Mechanisms

There are several means by which insects can become resistant to the effects of an insecticide.

- Metabolic resistance is the most common type of resistance. Insects possess a variety of enzymes, including oxidases, glutathione S-transferases, esterases and amidases, whose function is to degrade foreign compounds. Resistance to a toxicant can occur when the insects evolve an enhanced ability to detoxify or destroy the toxin, by alterations of these enzymes or of the amounts of enzymes produced in the body. As metabolic enzymes are directed at certain structural features of the toxicants, vulnerable sites in the molecule are affected. This type of resistance may not affect all members of a chemical class, and cross-resistance between compounds from different chemical classes can occur if they contain common chemical groups that are targeted by the same enzyme.
- Target site resistance is the second most common type of resistance, and involves a modification of the target protein structure or abundance, which usually confers some degree of cross-resistance to all compounds acting at that site. If the mutation involves a change in the structure of the pocket where the insecticide actually binds, the level of resistance can depend strongly on chemical structure and the modification could theoretically even favor the binding of certain analogs, leading to negative cross-resistance. Negative cross-resistance occurs when the insect's ability to develop resistance to one toxicant results in hypersensitivity to another.
- Behavioral resistance occurs when insects evolve the ability to detect and avoid the toxin or components of the formulation, or their behavior becomes modified so that they no longer come in contact with it, even if they can't detect it. An example of the latter is resistance of the horn fly to treatment with cattle ear tags. Fly populations evolved to avoid the animal's head, rendering them resistant to all insecticides applied by ear tags.
- Penetration resistance is where insects evolve to absorb the toxin through the cuticle or gut lining more slowly compared to susceptible insects.

Although it may not be possible to prevent resistance indefinitely, prudent application of a few proven insecticide resistance management (IRM) principles has the potential to delay the appearance of resistance or to maintain existing resistance traits at low enough levels that the efficacy of valuable insecticides can be sustained for a very long time. These principles of insecticide resistance management are described below.

Managing Insecticide Resistance

Economic Thresholds

Insecticides should be used only if insects are numerous enough to cause economic losses that exceed the cost of the insecticide treatment including application cost, or where there is a threat to public health. Exceptions are in-furrow, at-planting or seed treatments used for the control of early season pests that from experience are known to exceed damaging levels or economic thresholds annually. Farmers are always encouraged to consult local advisors about economic thresholds of target pests in their areas.

Integrated Control Strategies

Growers should incorporate as many different control strategies as possible, including the use of synthetic insecticides, biological insecticides, beneficial insects (predators/parasites), cultural practices, transgenic plants (where allowed), crop rotation, pest-resistant crop varieties and chemical attractants or deterrents.

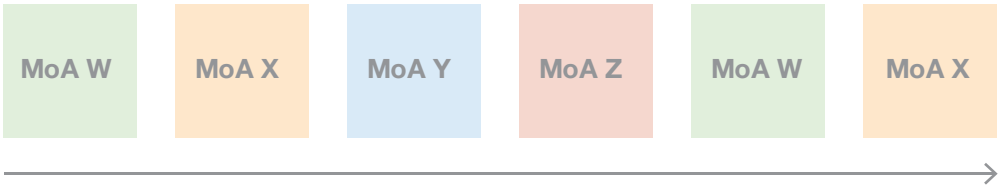
Applications of insecticide must always follow label guidelines and be timed correctly, targeting the most vulnerable life stage of the insect pest. The use of spray rates and application intervals recommended by the manufacturer and in compliance with the approved label and local regulations is essential.

It is important to mix and apply insecticides carefully. As resistance increases, the margin for error in terms of insecticide dose, timing, coverage, etc., assumes even greater importance. Recommendations from manufacturers and local advisors should be followed and be within the limitations of the approved label.

Growers should also consider crop residue options. Destroying crop residues can deprive insects of food and overwintering sites. This cultural practice can kill pesticide-resistant pests (as well as susceptible ones) and prevent them from producing resistant offspring for the next season. However, growers should review their soil conservation requirements before removing residues.

Rotation of Insecticides

A key element of effective resistance management is reducing selection pressure by rotating or alternating between insecticides with different modes of action in order to avoid selecting successive generations of insects for the same target site resistance mechanisms. As many different MoA groups as possible should be included in the rotation, as illustrated below. It is also important to avoid rotating between compounds that would be metabolized in the same way, but this is a complicated topic that is not yet codified by IRAC. Local resources (retailer or agronomic expert) should be consulted.



SEQUENCE OF INSECTICIDES THROUGH SEASON

It is important to consider the impact of pesticides on beneficial insects, and to use products at labeled rates and spray intervals, with attention to labeled precautionary and use guidelines in order to minimize undesired effects on pollinators, parasitoids and predators. Following are proven methods for resistance management using the IRAC MoA classification scheme:

1. Rotate compounds so that successive generations are not treated with compounds from the same MoA group.

2. In the event of a control failure, change to an insecticide with a different mode of action and to which there is no known cross-resistance.

3. Mixtures may offer a short-term solution to resistance problems, but each component must belong to a different IRAC mode of action group and be used at its recommended rate.

4. Different chemical families with the same mode of action are subgroups within a common IRAC MoA group. Rotate subgroups only when there is no alternative and no cross-resistance mechanism exists in the target populations.

5. Use bioassay and biochemical or genetic tests, if available, to identify and characterize resistance. Based on results from these tests, appropriate strategies can be developed.

Refugia

Some programs try to preserve susceptible individuals within the target population by providing a refuge or haven for susceptible insects, such as unsprayed areas within treated fields, adjacent refuge fields, or attractive habitats within a treated field that facilitate immigration. These susceptible individuals may out-compete and interbreed with resistant individuals, thereby diluting the impact of any resistance that may have developed in the population. A high-dose with refuge strategy is the only strategy recommended for crops expressing Bt toxins (pages 53-55).

Conclusion

While insects and mites have always been formidable competitors, global population growth continues to intensify the share of the earth's resources required by humans and the competition from these arthropod pests. Largely since the 1940s, successive generations of synthetic insecticides/miticides and microbially derived products have enabled the richness of low risk and effective pest management technologies available today. Lower toxicity insecticides/miticides are highly selective and specific in their actions, often taking advantage of small biochemical differences between pest species and their sometimes beneficial non-pest cousins that are not targeted. Development of such selective insecticides/miticides is expensive and increasingly difficult, and small genetic changes in the pests can lead to resistance and loss of effectiveness of the products - the biggest threat to our continued ability to control damaging arthropod pests. While resistance cannot be prevented, it can be significantly delayed with diligent use of resistance management strategies. In addition to minimizing insecticide/miticide usage by applying economic thresholds for treatment and integrated control strategies, rotation of compounds with different modes of action is a major component of resistance management, ensuring that target sites are not subject to undue selective pressure. Effective insecticide/miticide rotation requires an accurate classification of insecticides/miticides according to mode of action. This document explains the known modes of action of all insecticides/miticides on the market today, in the framework of the industry standard IRAC classification.

IRAC Group Index

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U.S. Environmental Protection Agency. http://www.epa.gov/

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