Instructor: Allan Felsot
afelsot@tricity.wsu.edu

Fall 2005

ES/RP 531
Fundamentals of Environmental Toxicology

Lecture 13
Neurotoxicity
Part II

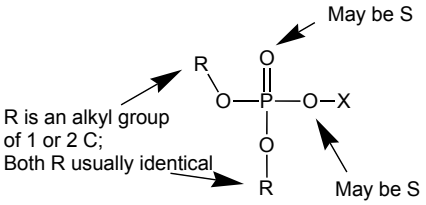
Where the Nerve Is Vulnerable

- Level of the ion channels (axon/dendrites)
- Level of the synapse
  - Inhibit acetylcholinesterase (AChE)
  - Mimic acetylcholine (ACh)
  - Inhibit GABA receptors
  - Agonize GABA receptors

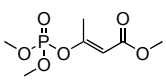
Pharmacodynamic Action at the Level of the Synapse

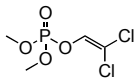
- Acetylcholine agonism (mimic)
  - Inhibit acetylcholinesterase, effectively increasing the concentration of ACh in the synapse
  - Cause prolonged stimulation of the ACh receptor
- Mode of action of organophosphorus (OP) and methyl carbamate (CB) insecticides

Generalized Structure of Organophosphorus (OP) Insecticides

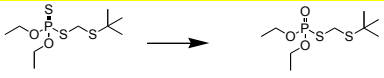


Organophosphoroates
Forms that bind to acetylcholinesterase (AChE) with high affinity

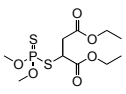

mevinphos

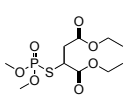

dichlorvos

Organophosphorodithioates
Forms that must be oxidized to oxons before binding with high affinity to AChE


terbufos

terbufos oxon


malathion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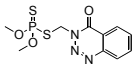
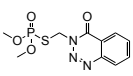
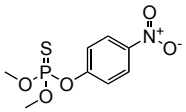
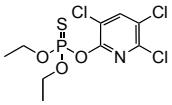

azinphos-methyl


azinphos-methyl oxon

Organophosphorothioates
Forms that must be oxidized to an oxon before binding with high affinity to AChE

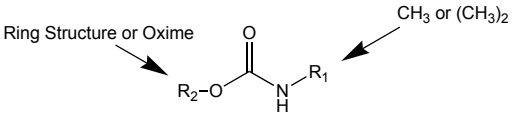


methyl parathion



chlorpyrifos

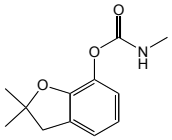
Generalized Structure of Methyl Carbamate (CB) Insecticides



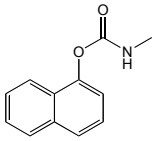
General Structure of A Methyl Carbamate Insecticide

Representative Methyl Carbamate Insecticides

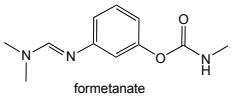
No activation need for high affinity binding to AChE



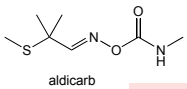
carbofuran



carbaryl



formetanate



aldicarb

oxime CB

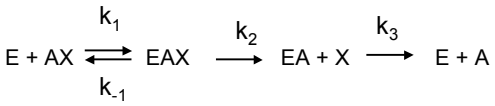
Differences Between OP and CB Insecticides with Regard to Target Affinity

- Both OP and CB insecticides bind to acetylcholinesterase and inhibit the enzyme from catalyzing the hydrolysis of acetylcholine
- OP insecticides must first be activated by P450 enzyme to an “oxon” form before binding to the AChE enzyme is appreciable
  - The Km for the oxon form is much lower than the Km for the thio form
- CB insecticides require no activation

Differences Between OP and CB Insecticides: Interaction with their Target

- CB is considered a completely reversible inhibitor, quickly disassociating from the enzyme as a result of its own hydrolysis
- OP insecticides are only slowly hydrolyzed and inhibit the enzyme over prolonged periods of time; thus they are considered to not be reversible inhibitors but over time that is not really true
- However, some OP insecticides are de-alkylated (i.e., the R-O- substituent is hydrolyzed) while on the enzyme, which causes essentially irreversible inhibition

Interaction of Ligands with AChE

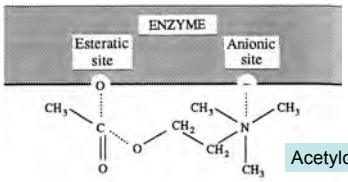


E = Acetylcholinesterase Enzyme

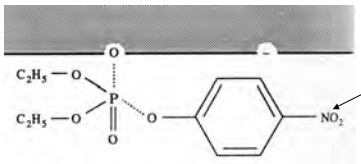
AX = Acetylcholine or Phosphate Ester or Carbamate Ester

X = Choline or Electronegative Leaving Group

A = Acyl group (or alkyl groups of OP or CB)



Acetylcholine (ACh)



Capable of H-Bonding

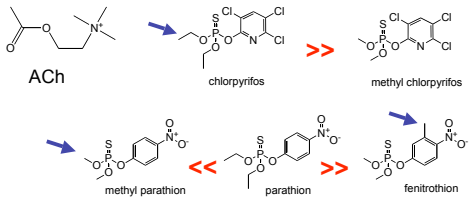
Interaction of AChE with the OP Insecticide Parathion

Relationship Between Structure, AChE Inhibition, and Toxicity

Compound	House Fly Head ChE Inhibition (I_{50}, µmoles)	LD50 (µg/fly)
Malathion	20	--
oxon	0.0046	--
Demeton	220	--
sulfoxide	3.60	2.0
oxon	0.024	0.7
Phorate	25	1.5
sulfoxide	3.7	5.5
oxon	0.50	1.1

What Makes a Good AChE Inhibitor?

- The Km for enzyme substrate interactions depends on having a very electronegative leaving group (the X in the generalized structure of the ligand)
- Also, for OP insecticides, the alkyl chain length of the phosphate ester constituents influences binding affinity



Effect of Structure on Toxicity of Homolgous OP Insecticides

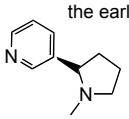
OP Insecticide	LD50 Rodent (Oral) mg/kg	LD50 Rodent (Dermal) mg/kg	LC50 Bluegill Sunfish µg/L	LC50 Daphnia magna (Water flea) µg/L
Ethyl parathion	2.7 - 10.8	No Data	161	3
Methyl parathion	4.5 - 24	6	1000	0.14
Fenitrothion	250 - 800	>890	2600	2.3

Typical Symptoms & Signs of AChE Inhibitors

- Symptoms
  - Headache, giddiness, nervousness, blurred, vision, weakness, nausea, cramps, diarrhea, discomfort in chest
- Signs
  - 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

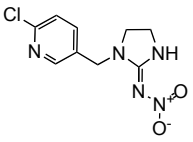
Acetylcholine Mimics

- Capable of binding to the ACh receptor on the post synaptic membrane, triggering an action potential when binding level is high enough
- Nicotine was one of the first ACh mimic discovered
  - Use as an insecticide prior to the 1950's
- Neonicotinoid (chloronicotinyl) insecticides
  - Imidacloprid was the first one commercialized in the early 1990's.



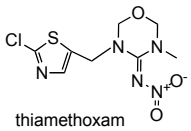
nicotine

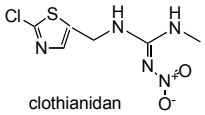
Rat oral LD50 = 53 mg/kg
Rat dermal LD50 =250 mg/kg

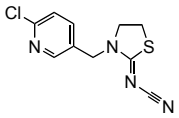


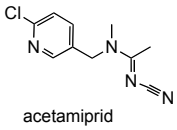
imidacloprid

Rat oral LD50 = 450 mg/kg
Rat oral LD50 = >5000 mg/kg


thiamethoxam


clothianidan


thiacloprid

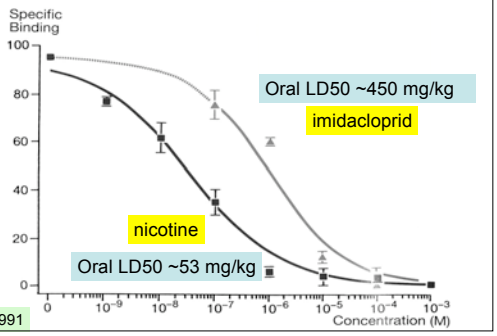

acetamiprid

Comparative Toxicity

Compound	Rat LD50 mg/kg	Bird LD50 mg/kg	Fish LC50 (µg/L)
Chlorpyrifos	135	68.3	3
Azinphos-methyl	13	74.9	20
Aldicarb	0.9	2.58	880
Cyfluthrin	869	>2000	0.68
Imidacloprid	450	31	211
Acetamiprid	182	180	>100
Thiamethoxam	1563	1552	>100

Why Are Chloronicotinyls Less Risky to Vertebrates than OPs and CBs

The key factor is specific binding potential with the nAChR



Abbink 1991

Why Is Imidacloprid "Safe" for Vertebrates But Not Invertebrates

Specific Binding (DPM/µg of tissue)

Species	Brain	Head	Whole body
Vertebrates	<0.01		
Insects			
House fly		16	1.4
Fruit fly			2.1
Cricket	42	0.6	<0.05
Honeybee		2.3	0.33
Cockroach	24		0.09

Liu & Casida (1993)

Specificity of chloronicotinyl insecticides for insect and vertebrate nicotinic receptors (nAChR)

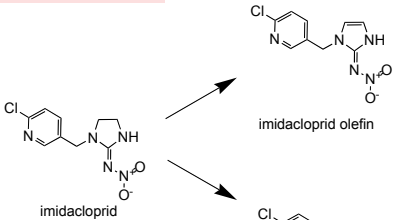
Tomizawa & Casida (2005)

Compound	IC50 (nM) Insect	IC50 (nM) Vertebrate	Selectivity Vert./Insect
Nicotine	4000	7.0	0.002
Imidacloprid	4.6	2600	565
Desnitroimidacloprid	1530	8.2	0.005
Thiamethoxam	5000	>100,000	>20
Acetamiprid	8.3	700	84

IC50 is the concentration of insecticide that displaces 50% of a pre-bound ligand from the nAChR

Potential for toxicity of imidacloprid metabolites

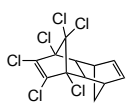
Retains toxicity to bees



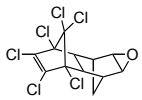
Toxic to rodents if injected

GABA Mimics

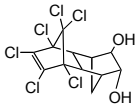
- Antagonists:
  - Bind noncompetitively to GABA receptor and block the channel from opening to allow diffusion chloride ions
- Polychlorinated cycloalkanes
  - Chlorinated cyclodienes
  - Chlorinated hydrocarbons



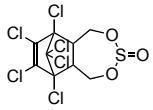
aldrin



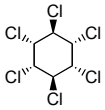
dieldrin



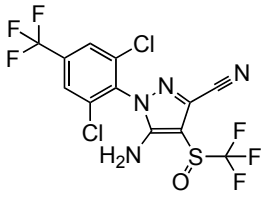
dieldrin trans diol



endosulfan



lindane



fipronil

Registered in the 1990's; greater selectivity for insects, but same MOA as banned chlorinated cyclodienes

Selectivity of Polychlorinated Cycloalkanes & Friponil

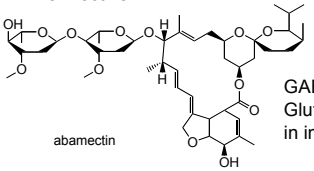
Ratra et al. 2001

Insecticide	Potency Mammals	Potency Insects	Selectivity Mammal/Inset
GABA R IC50 (nM)			
Endosulfan	28	10	2.8
Lindane	833	11	76
Fipronil	4300	2.3	1870
LD50 (mg/kg)			
Endosulfan	10	5.5	1.8
Lindane	40	5.5	7.3
Fipronil	32	0.25	128

Potency defined as the IC50 for displacement of ligand from GABA receptor

Natural Products Are Neurotoxins

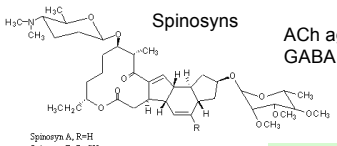
Avermectins



abamectin

GABA agonist in vertebrates; Glutamate receptor antagonist in insects

Spinosyns



Spinosyn A, R=H
Spinosyn D, R=CH_3

Spinosad

ACh agonist; interaction with GABA receptors

Both are produced in microbial fermentation cultures

Comparison of Acute Toxicity & Selectivity of Neurotoxic Insecticides

Geometric Mean LD50

Tomizawa & Casida 2005

Class	Rats (mg/kg)	Insects (mg/kg)	Ratio (rat/insect)
Chloronicotinyls	912	2.0	456
Organophosphate	67	2.0	33
Carbamate	45	2.8	16
Organochlorine	230	2.6	91
Pyrethroid	2000	0.45	4500

Neurodevelopmental Toxicity Current Concern for Human Health

- Recognized that brain development, including growth of neurons and synaptic connections, in the fetus depends on proper levels of acetylcholine and acetylcholinesterase
- Recognized that thyroid hormone plays a role in proper brain development
- Thus, any toxicant interfering with these biochemical systems can interfere with neonatal and later neurological development

Neurodevelopmental Toxicity Current Concern for Human Health

- Lead
- Methyl mercury
- Nicotine, chlorpyrifos (and possibly other OP insecticides?)
- PCBs