To who it may concern,

I am Gary Rondeau, a beekeeper and a scientist living in Eugene Oregon. I have studied the problem posed by insecticides on honeybees and other invertebrates beginning in about 2010 when I personally started to have trouble keeping my bees alive. I took on the task of identifying the most likely culprits for our bee declines and colony collapse that beekeepers across the country were experiencing. Beekeepers have been dealing with pesticides for many years, so I was at first not convinced that pesticides were the issue. However, the new class of pesticides that were becoming popular, the neonicotinoids, had some problematic properties that raised red flags. The issue that bothered me was what happened if you had low doses of the pesticide present for long periods of time. I looked at various research papers and concluded that this was an issue that needed further attention. I wrote a blog article on the subject that eventually became a published article: https://www.nature.com/articles/srep05566

Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termites

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***Scientific Reports* volume4, Article number: 5566 (2014)**

The article has been cited many times and I like to think of it as a chink in the armor that allowed the European Union to effectively ban the neonicotinoid pesticides throughout Europe.

In the process of learning about pesticides I have come to a much better understanding of their biological mechanisms and their environmental shortcomings. This has resulted in two blog articles that are not overly technical which I believe would benefit decision makers to understand the issues at hand. The links are here:

https://squashpractice.com/2014/06/15/the-mechanisms-of-neuro-toxic-pesticides/

https://squashpractice.com/2017/12/03/threshold-mechanisms-in-acetylcholine-pathway-insecticides-and-environmental-safety/

The point I wish to stress in the second article is that a key means to ensure environmental safety for chemical pesticides is that they exhibit a strong "threshold" type of non-linear dose-response action. Pesticides that exhibit strong threshold action include the organophosphate and carbamate classes of chemicals. Strong threshold action means that low residual doses of these chemicals are relatively benign. In contrast, chemicals without a strong threshold action begin to sicken target and non-target organisms at sub lethal doses and can pose unacceptable environmental risks at almost undetectable levels when organisms are continuously exposed to these nerve toxins.

Finally, recent studies have shown that the neonicotinoids not only attack synaptic nervous system receptors, but that these same receptors are commonly present on insect immune cells. These studies have provided the mechanism for what has been observed in the field, that

colonies exposed to low levels of neonicotinoids often succumb to a pathogens, often multiple pathogen species when colony collapse occurs. I reference several of these studies in the articles linked above.

The neonicotinoids are a very dangerous environmental hazard. They are likely a significant factor in the widely reported insect apocalypse where large fractions of the wild insect populations have disappeared. The neonics are water soluble so they move when it rains, eventually finding their way to the oceans. We need to stop using them immediately and hope that some of the lost insect diversity will recover.

Below are copies of the linked articles from my blog.

Thank you for your consideration.

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The Mechanisms of Neuro-toxic Pesticides

Gary RondeauJune 15, 2014Beekeeping, Ideas, Pesticides, Popular, ToxicsEdit"The Mechanisms of Neuro-toxic Pesticides"

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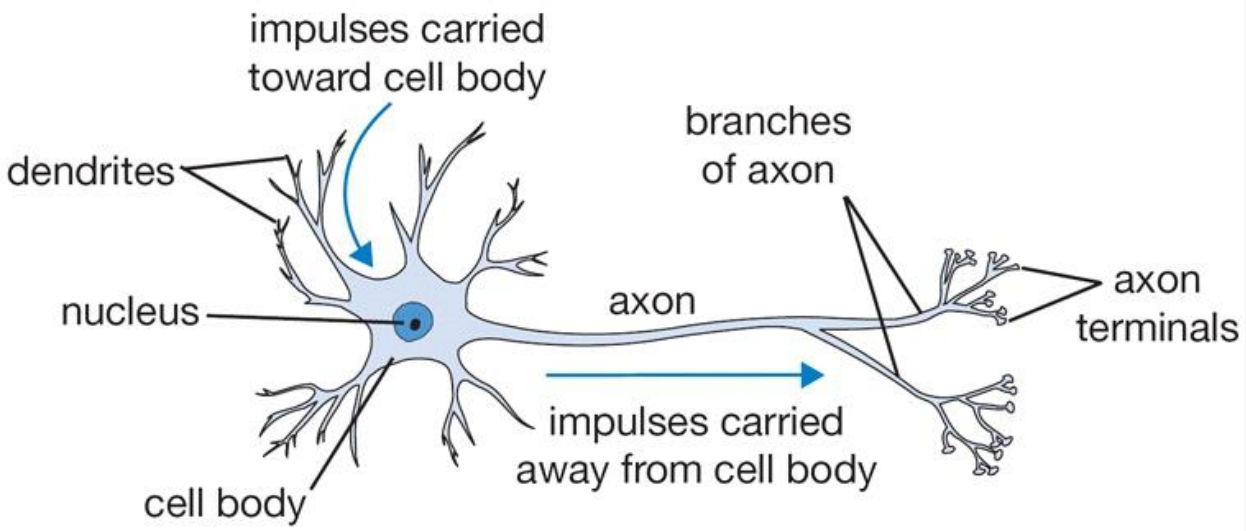
Agricultural pesticides have become part of the chemical landscape that we all live in. To be able to make intelligent decision about the use and regulation of these chemicals, it's important to understand how they work. Almost all modern pesticides are chemicals that interfere in some way with the nervous system. The characteristics of the chemical interaction with the nervous system function can shed light on the effectiveness of the pesticide and on its physiological effects at residual levels. We will start by looking at how some of the normal processes of the nervous system work, because it will be disruption of those processes that lead to toxic effect. Then we will look at the mode

of action for three major classes of pesticides and how they specifically interfere with normal function. In a future article we will look at how the specific mechanisms of action can effect dose scaling relationships.

Normal Neuron Function – Neurons, action potentials, sodium and potassium voltage gated ions channels, and ion pumps

The nervous system of insects and humans share many common features, starting with the basic structure of the neuron.



There are many variations on the same theme in different parts of the organism. Terminal branches can attach to dendrites of other neurons at synapses, or through motor synapses to muscle cells. Individual neurons are connected in complex, interacting networks by the synaptic connections. Information processing involves summing the inputs from many neurons and generating an output. When the summed stimulus is high enough, the neuron will generate an electrical pulse that is sent along the axon and which will, in turn, stimulate multiple downstream neurons connected through synapses to the axon branch terminals.

Neuronal signalling is accomplished by way of "action potentials", which are short electro-chemical pulse that travel along the neuron axon. The short pulse-like nature of the nerve signals are generated and maintained by way of "voltage-gated" ion channels and ion pumps. Ion pumps use the cellular energy store, ATP, to move sodium and potassium ions across the cell membrane, setting up a concentration gradient across the membrane that establishes a "resting potential" of about -70mV from the inside to the outside of the nerve cell. Once this gradient is established, then merely opening ion channels in the cell wall allows the sodium or potassium ions to move back across the membrane and move the potential closer to zero. Nature's trick, that turns this process

into a useful information processing network, is to open the ion channels which depolarize the neuron with a positive feedback action associated with the membrane potential. Once the membrane potential rises from its resting potential to a “threshold” the voltage gated channels open, steepening the rising edge into the action potential nerve pulse. The figure below is a nice schematic of the ‘anatomy’ of the action potential.

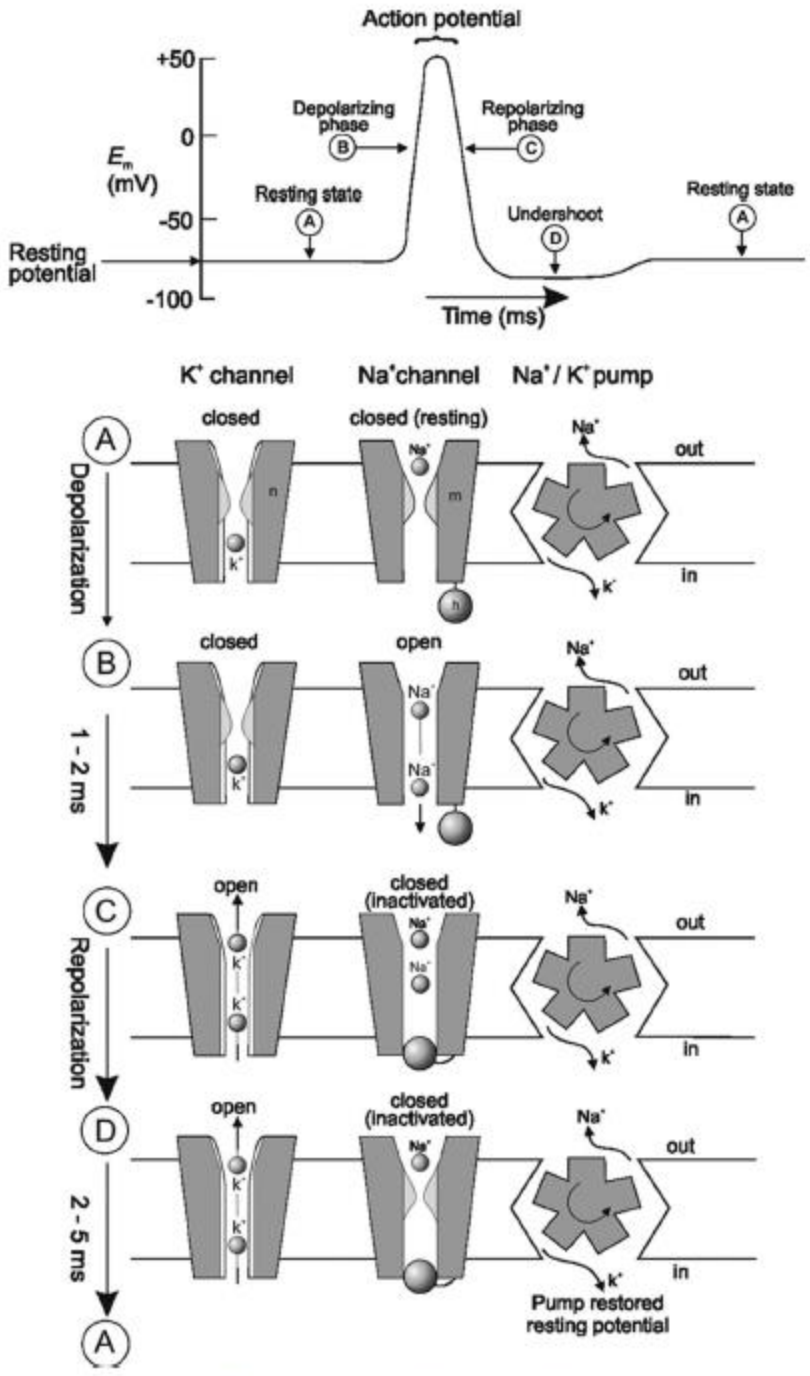
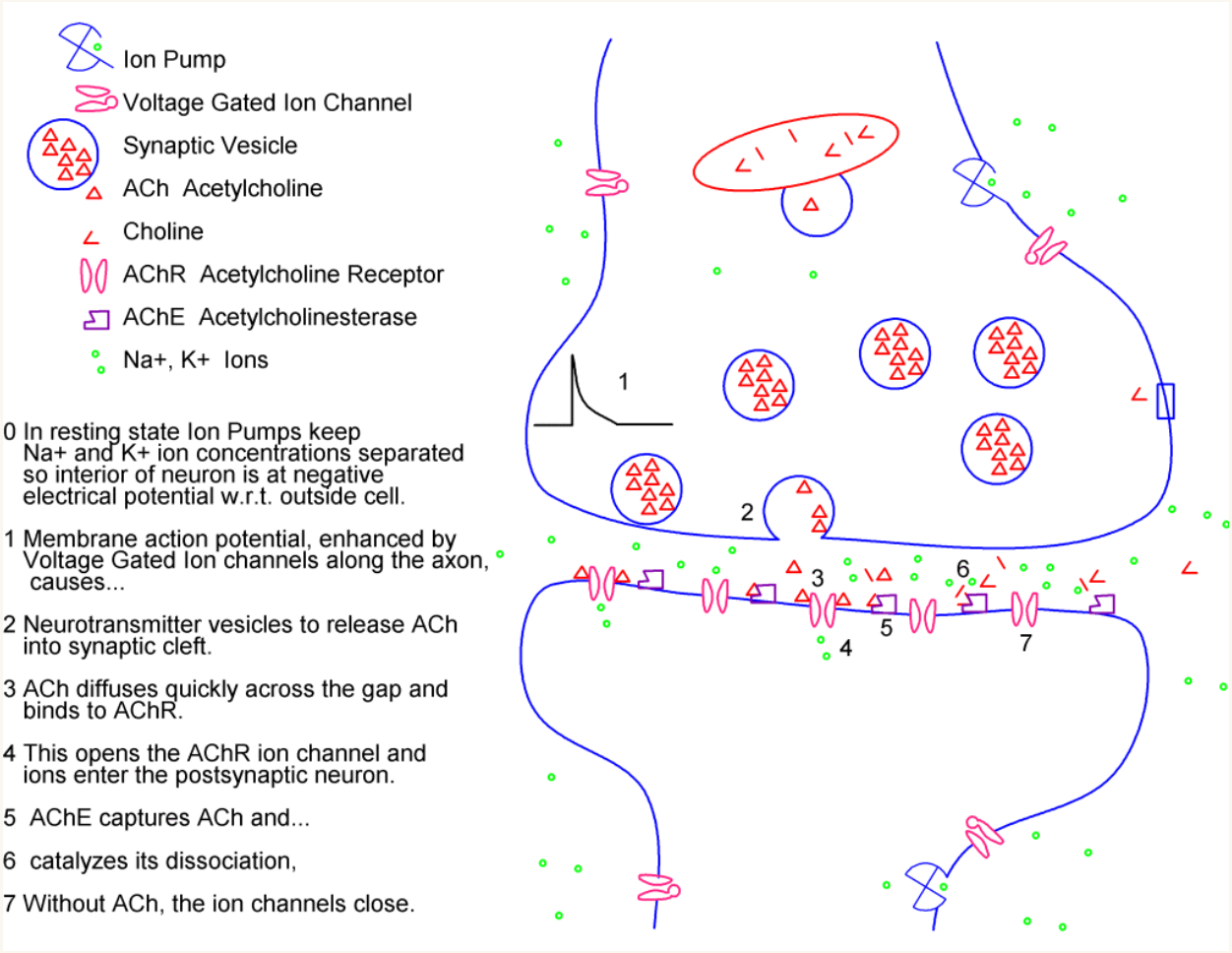


Figure 2. Generation of an action potential. The extracellular fluid surrounding the insect axonal membrane contains a high concentration of sodium ions (Na^+) and a low concentration of potassium ions (K^+), whilst the reverse is true for the inside of the nerve cell. At the resting potential (A) the axonal membrane is relatively permeable to K^+ but not Na^+. This makes the inside of the cell negative with respect to the outside, the difference in potential being around -60 mV. Nerve stimulation causes the axonal membrane to become permeable to Na^+ due to the sodium channel opening (B). This causes the inside of the axon to become transiently positive and generates the rising phase of the action potential. Sodium channel closure or inactivation (C) (usually within 1 ms) causes an efflux of K^+ as a result of opening of potassium channels and generates the falling phase of the action potential. The generation of the action potential results in sequential depolarization of neighbouring regions of the axon, resulting in a wave of depolarization along the axon. An ATP driven Na^+- K^+ pump maintains the ion gradient across the axonal membrane (D) and restores the resting potential. Continued transmission of the impulse across the synapse involves release of a chemical transmitter, which becomes attached to receptor sites at the postsynaptic membrane where it depolarizes the membrane to generate another action potential.

From Davies et al. 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. Signaling happens by way of the action potentials, which propagate along the axons and terminate at the synapse. There are several ways the action potential can be interact with cellular structures. We will concentrate on the acetylcholine mediated synaptic response because this is the target of several pesticide chemicals.

Normal Synapse Function – acetylcholine-mediated transmission



Acetylcholine (ACh) is a molecular neurotransmitter that conveys information across the synapse. In the figure above, the basic steps of the interaction are illustrated. Action potentials, those pulses of neural activity, cause synaptic vesicles containing ACh to release the ACh molecules into the synaptic cleft, the junction region between the two cells. The ACh quickly diffuses across the narrow junction region and is captured by acetylcholine receptors (AChRs) that are part of ion channel molecules. The AChRs that have captured an ACh molecule open the ion channel and allow Na+ ions to enter the post-synaptic neuron. The binding is transitory, however; the ion channels rapidly open

and close as the ACh molecules latch and unlatch from the AChR channel. Meanwhile, another ACh receptor is also present in the synaptic junction called acetylcholinesterase (AChE). This molecule is an enzyme which rapidly breaks apart the acetylcholine into choline and acetate, effectively ridding the synaptic cleft of the neurotransmitter almost as fast as it is made available. The result of all of this chemical activity is that the AChRs, as an ensemble, are open only for a few milliseconds. During this time, ions flood into the post-synaptic dendrite, depressing the potential in the down stream neuron, making it more likely to generate its own action potential.

This simplified discussion leaves out many details. There are many more specialized molecules that are part of cell membranes. Often molecules that are specific for one important function also are involved in unrelated functions. Nerve cells can be specialized and synaptic details can vary. Nevertheless, the basic picture we are painting is valid across much of the animal kingdom. These same basic process happen in the nervous systems of humans and bees alike. Now let us move on to discuss ways to interrupt these normal processes for insecticidal effect.

Insecticides targeting axonal voltage-gated ion channels

Two major classes of insecticides target the voltage gated ion channels shown in our cartoon. The organochlorines (e.g. DDT, dieldrin, chlordane) and pyrethroids (e.g. deltamethrin) act by opening these voltage gated ion channels. The molecules hold open the channels and allow ions into the axon that depolarizes the neuron. In the depolarized state the neuron is non functional, characterized by paralysis. In between the normal state and paralysis there is a range where the depolarization of the neuron is only partial. Partial depolarization leaves the neuron susceptible to “false triggering”. A small stimulus that would normally not trigger an action potential will produce one more easily as the resting potential gradually climbs to the threshold required to launch an action potential. Organisms in this state typically exhibit twitching and uncontrolled movements as the uncontrolled nerve impulses trigger muscles to move.

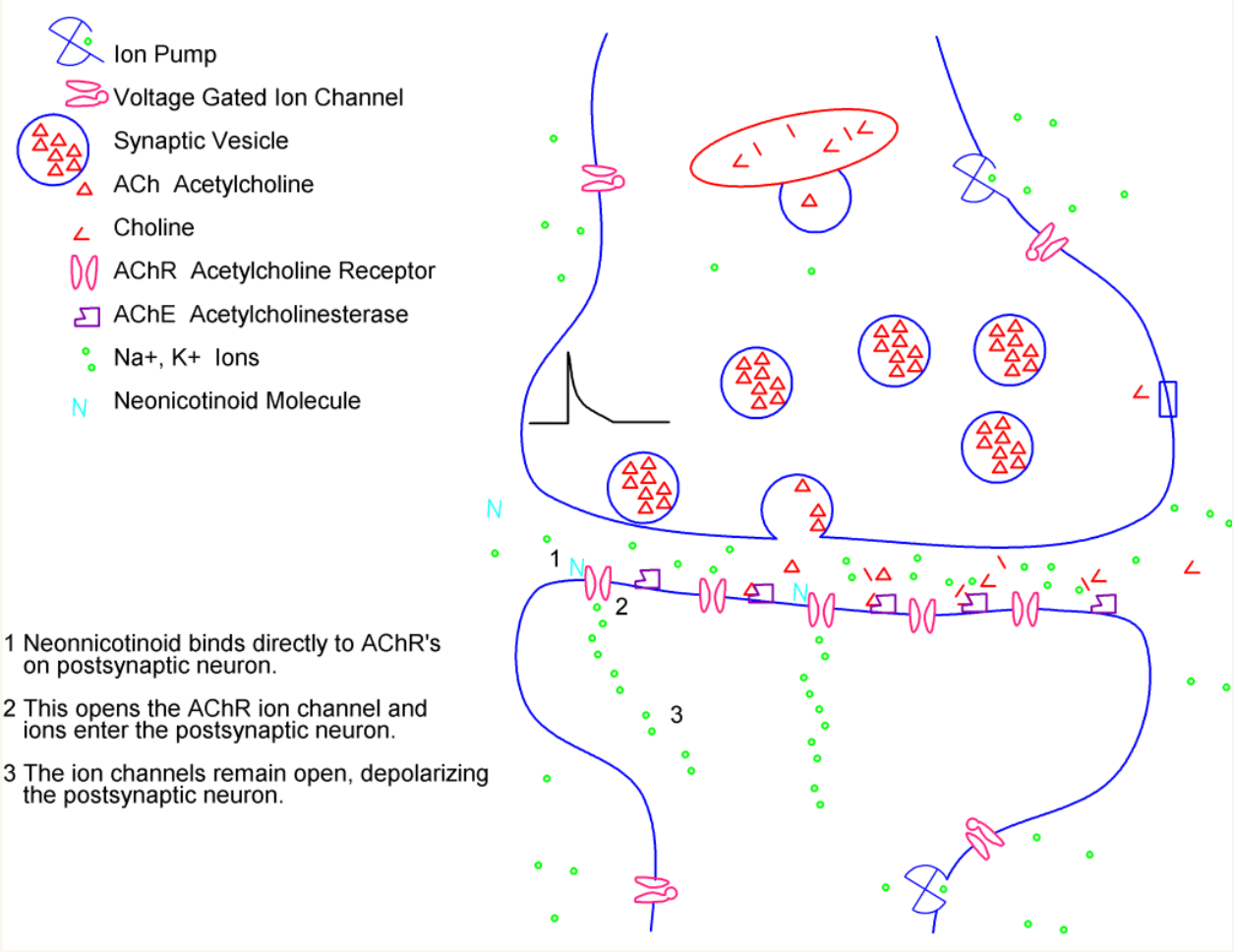
Nothing is static at the molecular scale. As organic molecules interact with one another, they can latch onto each other either very loosely or with tenacity depending upon the exact shape of the molecules involved and type of binding that happens. Binding that occurs via the covalent sharing of electrons is usually very strong, essentially permanent and irreversible. In contrast, many biological molecules interact through polar or Van Der Walls forces that are much weaker. Such interactions may last for a fleeting amount of time before thermal fluctuations pull them apart. Weak binding is reversible and can be characterized by a dissociation time, how long it takes to break the bond due to random and thermal fluctuations.

When dealing with pesticide chemicals, stronger bonds mean the insecticide is spending more time at the active site, so its potency is higher. Frequently it is just how tenacious the binding that determine the potency of the insecticide.

Chemical scavengers known as cytochrome P450 enzymes are always on the lookout for foreign chemicals which these enzymes break down into smaller parts in the process of metabolizing and eliminating unwanted molecules. Often, within a few hours much of a foreign chemical will be metabolized and eliminated from the organism. Bound molecules are not as easily digested by the cytochrome P450s so once toxins are bound to their site of action, they are more immune to detoxification.

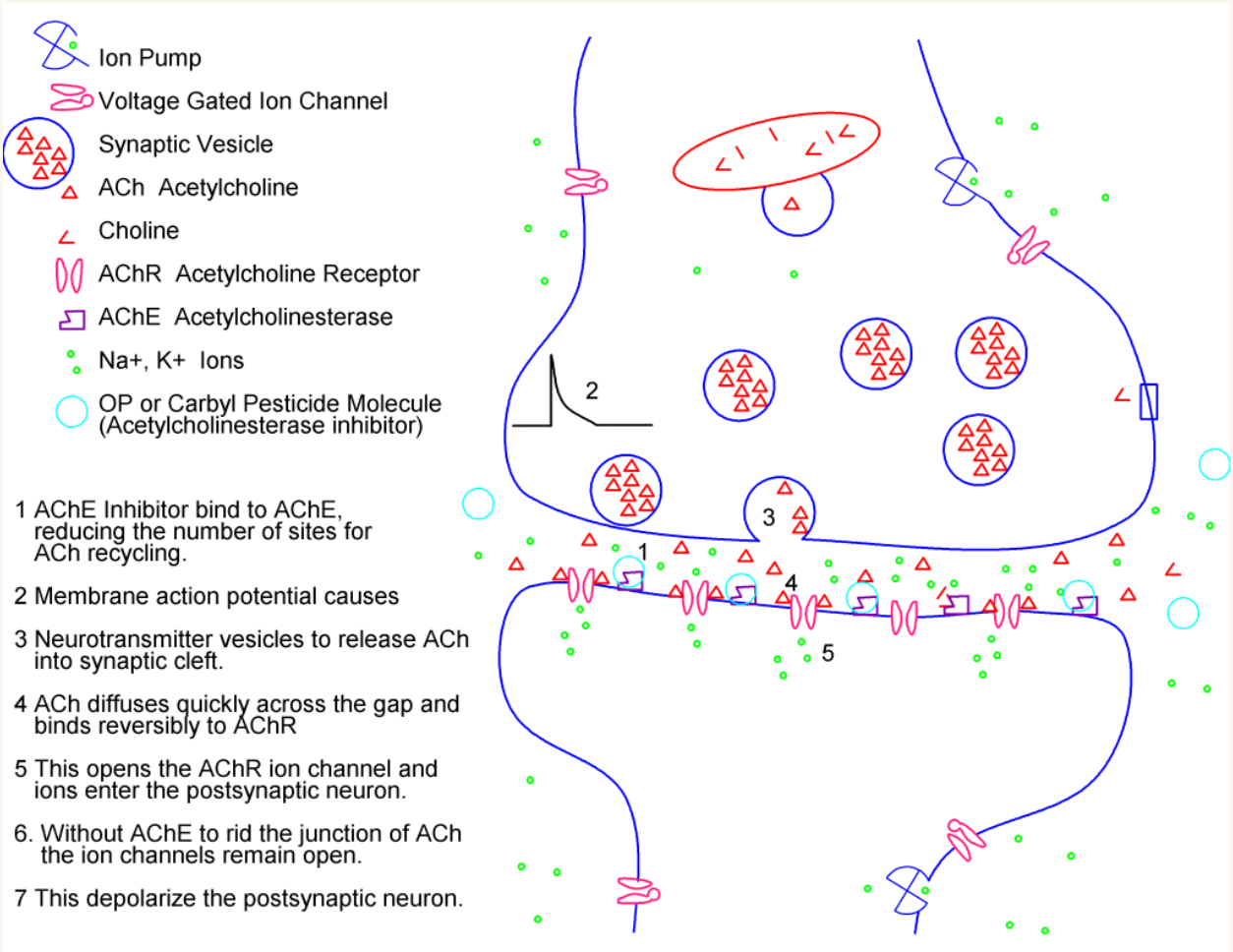
Insecticides targeting the acetylcholine pathway

There are several classes of pesticides that disrupt the acetylcholine pathway. We will start by looking at the neonicotinoids because they have the simplest mechanism, similar to the "direct action" of the pyrethroids discussed above.



The neonicotinoids bind strongly to the AChRs. Binding causes he ion channels to open so Na+ ions can flow into the neuron. Unlike the normal acetylcholine response where the channel is only open for about a millisecond, when the neonicotinoid binds the receptors never close. Hence, it takes only a relatively few open channels to eventually depolarize the neuron. If the ion pumps cannot keep up with the leakage through the nicotinoid-bound AChRs the cell will depolarize. Partial depolarization will make the neuron more excitable; complete depolarization leads to paralysis.

This situation is more complicated with acetylcholinesterase inhibitors such as the organophosphate and carbaryl insecticides. For these chemicals, the insecticide does not directly bind to neuronal receptors that open ion channels. Instead the chemicals bind to the acetylcholinesterase (AChE) enzymes which rid the synaptic junction of the ACh neurotransmitter that is released with normal activity. However, without the AChE to clear the junction, the ACh continues to bind with AChR ion channels. The figure below shows schematically what happens with these AChE inhibitors.



Insecticide molecules bind to the acetylcholinesterace (AChE) sites in the synaptic junction, preventing the naturally released ACh for being removed and recycled from the junction. The acetylcholine continues to activate receptors, keeping their channels open thereby depolarizing the post synaptic neuron. Again, poisoning symptoms begin with an over-excitable nervous system, characterized by uncontrolled twitching, similar to the other classes of neurotoxins we have looked at.

Neurotoxins are among the most potent biological chemicals known. The chemicals are targeted to interact with specific receptor molecules that are crucial for nervous system function. This means that very few pesticide molecules are required to have a large biological effect. Chemicals used as pesticides need to effectively poison target species while remaining benign to non-target organisms and humans. However, much of the cellular machinery is shared across the animal kingdom, so differentiating between target and non-target organisms is a challenge. Often only space and time are used to separate target and non-targets creatures from chemical exposure. The environmental effects of pesticide chemicals depends upon the success of various strategies to limit harmful exposure to non-target species. In many cases dilution is the solution, but as industrial agriculture and residential uses of potent chemicals become even more widespread, minute residual levels of toxins is inevitable. Next time we will see why this is more likely to be a problem with some classes of chemicals more than others.

See Threshold mechanisms in acetylcholine pathway insecticides and environmental safety

Threshold mechanisms in acetylcholine pathway insecticides and environmental s afety

Gary RondeauDecember 3, 2017Beekeeping, Ideas, Pesticides, ToxicsEdit"Threshold mechanisms in acetylcholine pathway insecticides and environmental safety"

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Overview

Previously we looked at some of the basic principles of nervous system function and how chemicals from several pesticide classes disrupt normal function. This time we will look in detail about what we can expect for a dose-response characterization of acetylcholine pathway insecticides based upon their mode of action and properties of the nervous system. This will get a little more technical than usual. The casual reader may want to skim over the equations but think about the explanations.

Several families of pesticides function by disrupting the synaptic acetylcholine pathway. Organophosphate pesticides and the carbamates block the enzyme acetylcholinesterase (**AChE**) such that the naturally released neurotransmitter, acetylcholine (**ACh**), is not broken down and recycled. Instead **ACh** builds up in the synaptic junction and over-stimulates the acetylcholine receptors (**AChR**) on the post-synaptic membrane.

The neonicotinoids act directly by bonding strongly to the nicotinic acetylcholine receptors (**nAChR**) in a manner that holds open the receptor ion channel.

Both classes of chemicals, the **AChE** inhibitors and the **nAChR** agonists, produce excessive numbers of activated acetylcholine receptors on the post synaptic membrane, which gives rise to a reduction in the post synaptic resting potential and a propensity to generate action potentials in the post synaptic neuron. Acute poisoning occurs when the general level of neural stimulation is sufficient to disrupt the normal physiological processes required to sustain life. Clinically, insects and animals poisoned with either class of chemicals are seen to lose muscular control, exhibit uncontrolled twitching, eventual paralysis, and death.

We will begin by considering a single synapse and come up with a relationship for the post synaptic stimulation as a function of the fractional lethal chemical level. We will also consider implications of acetylcholinesterase disruption for an entire neural network. Finally we will seek to understand the environmental implications of threshold versus non-threshold action with these chemicals.

Synaptic electro-chemical function

The nervous system is governed by neuronal generated “action potentials”, rapid electrical potential changes that travel rapidly along the neural axons and terminate in the branching tree of dendrites at synapses where they can cause the release of neurotransmitter into the synaptic cleft. The neurotransmitters rapidly diffuse across the synaptic junction and attach to receptors on the post-synaptic membrane. These transiently bound receptors change the permeability of the membrane and allow ion currents to flow across the membrane, thus altering the local cellular electrical potential in the post-synaptic neuron.

Action potentials are fast transients that last 1-3 milliseconds. Diffusion time of **ACh** across the junction is faster, measured in microseconds, and the decay of synaptic free-circulating **ACh** is normally around one millisecond. The time response of the excitatory post-synaptic potential is slightly slower, typically lasting several to 10’s of milliseconds.(1) This allows the post-synaptic neuron to be the summing junction from many synaptic inputs, doing some kind of dynamic averaging that determines whether or not the downstream neuron will produce its own action potential. We argue that changing the decay time of **ACh** in the synaptic junction relates directly to stimulation that will produce an action potential in the downstream neuron. Roughly, doubling the decay time is likely to double the likelihood of the downstream neuron generating its own action potential because the amount of post-synaptic charge transfer will be proportional to the length of time **ACh** holds open the **AChR** receptors, and this open time is within the typical averaging period of the neuron.

Acetylcholinesterase Inhibitors – Consider a single synapse

A normal stimulus, S_0, produced by a single action potential in the downstream neuron can be written as

[1] $$S_0 = kN_R N_A \tau_A$$

Where N_R is the concentration of **AChR**s, N_A is the concentration of **ACh** released by the action potential, k is a proportionality constant and τ_A is the lifetime of **ACh** in the synaptic junction.

The primary way acetylcholinesterase inhibitors act is by reducing the number of **AChE**molecules available to catalyze the destruction of **ACh** in the synaptic junction. It is reasonable to expect that decreasing the number of available **AChE** molecules will proportionally increase the time it takes for **ACh** molecules to be degraded. If we assume that a fraction, f, of the **AChE** is bound with inhibitor, then we estimate the ACh lifetime,τ, in the presence of **AChE** inhibitor as

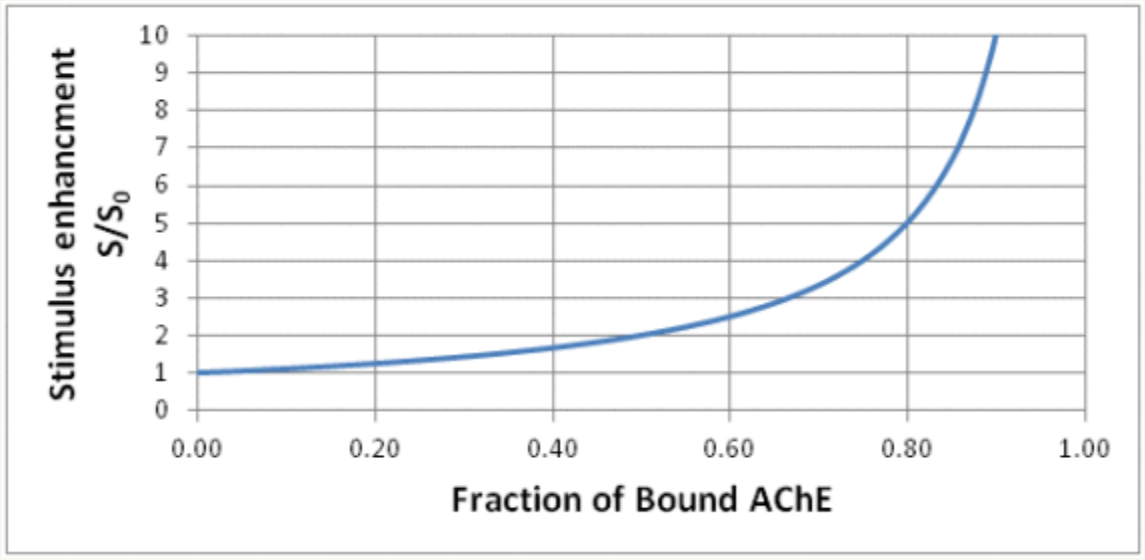
[2] $$\tau = \tau_A / (1 - f)$$

For a single action potential neither N_R nor N_A are affected by the **AChE** inhibitor, so we can express the excess stimulus as a function of the fraction of inhibited **AChE** as

[3] $$S = S_0 \left(1 - \frac{1}{(1-f)}\right)$$

As f increases, eventually the excess stimulus is lethal which we designate as S_L, occurring at f_L.

[4] $$S_L = S_0 \left(1 - \frac{1}{(1-f_L)}\right)$$



The graph shows what happens as the fraction of bound **AChE** increases. The stimulus enhancement rapidly gets large as most of the **AChE** becomes unavailable to catalyze the destruction of **ACh**.

With a little algebra you can show that the fraction of excess stimulation at the sub lethal limit compared to lethal over-stimulation can be expressed as

[5] $$\frac{S_\epsilon}{S_L} = \epsilon (1 - f_L)$$

Where $\epsilon = f/f_L << 1$ is the sub-lethal exposure as a fraction of the lethal level, and S_ϵ is the excess simulation associated with the small dose ϵ.

Example: If the lethal stimulus level is five times the normal background level of neuronal activity, then 80% of the **AChE** must be bound. If we ask what happens with an exposure that is 10% of the lethal level, (8% of **AChE** bound) then the increase in simulation is only 1.6% of the increase needed for lethality. In the residual limit, the stimulus increase is less-than-linear with exposure, with this "safe residual" effect strongest for chemicals where f_L approaches 1.

Studies with fish have shown that **AChE** inhibition levels need to be 60% to 90% (2), depending upon the chemical and species, to be lethal. This is more or less in accord with this model where lethality requires most of the **AChE** receptors to be out of commission, and would suggest that toxicity suppression for residual levels would be significant for these chemicals.

Acetylcholine Dynamics – Consider the complete network

Now we will take the nervous system as an ensemble of neurons with average properties. Specifically we are interested in the acetylcholine pathway, so we define several global average quantities and relationships between them. Acetylcholine activates receptor sites on the post synaptic membrane that stimulate the post synaptic neuron. We can express this globally averaged stimulus, S_{ACh}, as

[6] $$S_{ACh} = kN_{ACh}N_R$$

where k is a proportionality constant, N_{ACh} is the average concentration of synaptic acetylcholine, and N_R is the concentration of acetylcholine receptor sites. (Unlike the previous section, here N_{ACh} is an averaged network concentration, whereas N_A in Equation [1] described the total release of **ACh** caused by a typical action potential.) Acetylcholine is release into the synaptic junction by action potentials from stimulated neurons. It is then quickly degraded by acetylcholinesterase receptors located in the synaptic cleft. We can express this relationship as

[7] $$\frac{dN_{ACh}}{dt} = k'S_{ACh} - k_E N_{ACh} N_E$$

Where k' reflects the efficiency of the averaged stimulus at generating additional **ACh** due to stimulus-induced action potentials. N_E is the concentration of **AChE** that degrades **ACh** and k_E is a constant involving the efficiency for **AChE** destruction of **ACh**. Combining [6] and [7] and defining $kk' \equiv k_R$, we get

[8] $$\frac{dN_{ACh}}{dt} = \left(k_R N_R - k_E N_E\right) N_{ACh}$$

A solution of the differential equation is

[9] $$N_{ACh} = N_{ACh0}e^{t/\tau}$$

Where the growth rate, τ is

[10] $$\tau = 1/\left(k_R N_R - k_E N_E\right)$$

The growth rate, τ, must be negative or acetylcholine concentration will grow without bounds,

[11] $$N_E > \frac{k_R}{k_E} N_R$$

Hence, under normal conditions, the concentration of **AChE** must be sufficient to prevent runaway growth of the **ACh** concentration due to **ACh**'s ability to generally stimulate the neural network. Here we are not considering the many other neurotransmitters, both agonists and inhibitors, that are included in the network, nor are we considering external inputs. However, conditions that place the entire network in a rough dynamic balance enhance the network's ability to involve multiple neurons for information processing. Hence, one might suspect that the inequality [11] is only weakly maintained, at least in some portions of the neural network since this would lead to a network that would more optimal for information processing .

The complete network with AChE Inhibitors

Now let us consider what happens when we add **AChE** inhibitors and to this picture. The effect of chemical **AChE** inhibition will be to reduce the natural concentration **AChE**, N_{E0}, to an available active component

[12] $$N_E = N_{E0}(1 - f)$$

where f is the fraction of bound **AChE** receptors. Substituting [12] into [11] and solving for f, we find that there is a critical inhibition fraction that will result in uncontrolled growth of the ACh concentration.

[13] $$f_{Crit} = 1 - \frac{k_R N_R}{k_E N_{E0}}$$

One might consider this the threshold level at which AChE pesticides produce a lethal effect.

In the medical literature on OP poisoning, one comes across the notion of "cholinergic crisis" which suggests such a threshold-like condition (3). Although

experimentally it is found that relatively large fractions of the AChE must be inhibited to cause lethality, this network effect may play the role of the coup de grâce at the entire organism level.

Acetylcholine Receptor Agonists – Neonicotinoids

The acetylcholine receptor agonists such as the neonicotinoids will directly stimulate the post synaptic neuron. We can write the postsynaptic stimulation, S_{Nic}, due to the neonicotinoid as

[14] $$S_{Nic} = j_C f N_R$$

Where j_C is the single-receptor ion current stimulation, N_R is the **nAChR** concentration and f is the fraction of receptors bound with agonist. When only a few receptors are bound with agonist, the cell's ion pumps will attempt to restore the resting potential of the neuron. However, ion pumps are a slow energy-intensive process. Compared to an open **nAChR**channel, as a rough estimate, an ion pump will only generate ~10^{-5} as much current.(4) Put another way, for each open **nAChR** there needs to be ~ 10^5 ion pump channels in action to keep the cell in homeostasis. A normal functioning **nAChR** would remain activated only for a few milliseconds at most, so much less pumping is required to recover from normal activity because of the low synaptic duty cycle.

No obvious threshold mechanisms are present for this class of chemical. Instead, the excess stimulation is directly proportional to the amount of bound receptors, which is itself proportional to insecticide dose. If we go through the exercise like we did for equation [5] we discover that in the residual limit where $\epsilon << 1$,

[15] $$\frac{S_\epsilon}{S_L} = \epsilon$$

showing the stimulus is proportional to the residual dose.

Metabolic load for residual levels of these chemicals

Any depolarization of the post-synaptic neuron must be eventually be rectified by metabolic processes that pump ions uphill against the gradient to return the neuron to its normal resting potential. Chemicals that increase the post-synaptic stimulation beyond the natural level will require proportionately more metabolic effort to return the neuron to its resting potential. For the **AChE** inhibitors the excess stimulation is only operative when the synapse is stimulated by the action potential and **ACh** is present. If we wish to find an averaged excess stimulation of the post synaptic neuron, we need to multiply the instantaneous excess stimulation by the synaptic duty cycle, D. We can rewrite equation [5] for the averaged excess stimulation for a residual quantity of **AChE** inhibitor, ϵ_{OP} as

[16] $$\langle S_{\epsilon_{OP}} \rangle = \epsilon_{OP} \left(1 - f_L\right) D S_{L_{OP}}.$$

For the neonicotinoids, the stimulation is constant, with duty cycle equal to one when doing the time averaging.

[17] $$\langle S_{\epsilon_{NN}} \rangle = \epsilon_{NN} S_{L_{NN}}$$

If we assume that chemical pesticides are applied in the field at rates that are designed to produce a lethal effect in target organisms, then we can compare the relative effects of residual levels of the chemicals $\epsilon = \epsilon_{OP} = \epsilon_{NN}$, some small fraction of the lethal level, by normalizing to an application rate where $S_{L_{OP}} = S_{L_{NN}}$. Here the subscripts OP and NN refer to the organophosphate or neonicotinoid classes of chemicals respectively. With these assumptions, combining [16] and [17],

[18] $$\frac{\langle S_{\epsilon_{OP}} \rangle}{\langle S_{\epsilon_{NN}} \rangle} = \left(1 - f_L\right) D$$

The above comparison suggests that for similar residual levels of the two classes of chemicals, the neonicotinoids will produce a much larger average post-synaptic stimulation. We can make estimates for the synaptic duty cycle based upon observed average firing frequency, ~1 Hz, and typical action potential duration, ~2 ms. If we assume the threshold term $\left(1 - f_L\right) = 0.5$, then taken together the neonicotinoid chemicals will produce ~1000 times more averaged post-synaptic stimulation than would similar residual levels of organophosphate pesticides. For sub lethal doses of the pesticides, where nervous system function is not strongly impaired, the primary physiological effect one would expect to see would be a much higher metabolic drag on the organisms exposed to low levels of neonicotinoids.

Time Cumulative Effects

The time history of the the movement of the pesticide from its initial application, its interaction with target or non-target organisms, and its eventual dilution and degradation can have dramatic consequences in terms of both acute initial toxic effect and latent residual toxic effect.(5) An effective and safe pesticide should strongly attack the target organism yet remain benign to similar species that are *not* the target organisms. The best way to achieve a strong differentiation from initial application compared to residual pollutant is to use chemicals that have all of the following properties:

1. Rapidly degrade in the environment.
2. Rapidly disassociate at targeted biological binding sites.
3. Have a strong threshold action.

Lets look at these in turn. Persistent chemical pollutants have been the bane of the pesticide industry since DDT. None of the acetylcholine path insecticides are as bad as the organochlorines, but there is still quite a difference between members of this group. The neonicotinoids are said to have around a 1 year soil life, but experience suggests that to be an optimistic number. Where the chemicals have been used for many years, the contamination levels continue to increase. Since the neonicotinoids are water soluble, this suggests that what may appear as degradation is merely dilution and migration. Instead of the chemical disappearing, we find contamination far from the source of the application. (6,7,8) Chemicals that are persistent in the environment long after the crop is harvested and target insects are gone can only have deleterious consequences for unintended organisms. The severity of the consequences depends on the final two properties.

Insecticide chemicals that bind to targeted receptors can have a wide range of receptor affinity and binding strength. Chemicals that bind transiently (like the **ACh** molecule itself to **AChR**s) will remain in quasi chemical equilibrium with the extracellular fluid and will bind to target molecules at a rate that is proportional to the concentration of the chemical. However, some insecticide chemicals are designed to bind tenaciously to the desired receptor sites. In these cases, the molecules will become trapped at the target site even after most of the chemical has been rid from the organism's body by metabolic processes. In cases with very strong target binding, one can expect accumulation over time of molecules at the target sites as long as there is any continuing exposure to the chemical. How serious a problem this will be for non-target organisms depends on the last property, whether the chemical works with a threshold action or not.

Properties of Pesticide Classes

Chemical neuro-toxic pesticides have been widely used for more than 70 years. During that time several families of chemicals have been developed to target specific neurological receptors. The chart below lists several of these classes, includes a common example or two from each class and shows typical properties of these chemicals.

Pesticide Class	Example Chemical	Oral LD50 Honey-bees	Typical Soil half-life	Typical metabolic half-life	Typical binding dissocia-tion time	Typ. tox. time-scaling exponent	Toxic Mechan-ism	Comment
Neonic-otinoids	Imidacloprid	50 ng/bee	.5 – 3 yr.	4 hr.	>10 days	2	Synaptic nAChR agonist.	Often used as systemic insecticides
	Thiameth-oxam	20 ng/bee	30-300 days	2-6 hr. (rats)	?	2	Irreversible binding	Direct acting on nAChRs
Pyrethroids	Delta-methrin	60 ng/bee	11-72 days	2 hr.	Several seconds	2 ?	Keeps open voltage gated Na+ ion channels on axon	Direct acting on Na+ channels
Organo-chlorines	DDT	6190 ng/bee	2-15 yr.	6 yr.	Temperture dependant-- suggests less than a second.	?	Keeps open voltage gated Na+ ion channels on axon	Most of these chemicals have been banned by international treaty as persistent organic pollutants
	Dieldrin	133 ng/bee	5 yr.	9-12 mo. humans		?		
Organo-phosphate	Diazinon	370 ng/bee	15-200 days	17 hr.	16 days	1 ?	Irreversible AChE inhibitor	AChE inhibitors have inherent "threshold" action. A large fraction of AChE must be bound to have toxic effect Indirect acting on ACh
	Malathion	720 ng/bee	1-15 days	12 hr.	? days	0.5 (fish)		
Carbamates	Carbaryl (Sevin)	1540 ng/bee	4-30 days	8 hr.	short	1	Reversible AChE inhibitor	

It is worth looking at the typical chemicals in the table above in light of the requirements we identified as desirable for a safe pesticide. Note that the organochlorines failed badly because they were so persistent in the environment to the point they have been almost universally banned. They were largely replaced by the organophosphates with which we've continue to have an uneasy coexistence for the last half-century. Under scrutiny because of their potent effects on humans and other vertebrates, many of the organophosphate insecticides are being forced into retirement. The replacement has been the neonicotinoids, which have the benefit of relative specificity to invertebrate **nAChR** receptors making the chemicals less toxic to humans and other vertebrates. Unfortunately, the neonicotinoids fail with regard to all three of the properties for safe and effective pesticides.

From our discussion you can see that the safest chemicals are the carbamates. Typically it takes more chemical (compare LD50 for neonicotinoids, organophosphates, and carbamates) to kill the target insect, but the persistence of the chemical in the environment is short. It is metabolized relatively quickly, and acts reversibly with the target receptors. Finally, it is also an **AChE** inhibitor that has a strong threshold of action effect. Compare this with the neonicotinoids at the top of the chart. It takes much less neonicotinoid chemical to kill, but this is likely due to its tenacious persistence on the

target receptor sites. The chemicals do not degrade very quickly in the environment so they will continue to accumulate on target and non-target organism synaptic receptors long after the initial application. And finally, the neonicotinoids produce toxic effects at residual dose levels, unlike the **AChE** inhibitors. All of the tricks we have in the playbook to segregate between target and beneficial insects fail with the neonicotinoids.

Implications of threshold action for toxicity scaling

Change of sign of the acetylcholine growth rate provides a clear qualitative turning point for the organism. It is easy to understand how such a runaway event can lead to death. Hence, if you wish to model the toxicity scaling of a compound with such a distinct threshold action, all you have to do is follow the movement of toxin to receptor sites until the threshold is reached. This will naturally give you Haber's rule for substances that accumulate, such as most of the organophosphate insecticides. For insecticides that don't accumulate on receptors, such as carbamates, one would expect threshold action without a significant time dependence. Once pesticide concentrations reached levels where chemical equilibrium at receptor sites resulted it enough bound **AChE** to change the sign of the **ACh** growth rate, the threshold condition would be reached. However, at small residual concentrations of acetylcholinesterase inhibitors, the molecules disable a few AChE sites and hence slightly change the synaptic response, but otherwise remain largely benign to the organism. For this class molecules, there is a very large change in toxic effect with concentration. Despite the continued environmental issues and concerns with organophosphate pesticides, it should be recognized that they may be intrinsically environmentally safer because of their strong threshold action than the newer neonicotinoids.

For the neonicotinoids where there is no distinct threshold condition, the situation is more complicated. The transition from alive to dead is not accompanied by a convenient mathematical marker like the change in sign of a growth rate. Especially at the residual limit, we are left to speculate on the physiological impact of accumulate insults from the toxic chemical. Single molecules will open ion channels and begin to depolarize neurons. This abnormal state of affairs would be countered by energy-burning processes in the organism to mitigate the dysfunction. This is the definition of stress. It is likely that the residual-level stresses to non-target organisms is the Achilles' heal for the neonicotinoid insecticides. Very low concentrations of these pesticides have the potential to switch on compensatory physiological processes that are poorly understood, but likely stressful. One example was the discovery that very low levels of the neonicotinoid clothianidin reduced the immune response of honeybees to the point where deformed wing virus could replicate. Low levels of the acetylcholinesterase inhibitor chlorpyriphos, the molecules of which in our understanding would be rather benignly latched on to a few of the **AChE** sites, showed no such immune suppression effect.(9) The fact that **nAChR** channels are involved in less well studied immune system

and cellular signaling functions adds to the risk that disrupting these pathways will have unintended consequences.(10,11)

A key point is that at residual levels, **AChE** inhibitors are really doing nothing. A small fraction of the **AChE** sites my be out of commission, but even that effect is only apparent when the neuron fires and there is **ACh** to be swept away. During the neuron's quiet state the pesticide molecules are benign. Contrast this situation with what happens on the postsynaptic membrane with a few neonicotinoid molecules. Single neonicotinoid molecules hold open **nAChR** channels that will tend to depolarize the neuron. This happens even when the neuron is in an un-stimulated state. However, given the persistent depolarization by the open channel, it can't rest. Instead the cell must muster energetic processes in an attempt to restore the neuron's polarization so that it may still function.

Besides suppression of immune response as mentioned above, there are likely other detrimental effects from the energy sapping response required by residual neonicotinoid poisoning. Trade-offs between energy expenditures to maintain neurological function and more normal activities such as powering flight muscles may explain some of the observed effects of chronic low level exposure. (12) Another study shows epi-genetic changes to imidacloprid-exposed honeybee larva that that strongly affects genes involving metabolism. (13) The myriad effects that low level neonicotinoid exposure presents, such as impaired navigation, poor learning ability, reduced flight time, and immunological impairment may be better understood from the perspective of the metabolic stress caused by open nAChR channels than by direct neurological impairment.

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