September 29, 2003
Lecture 9: Neurotoxicity

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. Nerve Cell Morphology
   1. Single nerve cell is called a neuron
   2. A neuron consists of:
      a. Cell body containing the nucleus and numerous thin fibers extending from it.
         1. The fibers consist of a single long one called the axon and numerous shorter ones called the dendrites that are heavily branched. (See Figure 1)
         2. 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.
         3. The axon terminates at other nerve cells without touching them;
            a. Instead there is a small gap called the synapse between nerve cell axons and dendrites. (See Figure 2)

C. Electrical Physiology of the Nerve Cell
   1. The nerve signal conducts down the length of the axon in a one-way direction (See Figure 2).
      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. 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.

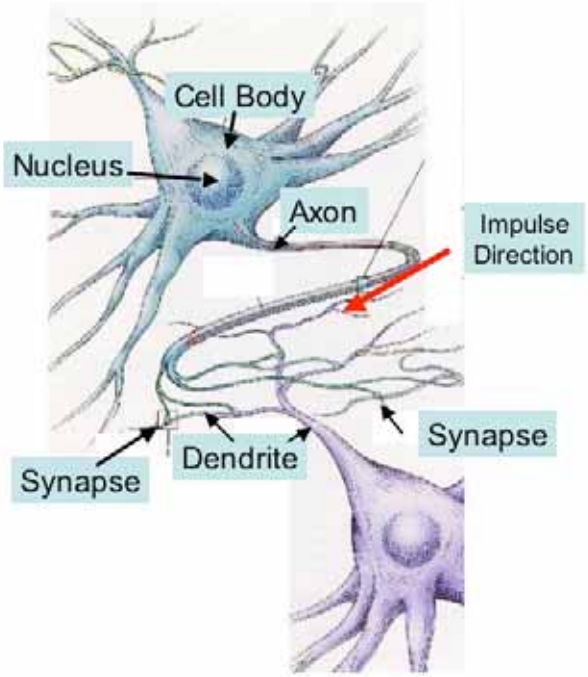


Figure 1. Nerve cell morphology (adapted from Scientific American)

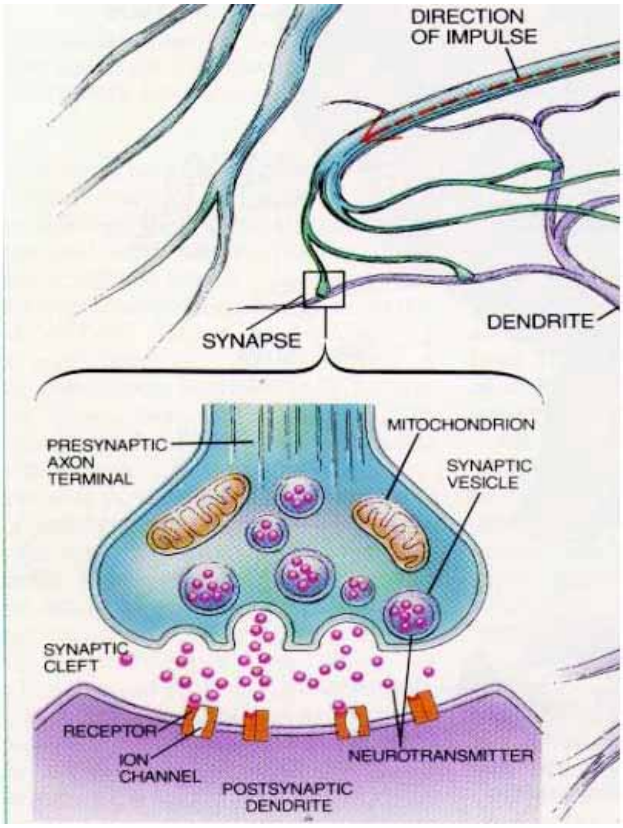


Figure 2. Nerve cell axon terminates prior to physically touching the dendrites. The gap is called the synapse. The nerve signal is transmitted across the synapse via a chemical signal called a neurotransmitter. (Adapted from Scientific American)

2. Permeabilty of the membrane to ions
    a. Cell membrane is semipermeable; allows some ions to freely diffuse across it, but other ions cannot;

3. Cell membrane and the resting potential
    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;
    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
        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

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.
   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. (Figure 3)

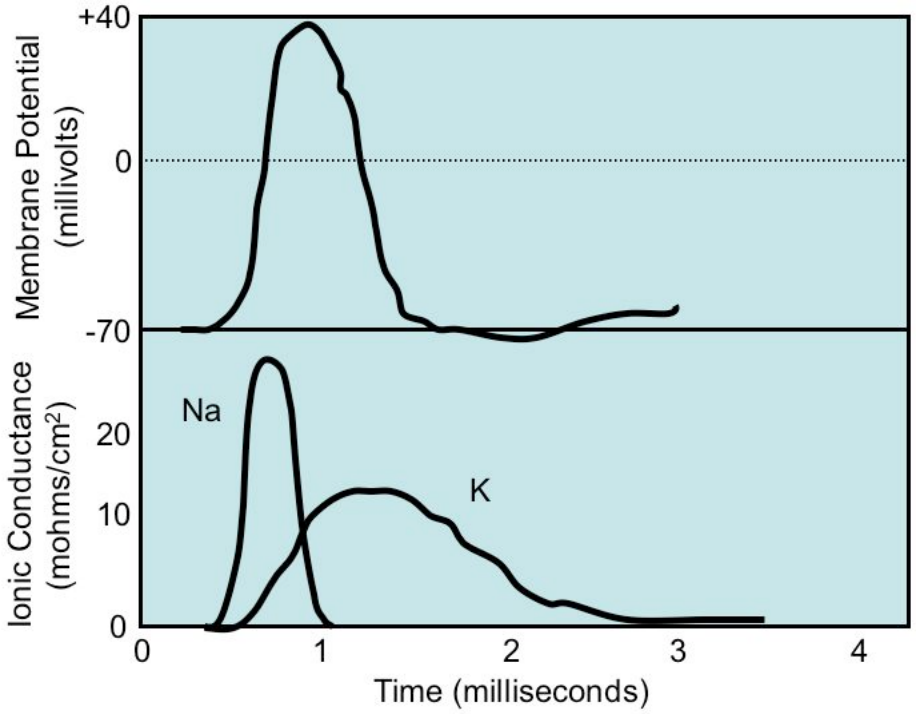


Figure 3. As membrane becomes partially depolarized, the sodium gates open, allowing sodium influx. About a millisecond later, the potassium gates open allowing potassium efflux.

2. The resting potential is depolarized and then hyperpolarized in the opposite direction to about +40 mV.
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 4)

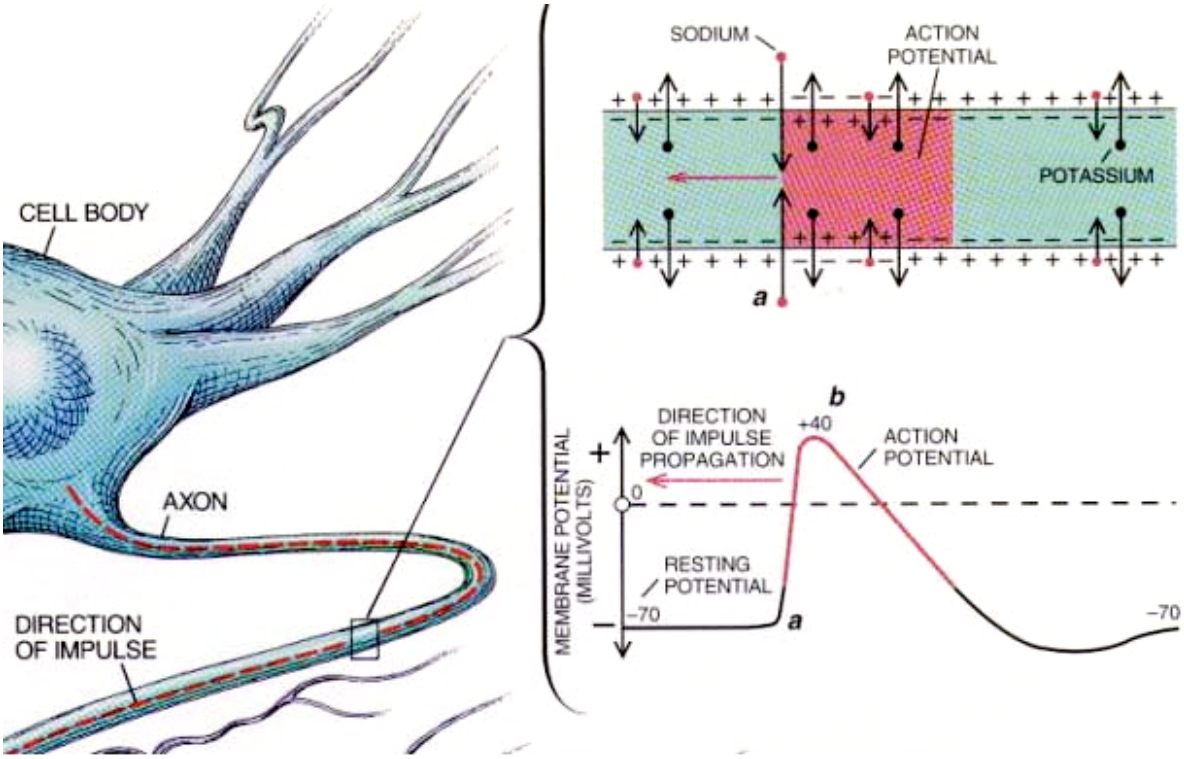


Figure 4. Illustration of the start of an action potential and its propagation along one direction of the nerve axon.

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.

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.

5. Repolarization and Return of Resting Potential
   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 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 to chemical energy;
   c. A neurotransmitter chemical called acetylcholine (ACh) is released from packets (vesicles) in the pre synaptic membrane;
   d. The acetylcholine molecules diffuse into the synapse (Figure 5);

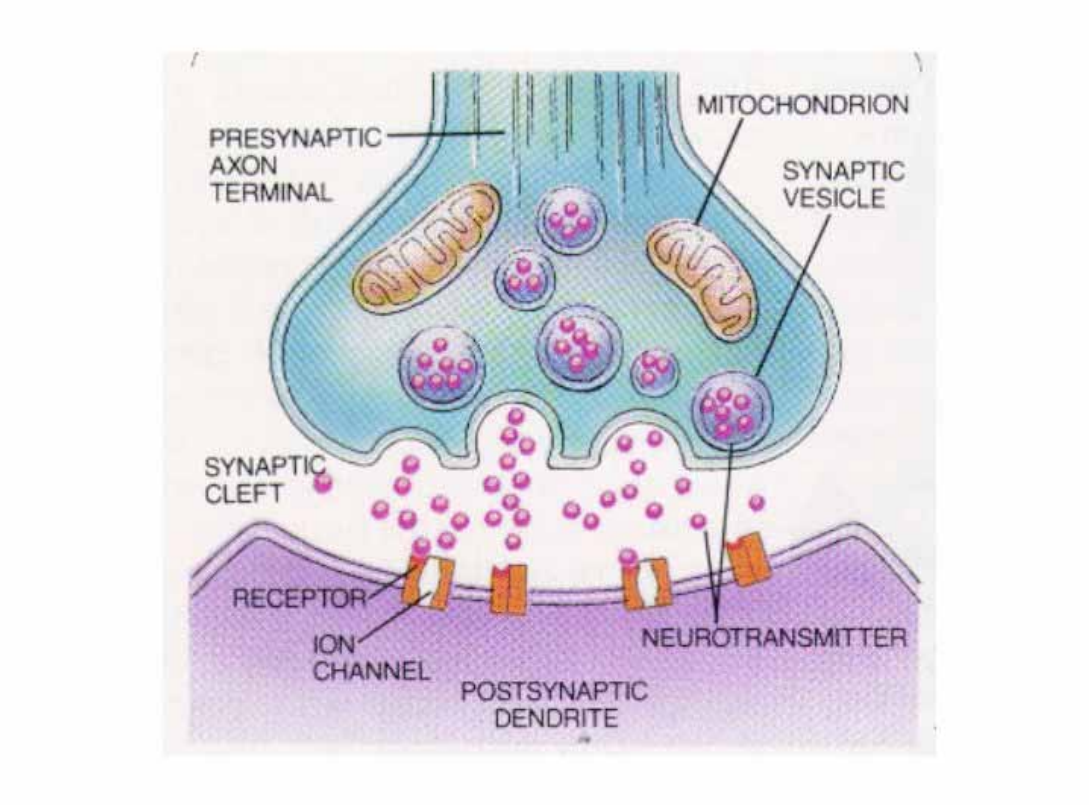
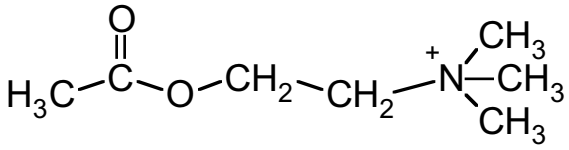


Figure 5. Release of acetylcholine neurotransmitter from the vesicle in the pre synaptic axon. ACh molecules diffuse across the synapse (the gap space is called the synaptic cleft) and binds to receptor proteins embedded in the post synaptic membrane. Not shown are acetylcholinesterase enzymes, also embedded in the post synaptic membrane.

   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 post synaptic membrane and act as ion channels, similarly to the sodium channels in the axon.

f. Binding of acetylcholine to the receptors than 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.
   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.

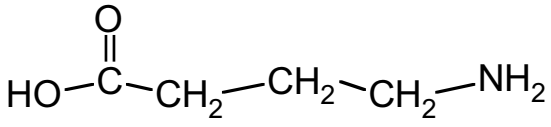


acetylcholine (ACh)

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 post synaptic membrane near the ACh receptors.
   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 verteberates, but glutamate is used at the neuromuscular junction.

7. Inhibition and Modulation of Signal Transmission
   a. Some neurotransmitters inhibit nerve-nerve or nerve -muscle transmission;
      1. GABA (gamma amine butyric acid) interacts with receptors on the post-synaptic 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)

b. Some nerves terminate on organs without a specific synaptic junction
1. These release chemicals called <u>neuromodulators</u> that 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>.

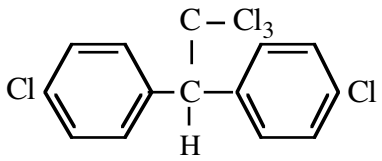
III. Vulnerability of Nervous System to Attack by Insecticides

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?

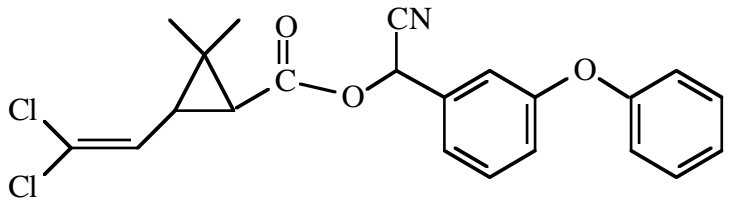
B. Basically, the nervous system is vulnerable to toxicosis at two levels:
1. Level of the Synapse
2. Level of the Ion Channels

IV. Toxicants Affecting the Ion Channels

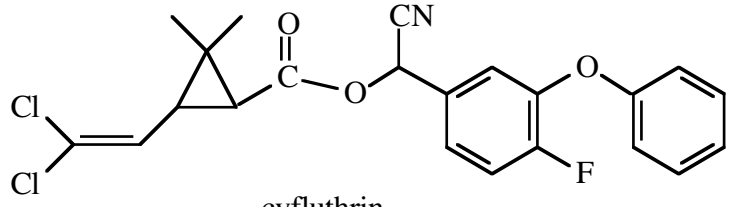
A. DDT and Pyrethroid Insecticides have an affinity for the sodium channels.
1. These insecticides block the closing of the sodium gate, thereby prolonging the sodium influx.
2. When the sodium channel is prevented from closing, the nerve is easily stimulated again to produce action potentials because now the threshold for response has essentially been exceeded.



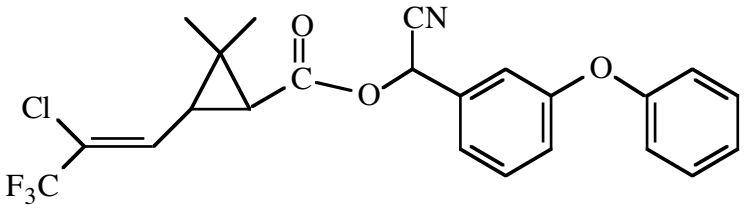
p,p'-DDT



cypermethrin



cyfluthrin

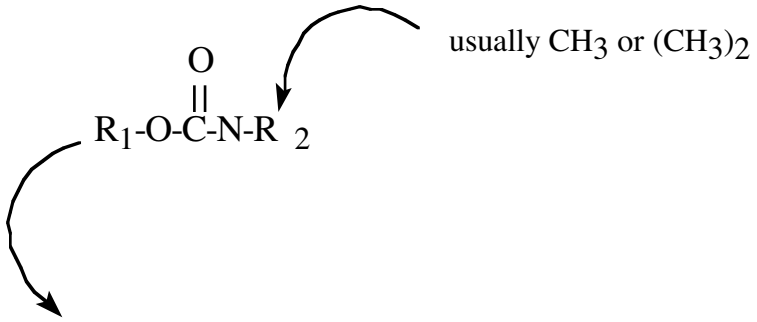


lambda cyahalothrin

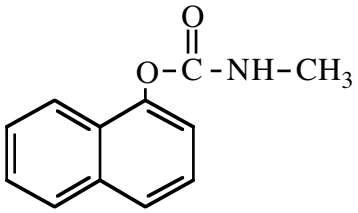
Examples of three pyrethroid insecticides. Pyrethroids are all esters and are quickly hydrolyzed in vertebrates, but not in invertebrates.

V. Toxicants Acting at the Level of the Synapse

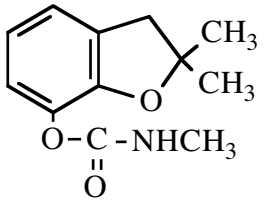
A. Several biochemical mechanisms are possible
   1. Acetylcholine agonism
      a. Inhibit acetylcholinesterase, effectively increasing the concentration of acetylcholine in the synapse
         1. The insecticide classes known as organophosphorus (OP) and methyl carbamates (CB) inhibit AChE, causing prolonged stimulation of the ACh receptor.
         2. Differences between OP and CB insecticides:
            a. OP insecticides must be in the oxon form to inhibit AChE; CBs can inhibit AChE without prior transformation (Table 2)



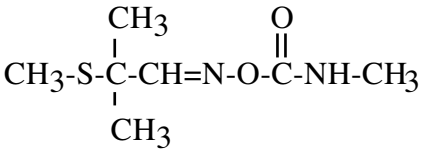
General Structure of a Methyl Carbamate Insecticide



carbaryl (Sevin)



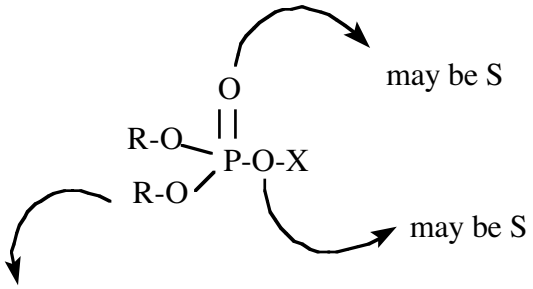
carbofuran (Furadan)



aldicarb (Temik)

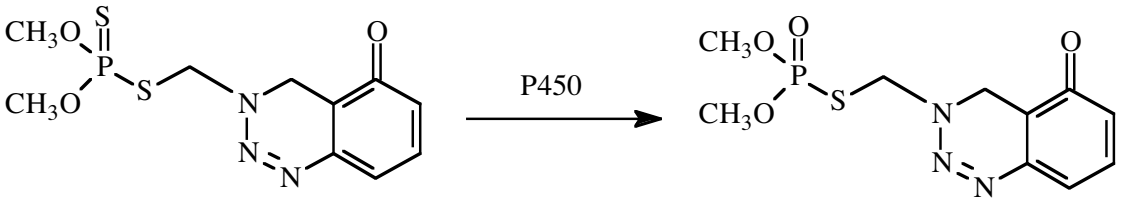
Three methyl carbamate insecticide frequently studied in the environmental toxicology literature. Note that aldicarb is called an oxime methyl carbamate insecticide. Carbamates are capable of inhibiting acetylcholinesterase without being activated (i.e., oxidized to an active form).

1. 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 bioative;
2. In other words, the Km for the oxon form is much lower than the Km form for the thio form.

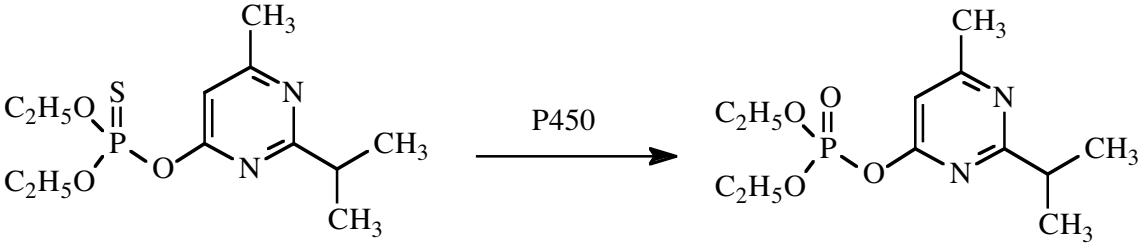


R is an alkyl group
usually of 1 or 2 C;
both R groups usually the same

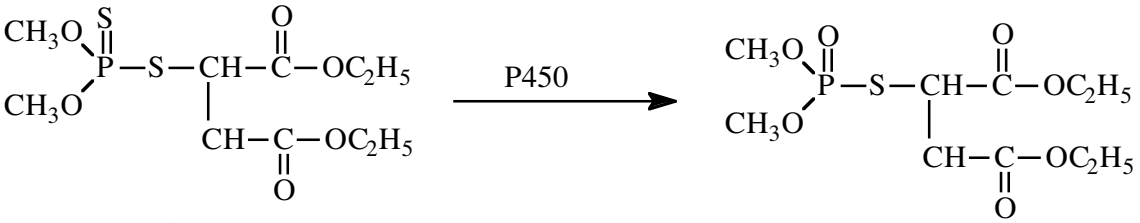
General structure of an organophosphorus insecticide. Most of the OP insecticides are in the P=S configuration and must be activated (oxidized via P45) to be able to bind and inhibit AChE.



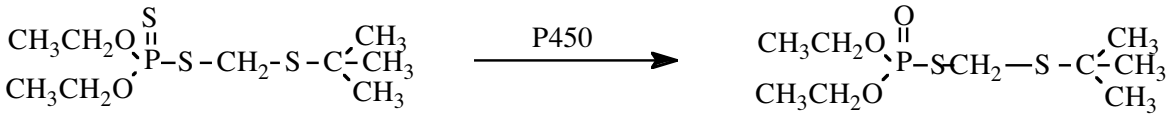
azinphos-methyl (Guthion) azinphos-methyl oxon



diazinon diazoxon



malathion malaoxon



terbufos (Counter) terbufos oxon

Four examples of OP insecticides studied in the environmental toxicology literature.

Table 2. 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)

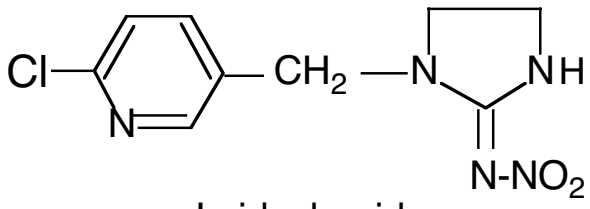
Compound	House Fly Head ChE Inhibition (I50, moles x 10^{-6})	LD 50 (μg/fly)
Malathion	20	Not determined
oxon	0.0046	
Schradan	150000	Not determined
oxide	0.34	
Demeton, thiono	220	Not determined
sulfoxide	3.60	2.0
sulfone	0.83	1.2
oxon	0.024	0.7
oxon sulfoxide	1.10	8.7
oxon sulfone	0.12	3.7
Demeton, thiolo	3.50	Not determined
sulfoxide	1.50	0.8
sulfone	0.60	1.2
Disulfoton	>100	Not determined
sulfoxide	70	
sulfone	3.50	
oxon	3.50	
oxon sulfoxide	1.50	
oxon sulfone	0.60	
Phorate	25	1.5
sulfoxide	3.70	5.5
sulfone	0.04	4.5
oxon	0.50	1.1
oxon sulfoxide	0.40	5.5
oxon sulfone	0.10	1.5

b. OP insecticides bind more tightly to AChE and do not release very quickly; CB binding to AChE is completely and quickly reversible.

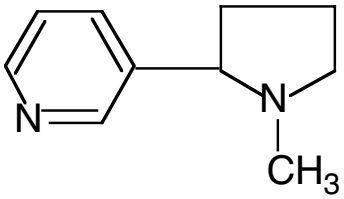
1. OP insecticides for the most part are reversible inhibitors, but the enzyme is dealkylated much slower than with CBs.
2. OP insecticides can undergo an aging process, cleaving off one of the alkyl groups of the P, leaving the enzyme phosphorylated for very long periods of time. Diethyl groups are much slower to age than deisopropyl groups.

3. Typical symptoms (mammals)
   a. Symptoms include headache, giddiness, nervousness, blurred vision, weakness, nausea, cramps, diarrhea, and discomfort in the chest.
   b. 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.
      1. The last four signs are seen only in severe cases but do not preclude a favorable outcome if treatment is prompt and energetic.
   c. Antidotes are available--atropine and 2-PAM;
      1. These essentially function as competitive inhibitors of the AChE enzyme, but these are hydrolyzed much more readily than the insecticides
      2. 2-PAM has been used as a tool to determine aposteriori whether vertebrate animals may have been poisoned by exposure to OP insecticides.
         (a) A homogenate is made of muscle tissue and then either exposed to 2-PAM or left untreated. The to reactivate acetylcholinesterase
         (b) If AChE was inhibited, the 2-PAM would "reactivate" it and the difference in AChE activity could be compared to the untreated "control" homogenate.

b. Mimic acetylcholine
   1. Capable of binding to the acetylcholine receptors on the post synaptic membrane, triggering an action potential.
      a. Nicotine is an ACh receptor agonist
      b. A comparatively new class of insecticides called neonicotinoids mimic ACh by binding to the receptor of insects.
         1. However, the vertebrate ACh receptor seems insensitive to the neonicotinoids (i.e., binding is very poor compared to that in insects)

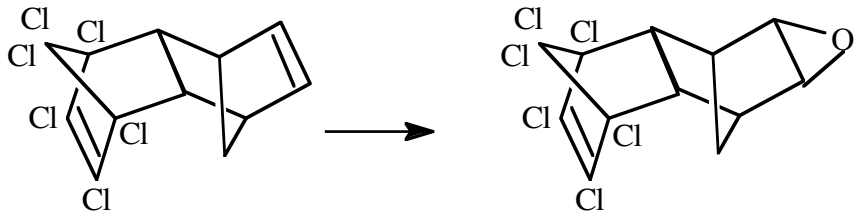


Imidacloprid

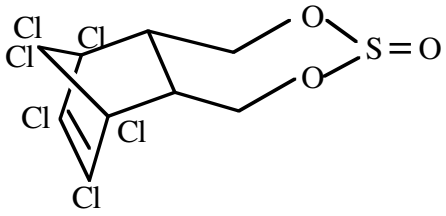


Nicotine

2. Inhibit GABA (i.e., gamma aminobutyric acid antagonism)
   a. Chlorinated cyclcodiene insecticides like dieldrin (banned since 1974) and endosulfan bind to the GABA receptor and inhibit the channel from opening to the influx of chloride ions.
      1. Dieldrin may require further metabolism to a trans-diol form



aldrin dieldrin



endosulfan

Chlorinated cyclodiene insecticides. Aldrin is oxidized in the environment to dieldrin. Aldrin and dieldrin were banned by EPA in ~1974. Endosulfan is still used today, mainly to control aphids and related pest species.

VI. Comparative Acute Toxicity of Major Insecticide Groups

Chlorinated Hydrocarbons & Cyclodienes

Compound	Rat oral LD_{50} mg/kg	Rabbit Dermal LD_{50} (mg/kg)	Fish LC_{50} μg/L, trout	Bird $LD_{50\ mg/kg}$ Calif. Quail
DDT	87	1931	4.1-11.4	595
DDD	3400	4000	70	
DDE	880-1240		32	
Chlordane	400-700	580	8.2-135	14.1
Dicofol	575-2000	4000	111	1.89
Endosulfan	18	74	1.1-2.9	80-160 (pheasant)
Methoxychlor	5000	2820	11-61	>2,000

Organophosphates, Carbamates, Pyrethroids

Compound	Rat oral LD_{50} mg/kg	Rabbit Dermal LD_{50} (mg/kg)	Fish LC_{50} μg/L, trout	Bird LD_{50} mg/kg Calif. Quail
parathion	3	6.8	1500	16.9
chlorpyrifos	135	2000	3	68.3
phorate	1.6	2.5	13	7.1 (pheasant)
azinphosmethyl	13	250	20	74.9 (pheasant)
diazinon	300	379	16000	4.33 (pheasant)
malathion	885	4000	170	167 (pheasant)
carbaryl	307	2000	1300	>2000
carbofuran	8	2550	280	4.15 (pheasant)
aldicarb	0.9	>5	880	2.58
propoxur	95	>1000	3700	30
cypermethrin	150	1600	1	2000 (chicken)

A Comparison of Acute Toxicities of Organochlorine, Organophosphate, Carbamate & Pyrethroid Insecticides--Concept of Selectivity (numbers in parantheses represent the no. of data values used to calculate the geometric mean LD_{50})--Data from Elliott 1977

Class	Rats (mg/kg)	Insects (mg/kg)	Ratio (rat/insect)
Carbamate	45 (15)	2.8 (27)	16
Organophosphate	67 (83)	2.0 (50)	33
Organochlorine	230 (21)	2.6 (26)	91
Pyrethroid	2000 (11)	0.45 (35)	4500