October 5, 2005

Lecture 12: Neurotoxicity I

I. Introduction: The Importance Neurotoxicity in the Context of Environmental Toxicology

A. Of all the environmental contaminants, the pesticides are perhaps the most studied from the perspective of environmental chemistry and toxicology
   1. With respect to the mode of action, i.e., how toxicity is manifested through biochemical interactions, pesticides are well studied because they must work against pests yet provide a margin of safety to the user.
   2. For the most part, the pesticides that have been most environmentally contentious are toxicants with biochemical modes of action in the nervous system.
      a. Nearly all of the pesticides with pharmacodynamics (toxicodynamic) action at the level of the nervous system are insecticides.
         1. The ultimate biochemical receptors are at different sites on the nerve cell.

II. Normal Nervous System Physiology

A. Because nerve physiology is interfered with by insecticides, a discussion of normal nerve physiology is necessary to understand mode of action of commonly used insecticides

B. General Overview
   1. The nervous system provides rapid coordination of sensory and mechanical functions (i.e., muscle movement) and interacts (i.e., carries information to) with the endocrine and immune system.
   2. Consists of electrically excitable cells (neurons) that can generate electrical signals long distances without degrading the strength of the signal.
   3. Functions similarly among all animals.
   4. Basic structures of the neuron
      a. Soma (cell body containing the nucleus and providing metabolic maintenance of the cell)
      b. Dendrites (receive and carry signals toward the soma)
      c. Axon (carries signals away from the soma)
      d. Supporting cells (glial cells, myelin sheath)

C. Nerve Cell Morphology
   1. A single nerve cell is called a neuron.
   2. A neuron consists of:
      a. Cell body (soma) containing the nucleus and numerous thin fibers extending from it.
         1. The cell body provides metabolic maintenance of the neuron.
      b. The fibers consist of a single long one called the axon extending in one direction and numerous shorter ones called the dendrites that are heavily branched. (See Figure 1)
      c. The entire nerve cell, including the cell body, axon, and dendrites, is surrounded by a membrane that also acts as electrical insulation. This insulating membrane is called the myelin sheath.

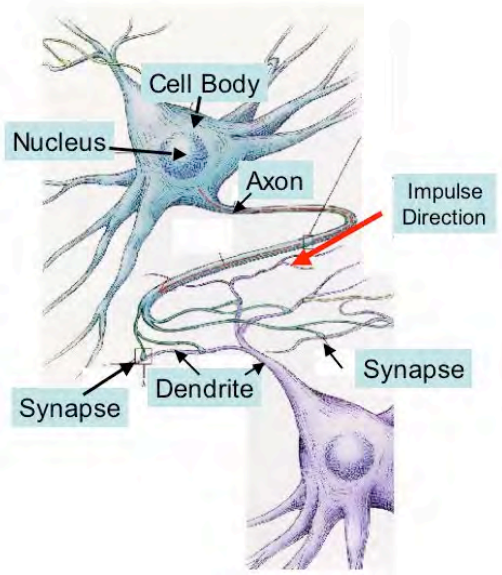


Figure 1. Nerve cell morphology (adapted from Scientific American)

   d. The axon terminates at other nerve cells without touching them;
      1. Instead there is a small gap called the synapse between nerve cell axons and dendrites. (See Figures 1 & 3)

D. Organization of the Nervous System
   1. Sensory neurons
      a. Transmit information collected from external stimuli
      b. Respond to endogenous stimuli
   2. Interneurons
      a. Link other neurons within the central nervous system (i.e., brain and nerve cord)
      b. Networked to exchange information and perform complex "computations" leading to behavior (or thought)
   3. Motor neurons
      a. Carry information (instructional signals) to effector organs
         1. An effector is a cell, tissue, or organ that acts to change the condition of an organism in response to neuronal or hormonal signals (for ex., contraction of muscles; sections by glands).
   4. Supportive Cells
      a. Provide structural, insulative, and metabolic functions to the neuron.

E. Transmission of Nerve Signals: Overview (see Figure 2)
   1. The plasma membrane of the soma and its dendrites receive "signals" from the terminals of other neurons.
      a. As will be explained later, these signals are in the form of neurotransmitter chemicals (most common form) or as electrical current (less common form), but all signaling is transduced to electrical information.
   2. All the signals received by one soma from other axons are integrated in a region called the axon hillock. The received electrical signals are actually membrane voltage changes.

a. Sufficient input will initiate a large enough change in the plasma membrane voltage potential to generate an action potential.
b. The action potential is what is commonly thought of as the nerve impulse or signal that travels outward from the axon hillock down the axon to its terminus adjacent to different neurons dendrites.

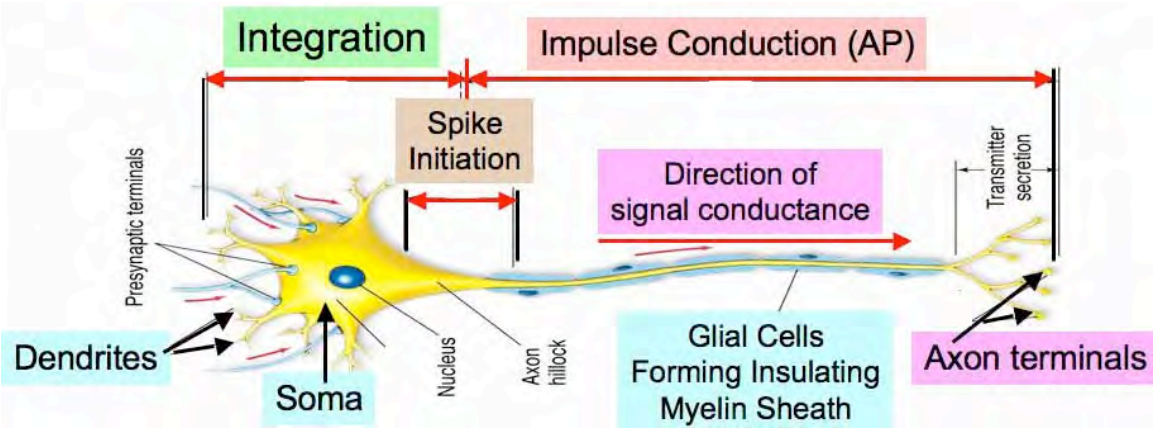


Figure 2. General overview of nerve cell and signal transmission pathway. Drawing from Randall, D., W. Burggren, and K. French. 2002. *Eckert Animal Physiology: Mechanisms and Adaptations*, 5th Ed. W.H. Freeman, NY.

F. Electrical Physiology of the Nerve Cell
1. The nerve signal conducts down the length of the axon in a one-way direction (See Figure 3).
   a. The nerve signal is actually an electrical signal that travels along the length of the axon until it reaches the synapse where the axon and dendrite are separated.
2. The ability of the nerve cell to conduct electrical signals down its axon is due to two factors:
   a. Permeability of the membrane to ions
      1. Cell membrane is semipermeable; allows some ions to freely diffuse across it, but other ions cannot;
   b. The membrane electrical potential (i.e., the membrane potential)
      1. Membrane potential is the charge separation, or potential difference, across the membrane inside surface and the outside surface.
3. Cell membrane and the resting potential (Figure 4)
   a. The membrane potential existing prior to conduction of the nerve impulse is called the resting potential.
   b. Cell membrane is freely permeable to potassium but not to sodium;
   c. In addition to sodium and potassium, chloride and organic anions are present; (See Table 1 for ion concentrations inside and outside of the membrane.
   d. The difference in distribution of the ions at equilibrium creates the resting potential, which is about –60 to -70 mV (millivolts) of electrical potential;
      1. The inside of the cell is negative with respect to the outside, i.e., it is POLARIZED.

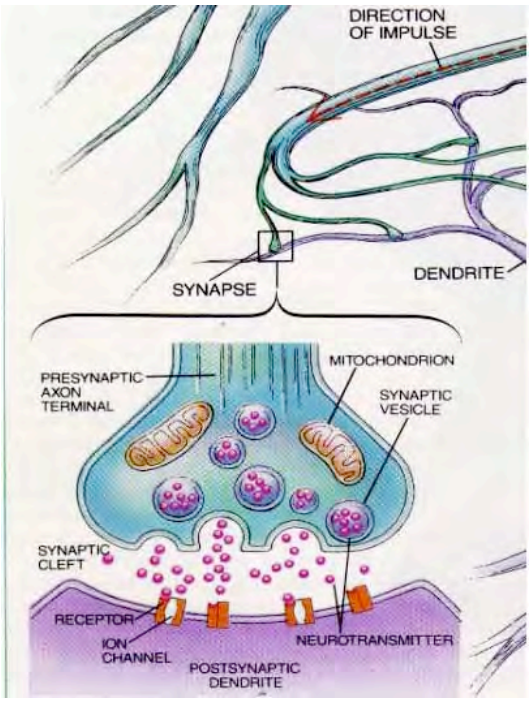


Figure 3. Nerve cell axon terminates prior to physically touching the dendrites. The gap is called the synapse. The nerve signal is transmitted across the synapse via a chemical signal called a neurotransmitter. (Adapted from Scientific American)

2. The concentration of sodium and chloride is much greater on the outside of the cell than on the inside;
3. The concentration of potassium and organic anions is much greater on the inside of the cell than on the outside

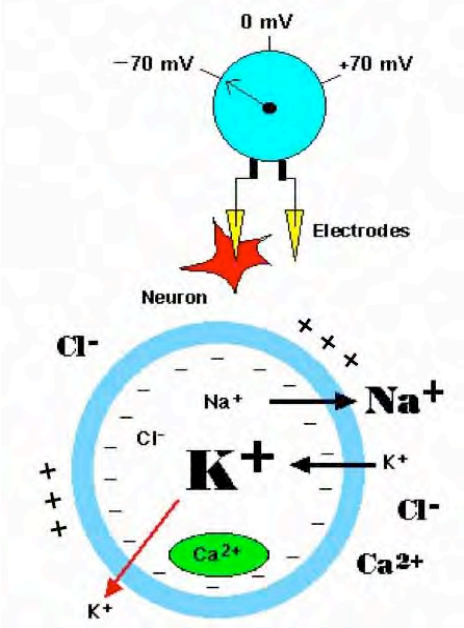


Figure 4. Schematic of resting potential of membrane due to differential permeability to cations and anions.

Table 1. Ion concentrations (millimoles per liter) in the nerve cell cytoplasm (axoplasm) and outside of the cell membrane in the blood. Seawater is shown for comparison to blood. The data are from experiments conducted in the 1950s with the giant nerve fibers of the squid. Note that nerve physiology is remarkably similar across numerous taxa, and thus the squid nerve made a convenient tissue to study because it is one of the largest axons in the animal kingdom. (Data presented in Schmidt-Nielsen 1997)

Ion	**Axoplasm (Inside of cell membrane)**	**Blood (outside of cell membrane)**	**Sea Water**
Potassium (K)	400	20	10
Sodium (Na)	50	450	470
Chloride (Cl)	40	570	550
Calcium (Ca)	0.0003	10	10
Magnesium (Mg)	10	55	54

e. The membrane is essentially impermeable to the movement of sodium and chloride ions.
f. On the other hand, the membrane allows potassium to diffuse across its concentration gradient, so some is always "leaking" out but without the movement of chloride ions.
    1. Thus, the resting potential is polarized with the inside of the membrane having a negative charge relative to the outside.

4. Depolarization and the Action Potential
    a. When the nerve is stimulated, proteins in the cell membrane change conformation allowing the diffusion of sodium ions through the membrane into the axoplasm.
        1. The proteins embedded in the cell membrane are called pores or channels. They function as selective gates that only allow specific ions to move through as they open and close in response to electrical signals.
            a. The sodium channel or gate is called a voltage-gated channel because it's opening is triggered by change in membrane potential (i.e., by partial depolarization). Partial depolarization opens the gate to allow sodium to pass to the inside of the membrane.
    b. As the sodium passes into the interior of the cell, the membrane potential moves in the positive direction and begins to undergo a full depolarization.
        1. Shortly after the sodium gate opens up, the cell becomes permeable to potassium ions (via opening of independent and separate potassium voltage-gated channels).
            a. The potassium efflux is much slower but more prolonged than the sodium influx. (Figures 5, 6)

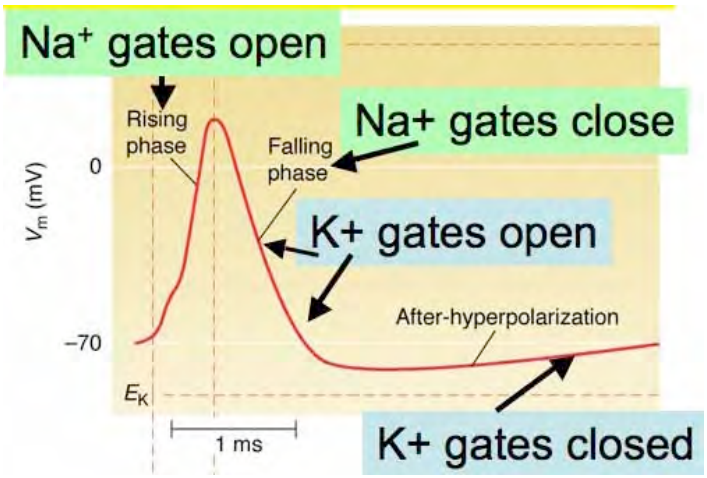


Figure 5. The generation of the action potential due to sequential opening and closing of sodium channels (gates), and opening and later closing of potassium gates. (graph from Randall et al. 2002)

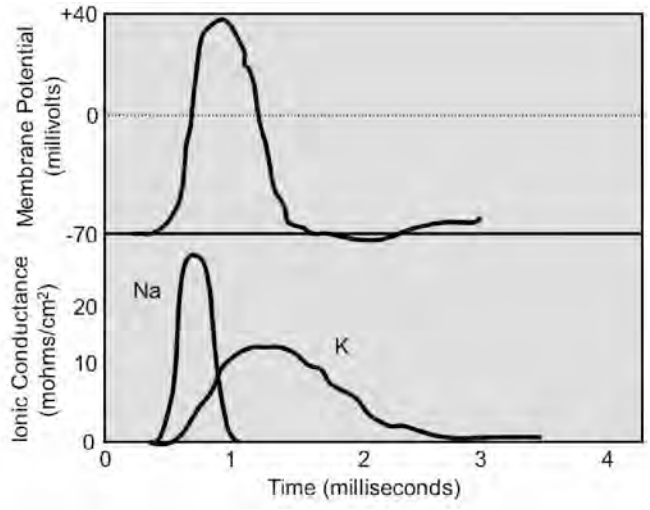


Figure 6. The timed correspondence of the action potential's change in membrane potential (top part of graphic) with the conductance of the membrane to sodium and potassium (bottom part of graphic). As membrane becomes partially depolarized, the sodium gates open, allowing sodium influx. About a millisecond later, the potassium gates open allowing potassium efflux.

2. The resting potential is depolarized and then hyperpolarized in the opposite direction to about +40 mV (Figures 5, 6).
3. This change in electrical potential causes the appearance of the action potential, an all-or-none electrical response, that is transmitted along the length of the nerve through a mechanism known as the local circuit current (i.e., a wave of depolarization). (Figure 7)

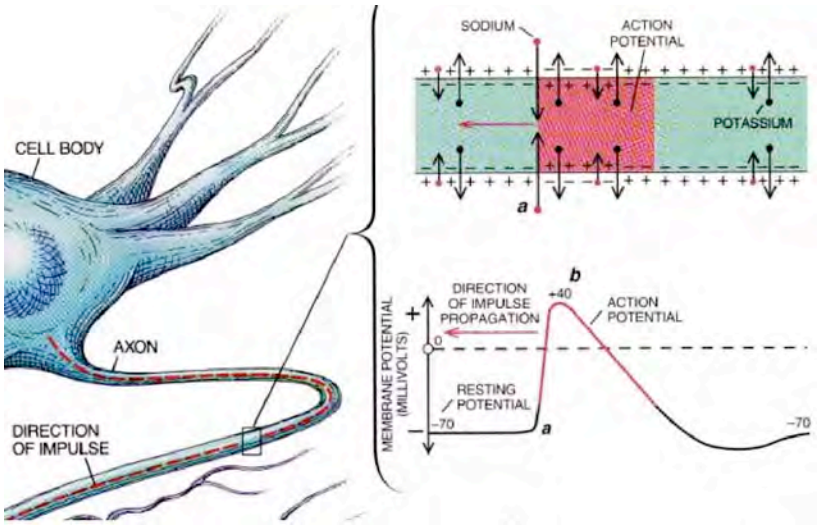


Figure 7. Illustration of the start of an action potential and its propagation along one direction of the nerve axon through the local circuit current mechanism. (copied from Scientific American)

a. As the action potential develops, the adjacent membrane is partially depolarized, opening up sodium channels in that region.
   1. Thus, nerve signal transmission is a positive feedback systems because the sodium channel opening allows even greater amounts of sodium to flow through, completely depolarizing and then hyperpolarizing the membrane (i.e., membrane potential falls from –70 mV to 0 mV and then to +40 mV).

b. As soon as the sodium channels open, the action potential develops. Thus, the action potential is propagated along the nerve.
   1. However, the initiating depolarizing stimulus has to reach a certain threshold before the action potential develops.
   2. Once the action potential develops, its magnitude is independent of the size of the stimulus. Thus, the generation of the action potential is an "all or none" mechanism.

c. The action potential propagates along the nerve axon without any loss in amplitude because the change in potential is strictly due to the movement of ions through the membrane channels.

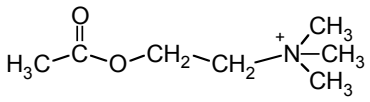
d. The action potential moves in only one direction down the axon. Its backward movement is prevented by a refractory period in the sodium gate that does not allow the channel to open again for a small period of time.

5. Repolarization and Return of Resting Potential (Figures 5, 6)
   a. After the electrical current passes a specific place along the membrane, the sodium gates close;
   b. Potassium efflux slows;
   c. The membrane potential rapidly falls to its polarized resting state.
6. Synaptic Transmission

a. At the terminus of the axon, there is a tiny gap (synapse) between the axon and the next nerve cell (or dendrite);
   1. The axon from which the nerve signal (i.e., action potential) is emanating is called the presynaptic membrane, and the dendrite of the next nerve cell is called the postsynaptic membrane.

b. At the synapse, transmission of the nervous electrical energy is changed (transduced) to chemical energy;

c. A neurotransmitter chemical called acetylcholine (ACh) is released from packets (tiny membrane bound vesicles in the axoplasm) that are positioned at the presynaptic membrane; (Note that there are other kinds of neurotransmitters, but ACh operates in the central nervous system and at the neuromuscular junction, and disruption of its function (via several different mechanisms) is a major pharmacodynamic effect of several groups of insecticides.



acetylcholine (ACh)

   1. The vesicles fuse with the plasma membrane upon stimulus from the action potential (i.e., depolarization) and the subsequent movement of calcium cations (Ca+2) through voltage gated channels.
   2. The Ca+2 ions interact with a protein that anchors the vesicle near the plasma membrane. Binding of Ca+2 with the protein causes it to change conformation. In this change, the vesicle membranes become fused with the pre-synaptic plasma membrane in a process called exocytosis.

d. The acetylcholine molecules diffuse into the synapse (Figure 7);
   1. Each vesicle contains between ~1000-10,000 molecules of ACh, and these are released in discrete quantities or quanta.

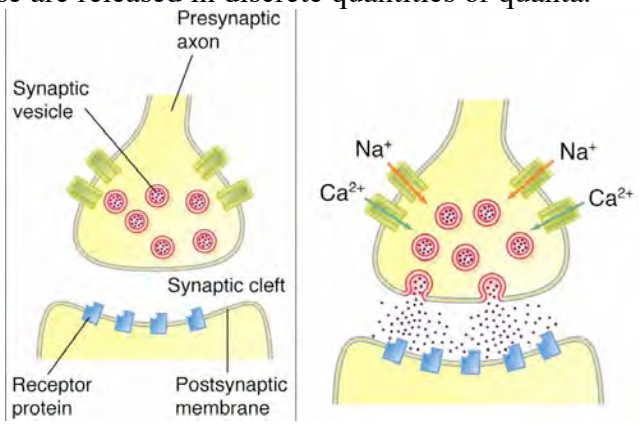


Figure 7. Release of acetylcholine neurotransmitter from the vesicle in the presynaptic axon upon depolarization and Ca+2 influx. ACh molecules are released from each fused vesicle (by exocytosis) and into the synaptic space (cleft) where they diffuse to the postsynaptic membrane and bind to embedded receptor proteins. (Drawing from Randall et al. 2002)

e. Some of the acetylcholine molecules interact with acetylcholine (ACh) receptors embedded in the post-synaptic membrane;
   1. The ACh receptors are proteins embedded in the postsynaptic membrane and act as ion channels, similarly to the sodium channels in the axon.

f. Binding of acetylcholine to the receptors then causes a depolarization in the post synaptic membrane by the local current circuit mechanism across the rest of the nerve;
   1. When ACh is bound to the receptor, the permeability to sodium is increased casing large influx of the ion through the ACh receptors (Figure 8).
   2. Unlike the action potential, which is an all or nothing response stimulated by change in membrane potential, the post synaptic potential is directly related to the number of ACh molecules binding to the receptor.
   3. ACh molecules are released quickly from the receptor and then become available to bind to other receptors on the post-synaptic membrane.
      a.

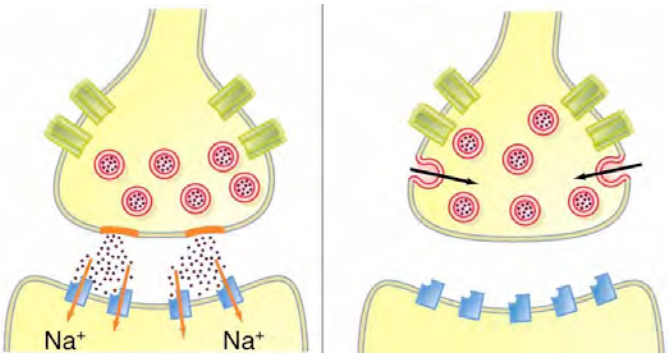


Figure 8. Opening of sodium gate in ACh receptor on postsynaptic membrane. Vesicles are recycled through endocytosis and "refilled" with ACh molecules to repeat the cycle of release. (Drawing from Randall et al. 2002)

g. The chemical transmission of the nerve signal across the synapse is terminated by enzymatic breakdown of the excess acetylcholine molecules in the synapse.
   1. The enzyme, acetylcholinesterase (AChE) is embedded in the postsynaptic membrane near the ACh receptors (Figure 9).
   2. The ACh has an equal probability of binding to AChE and to the ACh receptor.
   3. Thus, when ACh diffuses across the synapse, it will either bind to the receptor or be hydrolyzed by AChE.

h. Neuromuscular junction of vertebrates is where acetylcholine-based chemical transmission occurs; in insects, acetylcholine is used only in the

central nervous system as it is in vertebrates, but glutamate is used at the neuromuscular junction.

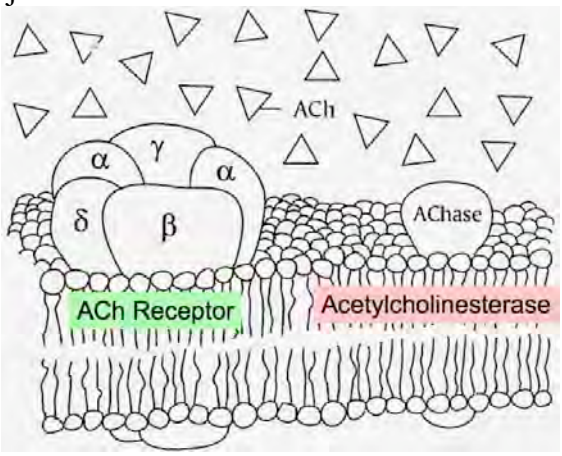


Figure 9. The neurotransmitter is rapidly hydrolyzed by the esterase enzyme called acetylcholinesterase. It is embedded in the postsynaptic membrane near the ACh receptors.

7. Types of Post Synaptic Receptors
   a. Thus, far we have discussed the acetylcholine receptor and direct binding and triggering by acetylcholine that results in increased permeability to sodium and potassium ions. (Note that the ion channel of the postsynaptic acetylcholine receptor allows passage of both sodium and potassium in contrast to the axon where the channels are distinct proteins in the membrane.)
   b. Actually, there are two types of acetylcholine receptors (as well as two types of other neurotransmitter triggered receptors): the direct acting ionotropic receptors (the ligand gated receptors, which produce a fast response) (**Figure 10**) and the indirect acting metabotropic receptors (which function through an intermediary signaling molecule or mechanism; these are slower responding receptors) (**Figure 11**).
      1. A single neurotransmitter can stimulate both types of receptors.

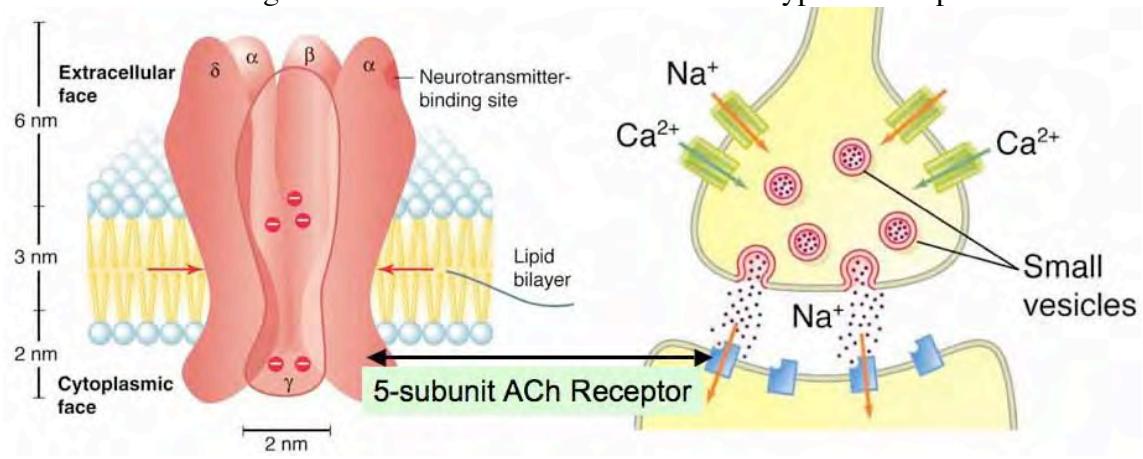


Figure 10. The fast acting ionotropic receptor. Direct binding of the neurotransmitter to this membrane bound 5-subunit protein opens up the sodium and

potassium channels that give rise to an excitatory post synaptic potential that spreads over the post-synaptic membrane to the axon hillock. These receptors function comparatively fast to transduce the signal to an electrical potential. The drawing illustrates an acetylcholine receptor that is called a cholinergic receptor. (Pictures taken from Randall et al. 2002)

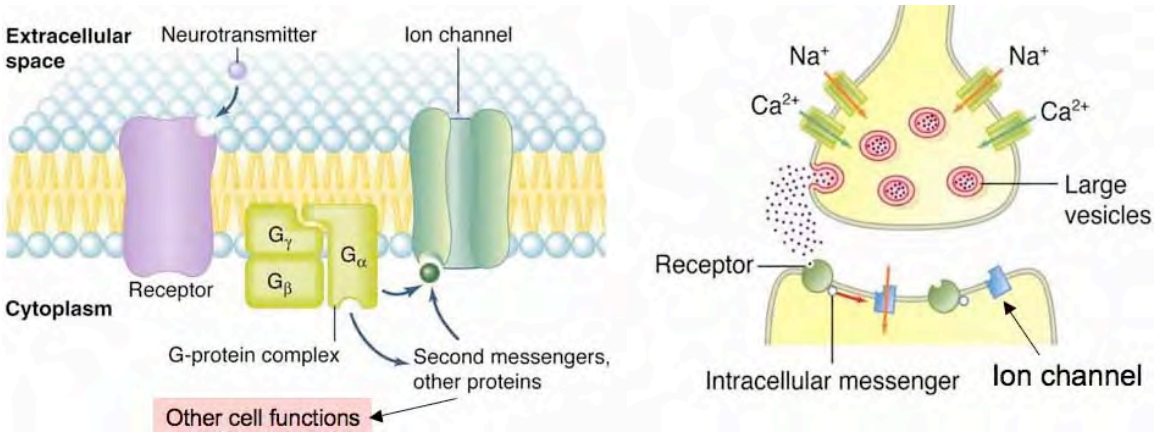


Figure 11. The slow-acting metabotropic receptors bind the neurotransmitter ligand, but the signal is transmitted via a membrane-associated protein that changes the conformation of a nearby ion channel and allows the flow of sodium ions in to the postsynaptic cell. Excitatory postsynaptic potential are produced as with the fast acting receptors, but the time course is slower. The picture represents a slow acting acetylcholine receptor that is called a muscarinic receptor. Note that both cholinergic and muscarinic receptors both bind acetylcholine, but the physiological effect is different depending on the receptor type. (Pictures taken from Randall et al. 2002)

8. Inhibition and Modulation of Signal Transmission—Role of Neurotransmitter Type
   a. There are several different types of neurotransmitters in addition to acetylcholine (glutamate was already mentioned above). All function by binding to either fast ionotropic receptors and/or slow metabotropic receptors. (see Table 2)

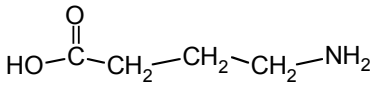
Table 2. Some neurotransmitters and corresponding receptor types in the vertebrate central nervous system (after Hill, R. W., G. A. Wyse, M. Anderson (2004) *Animal Physiology*. Sinauer Associates, Inc. Sunderland, MA.

	Receptor Type		
Neurotransmitter	**Direct (Ionotropic)**	**Indirect (Metabotropic)**	**Neurological Effect** [1/]
Amines			
Acetylcholine	X (nicotinic)		Excitation
Acetylcholine		X (muscarinic)	Slow excitation or Inhibition

Dopamine		X	
Noradrenaline		X	
Serotonin		X	
Amino Acids			
Glutamate	X		Excitation
GABA (gamma aminobutyric acid)	X	X	Inhibition
Glycine	X		Excitation
Peptides			
Various kinds		X	

1/ Excitation is caused by a depolarization of the nerve membrane potential that will eventually result in the generation of an action potential once a threshold is reached. Inhibition is caused by hyperpolarization, i.e., the membrane potential is driven in a more negative direction, making it more difficult for the action potential threshold to be reached.

b. Some neurotransmitters inhibit nerve-nerve or nerve -muscle transmission;
   1. GABA (gamma amino butyric acid) interacts with receptors on the postsynaptic membrane to keep the membrane in a hyperpolarized state, making generation of an electrical signal improbable.



gamma aminobutyric acid (GABA)

      a. The GABA receptors are proteins embedded in the membrane that also serve as chloride channels;
      b. When GABA binds to the receptors, the channel becomes permeable to chloride, allowing the negatively charged ion to flow through the membrane.
      c. The movement of chloride into the membrane causes it to become hyperpolarized in the negative direction (in other words the membrane potential becomes even more negative).
      d. Thus, even more acetylcholine would be required to stimulate production of the action potential.
      e. Therefore, GABA acts to inhibit (or dampen) the production of the action potential in the post-synaptic membrane).

c. Some nerves terminate on organs without a specific synaptic junction
   1. These release chemicals are called <u>neuromodulators</u>, and they modify the neurotransmitter signals of adjacent nerves;
      a. For ex., neuromodulators could produce finer gradations of movement in a muscle;
   2. An example of a neuromodulator at certain neuromuscular junctions in insects is called <u>octopamine</u>.

III. Vulnerability of Nervous System to Attack by Insecticides

A. Nerve physiology seems to be universally similar among all animals (vertebrates and invertebrates).
   1. Thus, pesticides whose pharmacodynamics action interrupts normal nerve functioning have a good probability of being toxic to a wide range of animal species.
      a. A question to ponder: Not all nerve toxicants are equally toxic (i.e., if toxicity were normalized for body weight); what mechanisms should be considered to explain differential or selective toxicity among nerve toxicants?

B. Basically, the nervous system is vulnerable to toxicosis at two levels:
   1. Level of the Ion Channels
   2. Level of the Synapse

IV. Toxicants Affecting the Ion Channels

A. DDT and Pyrethroid Insecticides (Figure 10) have an affinity for the sodium channels.
   1. Current status and use
      a. Although DDT was banned by the EPA in 1973, it is still used in many countries where malaria is endemic.
         1. It is used as a structural spray; i.e., it is sprayed on walls of buildings and kills mosquitoes when they land on the walls.
      b. Pyrethroid insecticides are current used in agriculture and for urban pest control.
         1. The tonnage of pyrethroid use in urban environments has increased significantly over the last few years as organophosphate insecticides have been suspended from both outdoor and indoor use in these areas.
   2. Both DDT and pyrethroid insecticides block the closing of the sodium gates in the axonal membrane, thereby prolonging the sodium influx and return of the membrane potential to its resting state.
   3. When the sodium channel is prevented from closing, the nerve is easily stimulated again to produce action potentials because now the threshold for response has essentially been exceeded.
   4. Depending on specific chemical structure, the pyrethroids have two different mechanisms of causing toxicity and characteristic signs of toxicity (signs taken from Ray, D. E. 2001. Chapter 59, Handbook of Toxicology. Academic Press).
      a. Type I pyrethroids lack a cyano (CN) group attached to the alcohol portion of the molecule (Figure 12).
         1. Signs of toxicity in mammals is characterized by severe fine tremor, marked reflex hyperexcitability, sympathetic nervous system activation, and paresthesia (a skin tingling following dermal exposure)
      b. Type II pyrethroids are characterized by a cyano group attached to the alcohol portion (typically a phenoxybenzyl alcohol) of the molecule.

1. Signs of toxicity in mammals include profuse watery salivation, coarse tremor, increased extensor tone, moderate reflex hyperexcitability, sympathetic activation, choreoathetosis, seizures, and paresthesia.
   a. (Choreoathetosis is a movement of intermediate speed, between the slower, writhing movements of athetosis, and the quick flitting movements of chorea that typically affects limbs and extremities)

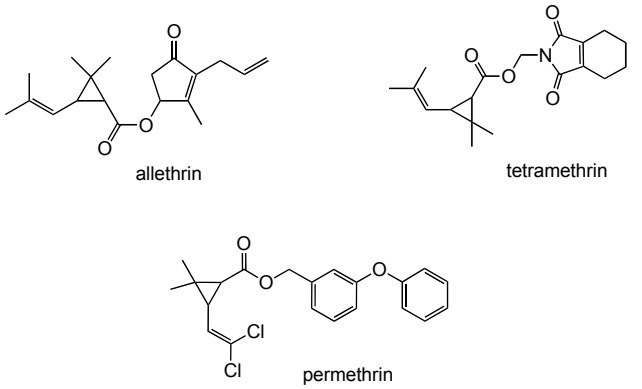


Figure 12. Examples of three Type I pyrethroid insecticides. Pyrethroids are all esters and are more quickly hydrolyzed in vertebrates than in invertebrates. However, differences in sensitivity are multifactorial, and perhaps more importantly is a difference in affinity for the sodium channel.

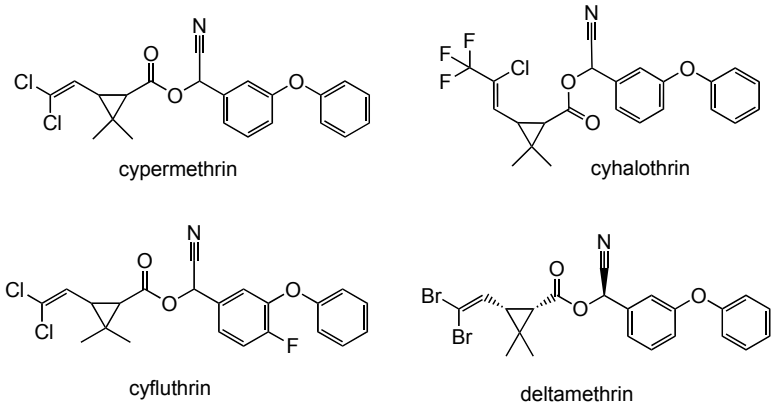


Figure 13. Examples of four commonly used Type II pyrethroid insecticides.

5. One difference between Type I and Type II pyrethroids is the length of time the sodium channel is held open. Type II pyrethroids hold the channel open much longer, thus producing a different array of toxic symptomology as describe above.
   a. For ex., permethrin prolongs the abnormal pyrethroid induced membrane current (owing to an open sodium channel) with a time constant of 7.3 msec; in contrast, deltamethrin has a time constant in the frog nerve fiber of 1772 msec) (Ray 2001)

6. Pyrethroids have multiple isomeric forms: both diastereomers (classified as R or S forms depending on groups bonded to an asymmetric carbon) and cis, trans isomerism.
   a. Technical grade pyrethroids that are used to formulate commercial insecticide products are racemic mixtures of the various isomers. However, usually only one isomer is the actual highly active toxicant molecule.
7. DDT has a mode of action more characteristic of a Type I pyrethroid.
8. An unusual characteristic of both DDT and pyrethroids is the inverse relationship between temperature and sodium channel interaction. Thus, these compounds tend to be more toxic at comparatively lower temperatures.
   a. Binding or interaction of the pyrethroid and DDT with the sodium channel may decrease with increases in temperature, but the end result is a slowing of the return of the membrane potential to its polarized resting state (i.e., a delay in "tail current decay"). (Figure 14)

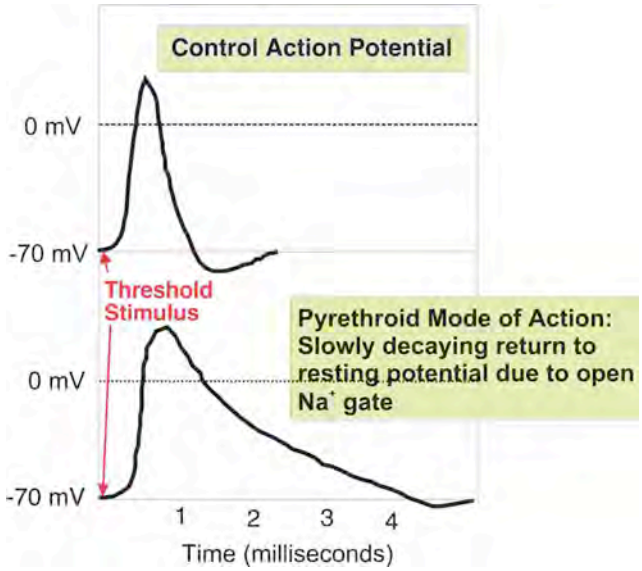


Figure 14. The normal action potential decays quickly, within around 1 msec due to a rapid closing of the sodium gate (top of graphic). However, pyrethroids bind to a subunit of the protein channel and prevent the gate from closing as quickly. The result is a slowly decaying action potential that makes the axon easily susceptible to further generation action potentials as excitatory postsynaptic potentials reach the axon hillock. The physiological effect is repeated erratic firing of the nerve rather than a return to the resting potential, a delay and then a new action potential.

9. Vertebrates are resistant to the effects of pyrethroid insecticides (i.e., at field use rates of product), but insects (and fish!) are very susceptible. The key differences between insects and vertebrates (except fish) in pharmacokinetics and pharmacodynamics of pyrethroids are given in Table 3.

Table 3. Factors Affecting the Differential Toxicity of Pyrethroids to Insects and Mammals (modified from Narahashi, T. 2001. Chapter 12, Handbook of Toxicology. Academic Press)

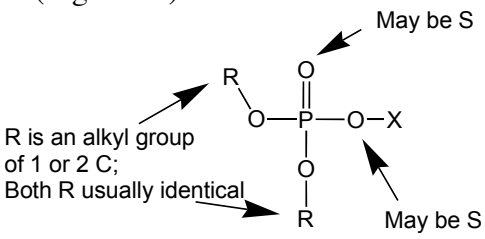
Selectivity Factor	Mammals	Insects	Differences (~X fold)
Potency on Nerve			
Due to temperature dependence	Low (body temp 37°C)	High (body temp variable, but cooler in morning and in shade)	5
Due to intrinsic sensitivity	Comparatively low	High	10
Recovery	Fast	Slow	5
Detoxification Rate			
Due to Enzymatic Action	High	Low	3
Due to Body Size	High	Low	3

V. **Toxicants Acting at the Level of the Synapse** (Several biochemical mechanisms are possible)

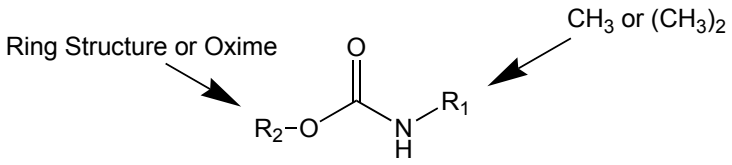
A. Acetylcholine receptor agonism (i.e., a mechanism that has effects similar to the effects of acetylcholine upon interaction with its receptor)

1. Inhibit acetylcholinesterase, effectively increasing the concentration of acetylcholine in the synapse.

a. The insecticide classes known as organophosphorus (OP) and methyl carbamates (CB) inhibit AChE, causing prolonged stimulation of the ACh receptor. (Figure 14)



Generalized Strucutre of an Organophosphorus Insecticide



General Structure of A Methyl Carbamate Insecticide

Figure 14. Generalized structures of an organophosphorus (OP) insecticide and a methyl carbamate (CB) insecticide. The basic OP structure is based on an alkyl phosphoric acid (P is pentavalent). The basic CB structure is based on methyl carbamate.

b. Differences between OP and CB insecticides:
   1. OP insecticides must be in the oxon form to inhibit AChE; CBs can inhibit AChE without prior transformation (Structures of some OP and CB insecticides shown in Figures 15, 16).

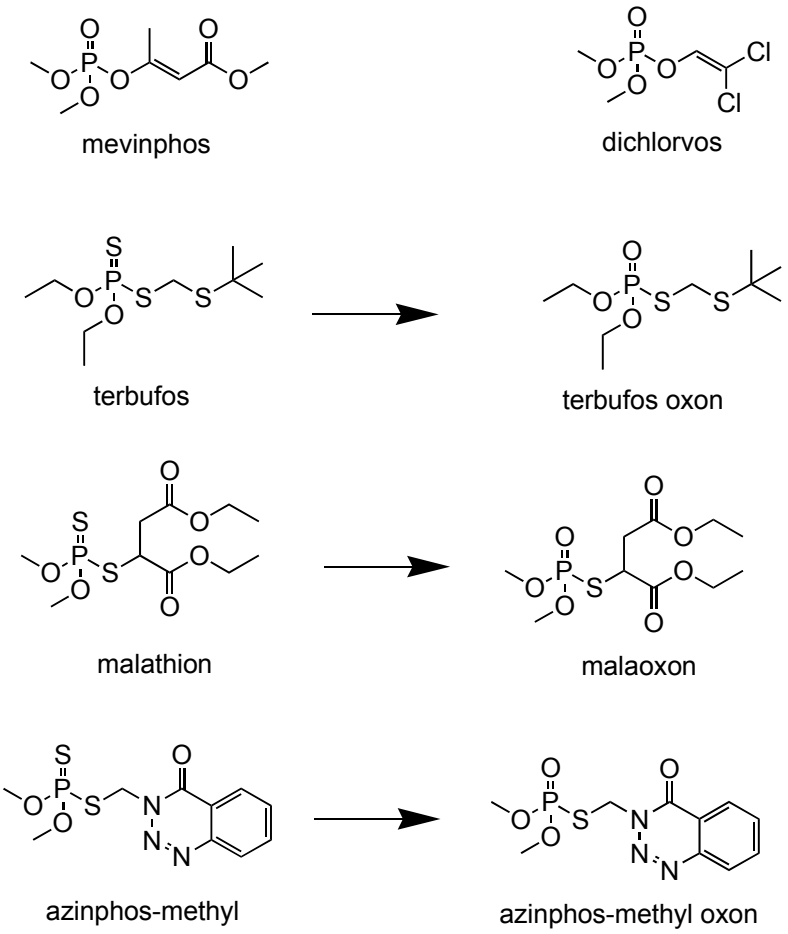


Figure 15. Some of the older OPs that are no longer used (mevinphos) or have very limited urban/commercial establishment structural uses (dichlorvos) are based on phosphate and therefore direct inhibitors of acetylcholinesterase (i.e., they do not have to be metabolized). However, most OP insecticides in commercial use are phosphorothioates (see earlier lecture with chlorpyrifos structure) or phosphorodithoates (represented by terbufos, malathion, and azinphos-methyl). These compounds are metabolized by the cyt. P450 dependent microsomal oxidases to an oxon form that is the highly active ligand inhibitor of acetylcholinesterase.

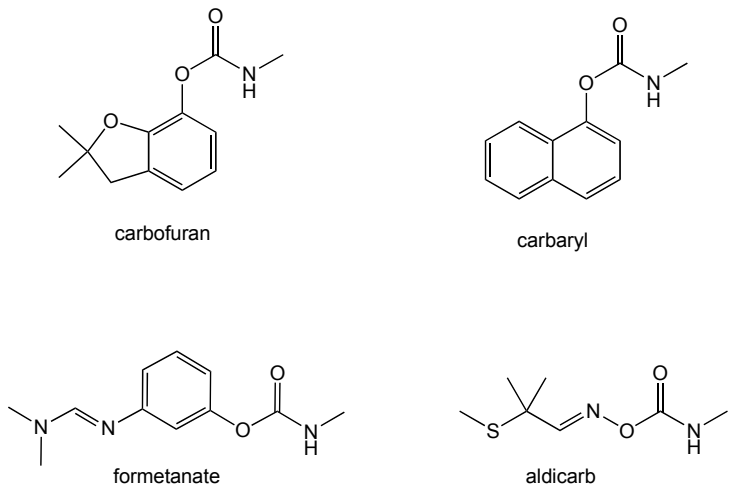


Figure 16. General structure of a methyl carbamate insecticide (top) and three methyl carbamate insecticide frequently studied in the environmental toxicology literature. Note that aldicarb is called an oxime methyl carbamate insecticide. Carbamates are capable of inhibiting acetylcholinesterase without being activated (i.e., oxidized to an active form).

a. OP insecticides in the thio form, i.e., P=S, have to be activated (i.e., metabolized) to the oxon (P=O) form to be significantly bioactive (Table 4).
   1. Note differences between the parent insecticide and its oxon form in the IC50 parameter. The IC50 is the concentration required to inhibit 50% of the acetylcholinesterase derived from house fly head homogenates.
b. In other words, the Km for the oxon form is much lower than the Km for the thio form of the OP.

Table 4. Acetylcholinesterase (AChE) Inhibition and Toxicity of Organophosphorus Insecticides and Metabolites. OP insecticides must be in the oxon form for effective binding with AChE. The LD50 generally decreases with the increase in binding, although the relationship is not necessarily linear. (From Felsot & Pedersen (1991, Am. Chem. Soc. Symp. Ser. 459)

Compound	**House Fly Head ChE Inhibition (I50, moles x 10^{-6})**	**LD 50 (μg/fly)**
Malathion	20	Not determined
oxon	0.0046	
Schradan	150000	Not determined
oxide	0.34	
Demeton, thiono	220	Not determined
oxon	0.024	0.7
Disulfoton	>100	Not determined
oxon	3.50	
Phorate	25	1.5
oxon	0.50	1.1

2. OP insecticides bind more tightly to AChE and do not release very quickly; CB binding to AChE is completely and quickly reversible.
   a. OP insecticides for the most part are reversible inhibitors, but the enzyme is dephosphorylated much slower than with CBs.
   b. Furthermore, OP insecticides can undergo an aging process, cleaving off one of the alkyl groups of the P, leaving the enzyme phosphorylated for very long periods of time. Diethyl groups are much slower to age than diisopropyl groups.

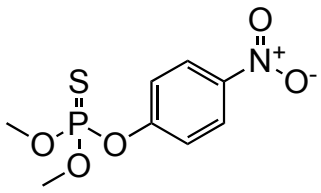
c. Typical symptoms (mammals)
   1. Symptoms include headache, giddiness, nervousness, blurred vision, weakness, nausea, cramps, diarrhea, and discomfort in the chest.
   2. Signs include sweating, miosis, tearing, salivation, excessive respiratory tract secretion, vomiting, cyanosis, papilledema, uncontrollable muscle twitches followed by muscular weakness, convulsions, coma, loss of reflexes, and loss of sphincter control.
      a. The last four signs are seen only in severe cases but do not preclude a favorable outcome if treatment is prompt and energetic.
   3. Antidotes are available--atropine and 2-PAM;
      a. These essentially function as competitive inhibitors of the AChE enzyme, but these are hydrolyzed much more readily than the insecticides
      b. 2-PAM has been used as a tool to determine a posteriori whether vertebrate animals may have been poisoned by exposure to OP insecticides.
         1. A homogenate is made of muscle tissue and then either exposed to 2-PAM or left untreated. The to reactivate acetylcholinesterase
         2. If AChE was inhibited, the 2-PAM would "reactivate" it and the difference in AChE activity could be compared to the untreated "control" homogenate.
   4. What makes a good acetylcholinesterase (AChE) inhibitor?
      a. OP and CB insecticides inhibit AChE by acting as alternative substrates to the neurotransmitter, ACh. The reaction of ACh with AChE proceeds as follows (where AX is acetylcholine, A is the acetyl group and X is the choline group, E is the enzyme, and k's are rate constants for the reaction).

$$E + AX \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EAX \xrightarrow{k_2} EA + X \xrightarrow{k_3} E + A$$

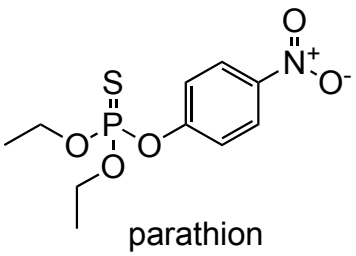
         1. Acetylcholine forms an enzyme-substrate complex with E, the active site of the enzyme is acylated, and then the acyl group is very quickly hydrolyzed, "recycling" E for the next reaction with AChE. In the process of acylating the enzyme, the choline (designated X and referred to as a 'leaving' group) diffuses back into the synapse and is taken up by the

presynaptic cells for synthesis of new ACh. Thus, the reaction kinetics denoted by k2 and k3 are very fast for ACh.

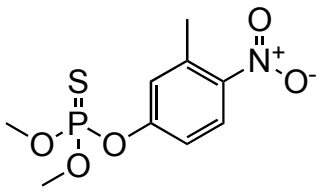
2. OP and CB insecticides also form a substrate-enzyme complex.
   (a) However, OP induced phosphorylation of the active site occurs in a reaction that creates a covalent bond, with X (the leaving group analog of choline) diffusing away from the reaction site.
   (b) The dialkyl phosphate group is only very slowly hydrolyzed, thereby freeing up the AChE. But, while AChE is phosphorylated, ACh cannot bind to it. Thus, the "excess" ACh is free to bind with the postsynaptic membrane receptors, continually stimulating the generation of action potentials in the postsynaptic axon.
      (1) For some phosphorylated AChEs, the dialkyl phosphate loses one of its alky groups in a process called aging. An aged AChE is not likely to recover, essentially inhibiting the enzyme over a very long time.
      (2) Thus, OP insecticides have a moderately fast k2 but a very slow k3 reaction rate.
3. In contrast to Ops, CBs have a very fast k3 (although slower than for AX). Thus CB intoxication is not as severe as OP intoxication because the enzyme is quickly "reactivated".
4. Among OPs, there differences in binding kinetics and affinity and hydrolyzability that influence in vivo toxicity.
   (a) As a rule of thumb, OPs with methyl groups attached to the P center are less toxic to mammals than OPs with ethyl groups.
   (b) As a rule of thumb, the electrophilic character of the leaving group X determines the affinity for the enzyme. The more electrophilic, the greater the affinity for the enzyme with subsequent phosphorylation (by loss of X).
   (c) Note for the homologous OPs below (methyl parathion, parathion, and fenitrothion), mammalian and fish toxicity decreases in the order parathion, methyl parathion, and fenitrothion. However, there is little difference in toxicity to Daphnia in flow-through studies.



methyl parathion



parathion



fenitrothion

Table 5. Comparative toxicity of analogous organophosphorothioate insecticides.

Insecticide	**Rodent Oral LD50 (mg/kg)**	**Rodent Dermal LD50 (mg/kg)**	**Bluegill Sunfish LC50 (μg/L)**	**Daphnia magna (Water flea) LC50 (μg/L)**
Parathion	2.7 – 10.8	No Data	161	3
Methyl Parathion	4.5 – 24	6	1000	0.14
Fenitrothion	250 - 800	>890	2600	2.3