October 10, 2005
Lecture 13 Neurotoxicity II

I. Toxicants Acting at the Level of the Synapse (continued)

A. Another mechanism of acetylcholine receptor agonism is mimicking acetylcholine by binding directly to the ACh receptor.
   1. The toxicant ligand has sufficient affinity with the receptor to bind with it and stimulate excitatory postsynaptic potentials (EPSPs) that eventually can surpass the threshold needed for generating an action potential at the axon hillock.
      a. Nicotine exemplifies a naturally occurring ACh receptor agonist. It specifically binds to nicotinic ACh receptors (indeed, this known binding biochemistry and subsequent neurophysiological effects is why the ACh receptors that are bound directly by acetylcholine to open the sodium channel are called nicotinic receptors).
         1. Nicotine was used as an agricultural insecticide prior to the 1940's and advent of DDT.
            a. Although rightfully considered a botanical insecticide, nicotine is far from innocuous even in terms of acute toxicity. The rodent acute oral LD50 is~53 mg/kg bw, and the dermal toxicity (based on the rabbit dermal toxicity assay) is 250 mg/kg bw.
   2. A comparatively new class of insecticides called neonicotinoids (or chloronicotinyls) mimics ACh and binds with high affinity to the ACh receptor of insects (Figure 1). Thus, these compounds also mimic nicotine in its mode of toxic action.

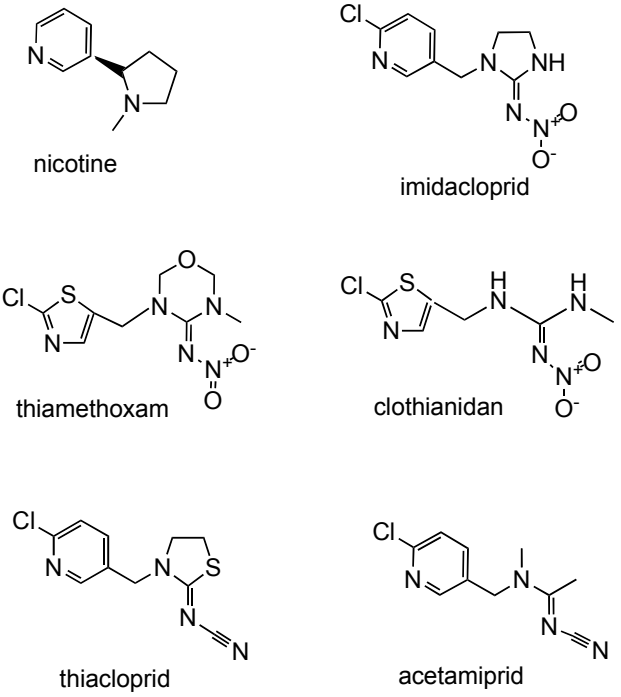


Figure 1. Major chloronicotinyl insecticides. Imidacloprid was the first one registered and has gained usage in both the urban and agricultural markets. It is very toxic to soft-bodied sucking insects like aphids and whiteflies.

a. However, the vertebrate ACh receptor seems insensitive to the chloronicotinyls compared to nicotine (i.e., binding is very poor compared to that in insects) (Figure 2).

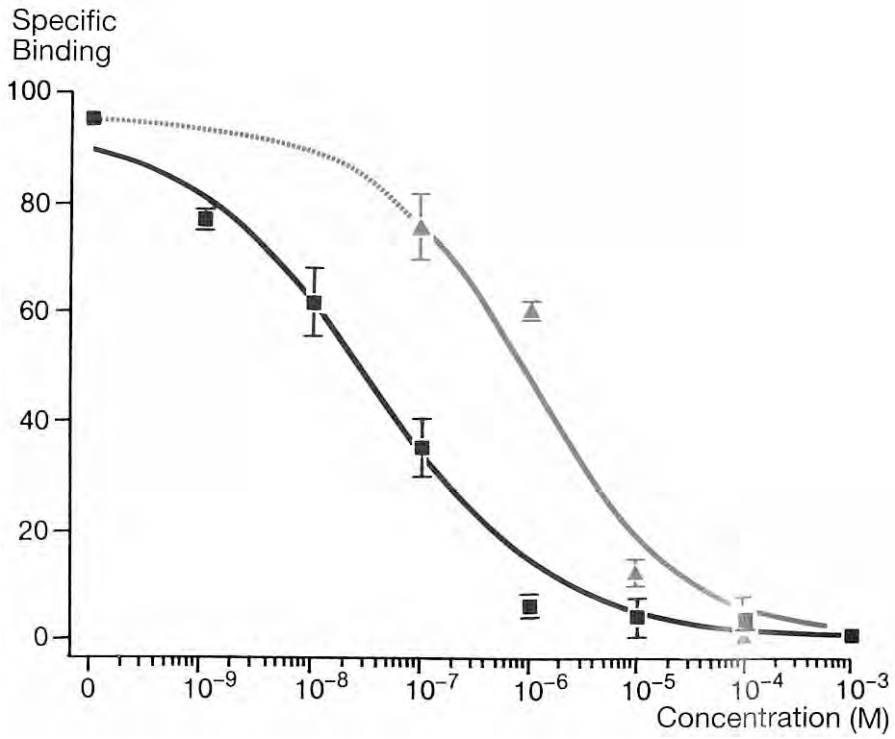


Figure 2. Binding of nicotine (dark line) and imidacloprid (light line) to acetylcholine receptors in vitro. Note that when the binding curve is displaced to the left on the graph, such as nicotine is relative to imidacloprid, then the interpretation is that binding occurs more readily or with greater affinity. Thus, the binding of nicotine to the receptor at a much lower concentration than imidacloprid is consistent with the greater acute oral toxicity of nicotine. (Graphic from Abbink, J. 1991. The biochemistry of imidacloprid. Pflanzenschutz-Nachrichten Bayer 44:183-195.)

b. The comparatively poor binding of imidacloprid to the acetylcholine receptor explains its much lower acute toxicity compared to nicotine. For example, the rat acute oral LD50 of imidacloprid is~ 450 mg/kg bw and the rat dermal LD50 is >5000 mg/kg.
   1. In addition to the poor binding to the ACh receptor, chloronicotinyls do not accumulate in fatty tissue nor do they cross easily the blood brain barrier (Sheets 2001, Chapter 54, Handbook of Pesticide Toxicology, Academic Press).
      a. Thus, imidacloprid (and other chloronicotinyl insecticides) are selectively toxic to insects because of pharmacodynamic and pharmacokinetic limitations to interaction with the target ACh receptors in mammals (see Tables 1 & 2).

Table 1. Binding of imidacloprid (measured as the radioactivity or dpm [disintegrations per minute]) to the acetylcholine receptor from different body regions in vertebrates and invertebrates. The higher the dpm number, then that more the binding (Liu and Casida. 1993. High affinity binding of [^{3}H]imidacloprid in the insect acetylcholine receptor. Pesticide Biochemistry & Physiology 46:40-46.)

	Specific Binding as dpm/μg protein plus (% of total tissue binding)		
Species	**Brain**	**Head**	**Whole Body**
Vertebrates (including human brain, dog. Mouse, chicken)	<0.01 (0)	--	--
Insects			
House fly		16 (92)	1.4 (65)
Fruit fly			2.1
Cricket	42 (82)	0.6 (19)	<0.05 (0)
Honeybee		2.3 (62)	0.33 (37)
American cockroach	24 (86)		0.09 (9)
German cockroach		3.8 (50)	
Milkweed bug		2.5 (43)	

Table 2. Specificity of chloronicotinyl insecticides, an imidacloprid metabolite (desnitroimdacloprid), a thiacloprid metabolite (descyanothiacloprid) and nicotine for insect and vertebrate nicotinic receptors. (Tomizawa, M. and J. E. Casida. 2005. Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. **45**: 247-268.)

Compound	**IC50 (nM) [1/] Insect**	**IC50 (nM) [1/] Vertebrate**	**Selectivity Ratio (Vertebrate/Insect)**
Nicotine	4000	7.0	0.002
Imidacloprid	4.6	2600	565
Desnitroimidacloprid	1530	8.2	0.005
Thiamethoxam	5000	>100,000	>20
Clothianidin	2.2	3500	1591
Thiacloprid	2.7	860	319
Descyanothiacloprid	200	4.4	0.022
Acetamiprid	8.3	700	84

1/ The IC50 is the insecticide concentration that displaces 50% of a pre-bound ligand on the acetylcholine receptor. For experimental purposes, either tritiated imidacloprid or nicotine was bound to the insect and vertebrate (rat) nicotinic ACh receptors, respectively. Thus, a lower IC50 signifies a very strong interaction of the insecticide with the receptor, and a comparatively higher IC50 indicates a weaker interaction (i.e., it takes a much higher concentration to displace the pre-bound ligand).

3. Some metabolites of certain chloronicotinyl insecticides retain their toxicity against insects.
   a. For example, the olefin metabolite of imidacloprid is still nearly as toxic to honey bees as is the parent compound (Nauen 2001). Receptor binding studies show a high affinity for nicotinic acetylcholine receptors. (see Figure 3 for the structure)
   b. Desnitroimidacloprid (Figure 3), a putative metabolite of imidacloprid in mammals, is actually much more toxic to mice than imidacloprid is (see Table 2 for receptor binding information and the selectivity ratio).

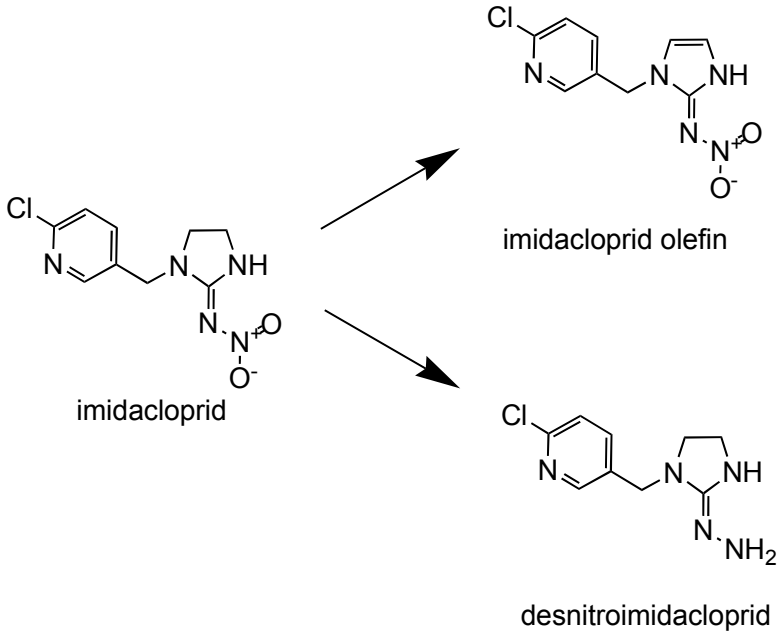


Figure 3. Toxic metabolites of imidacloprid. The olefin metabolites retains toxicity to honey bees, and the desnitroimidacloprid is toxic to mice but the parent exhibits very low toxicity. However, desnitrimidacloprid has only been tested by intraperitoneal injection. It seems to be able to cross the blood-brain barrier, but whether the desnitroimidacloprid is formed in high enough concentrations to be toxic in mammals exposed through normal routes (oral or dermal) remains untested.

4. Once a chloronicotinyl insecticide binds to the nicotinic ACh receptor, it can trigger an action potential if its concentration is high enough. Thus, chloronicotinyls may be relatively innocuous to invertebrates at normal environmental use rates, but as with all other chemicals, "dose makes the poison".
   a. For example, at high enough doses, imidacloprid causes typical symptoms of nervous system toxicity.
      1. For example, rats given an oral acute (single) lethal dose show typical nervous system poisoning symptoms not too different from the havoc caused by overdoses of organophosphate insecticides—diarrhea,

emaciation, lethargy, labored breathing, incoordination, staggering, trembling, and spasms.

2. Interestingly, the Material Safety Data Sheet (MSDS) for imidacloprid states, "no specific symptoms of acute overexposure are known to occur in humans", suggesting an acceptable level of safety to humans who handle the material.
3. In acute neurotoxicity studies, the objective is to determine whether high, but nonlethal single doses to rats, cause long-term neurological impairment, including limb paralysis and/or behavioral impediments.
   a. The highest single dose of imidacloprid (307 mg/kg) given orally resulted in some death, and survivors had decreased motor skills and response to auditory stimuli. However, symptoms in surviving rats subsided 5 days after exposure. At the lowest dose (42 mg/kg), females but not males exhibited reduced locomotor activity in a maze behavior study.

5. Imidacloprid, as well as other chloronicotinyls, are considered by EPA to have a very favorable ecological toxicity profile, i.e., they seem to be of comparatively low toxicity to nontarget organisms, especially when environmental concentrations are considered. (Note that in commercial use of the chloronicotinyls, rates of application to obtain efficacy against insect pests are ~10 fold lower than needed for the more conventional OP and carbamate insecticides.
   a. Table 3 compares the acute oral LD50's of nicotine and the chloronicotinyls among mammals, birds, and fish. Tables 4 and 5 show similar comparisons for OP, carbamate, pyrethroid, and polychlorocycloalkane insecticides.

Table 3. Comparison of the acute toxicity and no observable adverse effect level (based on chronic toxicity studies) of several chloronicotinyl insecticides and nicotine to rodents, birds, and fish (Tomizawa and Casida 2005).

Chloronicotinyl	Rat Acute Oral LD50	NOAEL (mg/kg/day)	Bird Acute Oral LD50 (mg/kg)	Fish LC50 (mg/L)
Nicotine	50-60	--	"Toxic"	4
Imidacloprid	450	5.7	31	211
Thiamethoxam	1563	0.6	1552	>100
Clothianidin	>5000	9.8	>2000	>100
Thiacloprid	640	1.2	49	31
Acetamiprid	182	7.1	180	>100

1. For further information about imidacloprid hazards and risks to nontarget organisms see **Felsot, A. S.** 2001. Admiring risk reduction. Does imidacloprid have what it takes? Agrichemical & Environmental News (October) 186:1-12. http://aenews.wsu.edu

Table 4. Acute toxicity of organophosphate, carbamate, and pyrethroid insecticides to rodents, rabbits (via dermal route), fish, and birds.

Compound	Rat oral LD_{50} mg/kg	Rabbit Dermal LD_{50} (mg/kg)	Fish LC_{50} μg/L, trout	Bird LD_{50} mg/kg Quail
Organophosphates				
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)
Carbamates				
aldicarb	0.9	>5	880	2.58
carbaryl	307	2000	1300	>2000
carbofuran	8	2550	280	4.15 (pheasant)
propoxur	95	>1000	3700	30
Pyrethroids				
allethrin	1320	2500 (rats)	2.6 (salmon)	2030
cylfluthrin	869	>5000	0.68	>2000
cypermethrin	150	1600	1	2000 (chicken)
permethrin	430	4000	5.4	15,500

Table 5. Acute toxicity of chlorinated hydrocarbon and cyclodiene insecticides (i.e., polychlorocycloalkanes) to rodents, rabbits (via dermal route), fish, and birds. Note that only dicofol and endosulfan remain registered today. The registration of all other compounds were revoked.

Compound	Rat oral LD_{50} mg/kg	Rabbit Dermal LD_{50} (mg/kg)	Fish LC_{50} μg/L, trout	Bird LD_{50} mg/kg 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
Dieldrin	46.8	60-90	1.1-2.4	8.7
Lindane	88-190	300	1.7	2000 (duck)
Endosulfan	18	74	1.1-2.9	80-160 (pheasant)
Methoxychlor	5000	2820	11-61	>2,000

B. Inhibition of GABA receptor (i.e., gamma aminobutyric acid receptor antagonism)

1. Chlorinated cyclcodiene insecticides like dieldrin (banned since 1974) and endosulfan, and the chlorinated cyclohexane lindane bind to the GABA receptor and inhibit the channel from opening to the influx of chloride ions. (see Figure 4 for structures). These compounds are also called polychlorocycloalkanes.
2. Dieldrin may require further metabolism to a trans-diol form

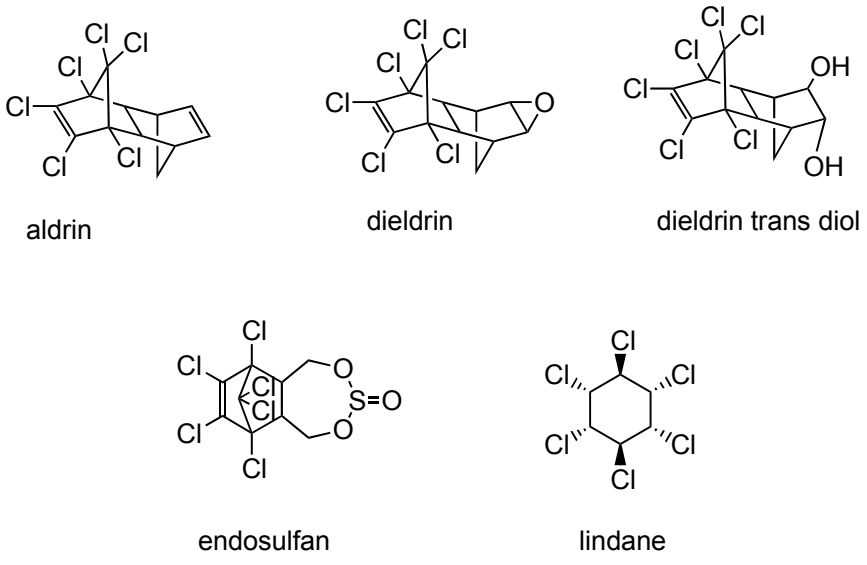
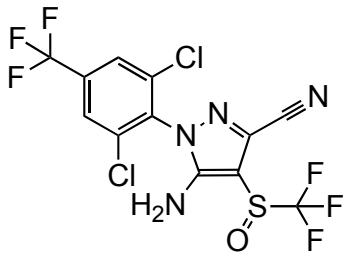


Figure 4. Chlorinated cyclodiene insecticides. Aldrin is oxidized in the environment to dieldrin. Aldrin and dieldrin were banned by EPA in ~1974. Endosulfan is still used today, mainly to control aphids and related pest species.

3. The GABA receptors occur on inhibitory neurons that form synapses on excitatory neurons. When GABA binds to the receptor, chloride channels are opened up allowing an influx of chloride ion. The postsynaptic membrane hyperpolarizes (i.e., becomes even more negative), making difficult the generation of an action potential because the threshold is harder to reach. Thus, inhibitory neurons modulate the activity of the excitatory neurons, and the chlorinated hydrocarbon cyclodienes (and lindane, see Table 5) noncompetitively antagonize the receptor.
   a. In other words, these insecticides bind to the receptor at a different domain than GABA, and they prevent the chloride channel from opening by essentially blocking the pore through which chloride would diffuse. The result is analogous to causing too much release of acetylcholine.
   b. The registrations of most of the chlorinated cyclodienes (except endosulfan) were revoked in the 1970's, and lindane is only used as a seed treatment (especially on wheat seeds) and as a pediculicide drug (by prescription only). However, fipronil (Figure 5) is a comparatively new insecticide that has the same mode of biochemical action as the older polycyclochloroalkanes.

1. In comparison to other polyhalogenated cycloalkane insecticides, fipronil is more much more selective for the insect GABA receptor than for mammalian receptor (Table 6), and it degrades in the environment much more quickly then the banned chlorinated cyclodienes and thus does not bioaccumulate in food webs.



fipronil

Figure 5. Structure of fipronil, a phenylpyrazole insecticide (rat acute oral LD50 = 97 mg/kg; rabbit acute dermal LD50 = 354 mg/kg)

Table 6. Selectivity of endosulfan, lindane, and fipronil between mammals and insects as GABA receptor antagonists and toxicants (Ratra, G. S., S. G. Kamita and J. E. Casida. 2001. Role of human $GABA_A$ receptor B3 subunit in insecticide toxicity. Toxicology Applied Pharmacology **172**: 233-240.)

Insecticide	Potency Mammals	Potency Insects	Selectivity Mammal/Insect
GABA Receptor 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

C. Natural product insecticides also have neurotoxic modes of action
   1. Avermectins are macrocyliclactones (Figure 6) isolated from the fermentation products of the soil acintomycete, Stlreptomyces avermitilis. Avermectins are particularly toxic to parasitic helminth worms and to mites. The antiparasitical avermetiin is ivermectin, and the insecticidal form (actually acaricidal) is abamectin.
   2. Avermectins are GABA agonists. They bind to the GABA recognition site on mammalian receptors and partially stimulate the opening of the chloride channels in the post synaptic membrane. The actual oral LD50 for rats is ~11 mg/kg, but because avermectins are extremely toxic to mites, they are used at low environmental application rates (~0.15 lbs/acre).

a. Ivermectin has been used as a therapeutic for parasitic worms in humans, and thus it (and abamectin) are generally considered safe. The selectivity is conferred by the blood-brain barrier and the presence of the P-glycoprotein that inhibits diffusion of the avermectins into the central nervous system.

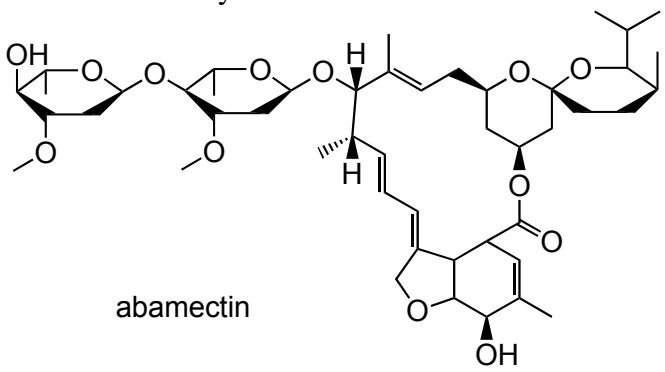


Figure 6. Abamectin is the acaricidal form of the avermectins.

3. Interestingly, avermectin in insects binds to the glutamate receptors and blocks chloride channels. The glutamate receptors in insects are inhibitory receptors (like the GABA receptor). Thus, the mode of action in insects and vertebrates are different. (Stevens and Breckenridge 2001, Chapter 56, Handbook of Pesticide Toxicology, Academic Press)

4. Spinosad is a type of spinosyn that is a biochemical product of fermentation cultures of the actinomycete *Saccharopolyspora spinosa*.
   a. Spinosad is used at extremely low rates of application (<0.1 lb/acre) and has very low toxicity to mammals, birds, fish, and aquatic invertebrates.
   b. Spinosad is considered a natural insecticide, and one formulation of it has gained approval for use in certified organic agriculture.

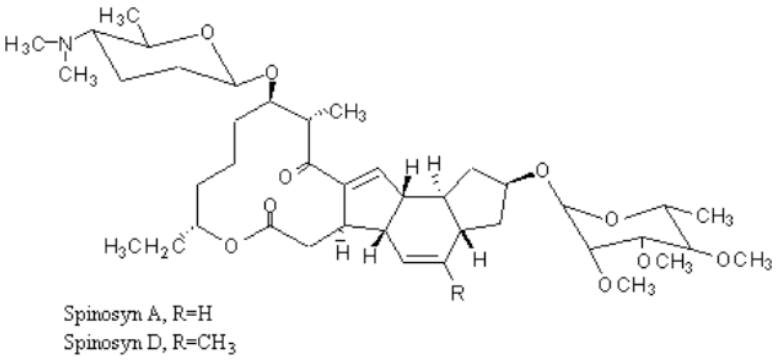


Figure 7. Spinosad, the insecticidal form of spinosyn, a microbial fermentation product.

c. The activity of spinosad is characterized by an excitation of the nervous system in insects consistent with activation of nicotinic ACh receptors but also with effects on GABA receptor function. The site of binding of

spinosad seem different from that of imidacloprid and other nicotinic receptor ligands. (Hanley, T. R., Jr., W. J. Breslin, J. F. Quast and E. W. Carney. 2002. Evaluaton of spinosad in a two-generation dietary reproducion study using Sprague-Dawley rats. Toxicol. Sci. **67**: 144-152

1. The oral and dermal LD50 in rats and rabbits, respectively are >3000 mg/kg and >5000 mg/kg.

D. Historical Overview & Summary of Insecticide Neurotoxicity
   1. Polychlorocycloalkane (PC) insecticides like DDT and dieldrin were developed in the 1940's and widely used throughout the 1950's.
   2. Early in the 1950's, the first OP insecticides were introduced. From an acute toxicity standpoint, the OP insecticides were much more toxic than the polychlorocycloalkanes, but they biodegraded quickly both in tissue and in the environment, whereas the PC insecticides were very persistent and tended to bioaccumulate within food webs.
   3. By the late 1950's and early 1960's, the first carbamate (CB) insecticides were introduced; their acute toxicity was somewhat less than the OPs.
   4. In the early 1970's the photostable synthetic pyrethroids, based on the structure of the naturally occurring botanical pyrethrins, were introduced with even greater selectivity toward insects relative to mammals and birds.
   5. Natural insecticides from microbial fermentation cultures are now used and in some cases gaining greater market share. However, the two examples given here, spinosad and avermectin, still have biochemical toxicity at the site of the nervous system.
   6. Table 7 given a comparison of the toxicity of the major groups of insecticides and their selectivity based on the geometric means of large datasets (Tomizawa and Casida 2005).

Table 7. A Comparison of Acute Toxicities of Organochlorine, Organophosphate, Carbamate & Pyrethroid Insecticides--Concept of Selectivity (numbers in parentheses represent the no. of data values used to calculate the geometric mean LD_{50})--Data from Elliott 1977 and Tomizawa and Casida 2005)

Class	Nerve Target	Rats (mg/kg)	Insects (mg/kg)	Ratio (rat/insect)
Neonicotinoids	nAChR	912	2.0	456
Organophosphate	AChE	67 (83)	2.0 (50)	33
Methylcarbamate	AChE	45 (15)	2.8 (27)	16
Polychlorocycloalkanes	Na^+ or Cl^- Channels	230 (21)	2.6 (26)	91
Pyrethroid	Na^+ Channels	2000 (11)	0.45 (35)	4500

II. Neurodevelopmental Toxicity

A. One toxicological issue that you should be aware of is the potential effects of certain contaminants on neurological development of the fetus and children.
   1. The contaminants most studied, but not necessarily with any definitive mechanism or specific receptor (as in our understanding of the

pharmacodynamics of insecticides) include lead, mercury (specifically methylmercury), and PCBs.

a. In addition to the above list, chlorpyrifos (an OP insecticide) has been studied in neonatal rats (dosed at ~2 days from birth) and results have suggested possible effects on proper brain biochemical function and structure (although the doses tend to be comparatively high compared to estimates of dietary or residential intake) (Slotkin, T. A. 1999. Developmental Cholinotoxicants: Nicotine and Chlorpyrifos. Environmental Health Perspectives **107**[Supplement 1]: 71-80).

B. Focusing on PCBs as an example, a team of researchers has used epidemiological studies to hypothesize that early natal exposure to PCBs (from the mother's dietary intake of fish highly contaminated with PCBs) has retarded reading ability in a cohort of young children.

1. In utero exposure to PCBs has been linked to adverse effects on neurological and intellectual function in infants and young children (Jacobson and Jacobson 1996, New England J. of Medicine 335:783 among many other studies). The hypothesis was studied in school age children to determine if the putative effects at an early age persisted and impaired acquisition of reading and arithmetic skills.
2. The Jacobsons had been following cohorts of children since birth that were classified by PCB exposure based on mother's ingestion of contaminated fish.
   a. These studies and several others could deconvolute prenatal and post natal exposure (studies reviewed in Longnecker et al. 2003)
      1. For example, by measuring PCBs in mother's milk and multiplying how long a baby was breast fed, postnatal exposure could be estimated.
      2. By measuring PCBs in mother's plasma, cord sera, and mother's milk, prenatal exposure could be estimated.
         a. One experimenter gave one cohort of mothers special formula that was shown to contain no detectable levels of PCBs; thus any infant exposure in this group were strictly due to prenatal exposures (i.e., during gestation).
3. Jacobson and Jacobson (1996) tested 212 children, recruited as newborns to over represent infants born to women who had eaten Lake Michigan fish contaminated with PCBs. A battery of IQ and achievement tests were administered when the children were 11 years of age. PCB concentrations in maternal serum and mild at delivery were slightly higher than in the general population.
   a. "Prenatal exposure to PCBs was associated with lower full-scale and verbal IQ scores after control for potential confounding variables such as socioeconomic status ($P=0.02$). The strongest effects related to memory and attention. The most highly exposed children were three times as likely to have low average IQ scores ($P<0.001$) and twice as likely to be at least two years behind in reading comprehension ($P=0.03$). Although larger quantities of PCBs are transferred by breast-feeding than in utero, there were deficits only in association with transplacental exposure,

suggesting that the developing fetal brain is particularly sensitive to these compounds."

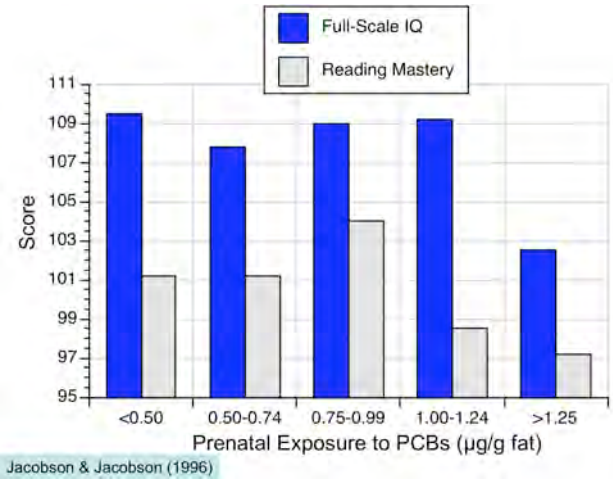
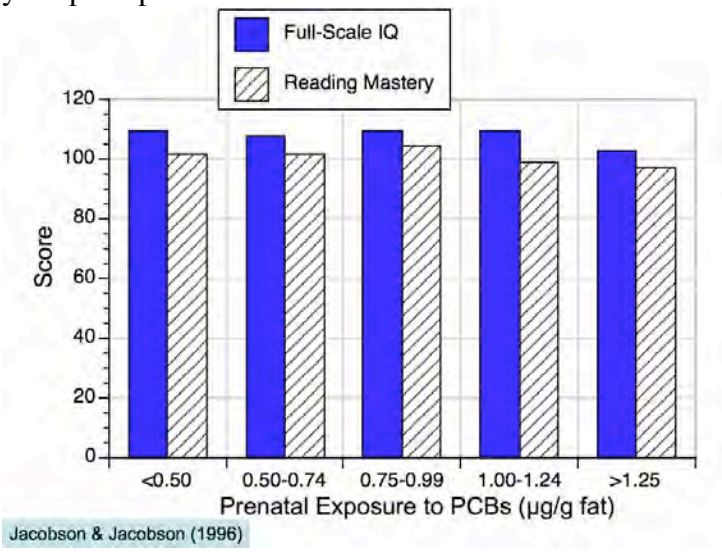


Figure 8. IQ and reading memory score of children exposed prenatally to PCBs.

1. Note in the Figures 8 & 9 the use of contingency groups to express the prenatal exposure-response relationship (the exposure is to breast milk, fat basis; thus, assuming that breast milk concentration would relate to higher prenatal exposures; however, this corresponded to 4.7 ng/mL I cored serum or 9.7 ng/mL in maternal serum).
2. Note that a regression analysis of exposure and outcome yielded a maximum regression coefficient of –0.17 for the relationship between intelligence or work comprehension and exposure.

b. Conclusion: "In utero exposure to PCBs in concentrations slightly higher than those in the general population can have a long-term impact on intellectual function."

c. Skeptic's birds-eye view: it's interesting what graphical scaling can do for your perception of data.



4. A recent study with rats exposed during gestation to PCBs (as Aroclor 1254; doses of 0, 1, 6 mg/kg) failed to produce significant effects on performance in

behavioral tests during development and adulthood (Bushnell et al., Toxicol. Sciences 68:109-120 [2002]).

5. Nevertheless, the various neurobehavioral toxicity studies, based on epidemiology, suggests that at least at the highest exposures, there may be some cognitive deficits.
   a. More importantly, neurobehavioral developmental deficits were only suggested when exposure to PCBs was prenatal rather than post natal.
6. A recent review by Seegal (2003) [Seegal, R. F. 2003. Effects of polychlorinated biphenyls on neuronal signaling. Chapter 10 in Dioxins and Health, 2nd ed., A. Schecter, T. A. Gasiewicz, ed. John Wiley & Sons, Inc. New Jersey. Pp. 433-455] reports the results of in vitro and some in vivo rodent students that shows non coplanar PCBs can be neurotoxic (as determined by several assays). These studies give some mechanistic plausibility for the epidemiological observations of neurobehavioral effects at the highest exposure levels.
   a. However, the neurotoxicity assays tended to show effects at only the highest doses.
   b. The hypothetical mechanism for neurotoxicity of PCBs is due to alterations in calcium homeostasis.
      1. For example, neurotransmitters are released when axon depolarizations are great enough at neuronal presynaptic junctions to open calcium channels in the membrane. The influx of calcium then stimulates the release of neurotransmitter.
      2. Calcium influx is not stimulated by coplanar PCBs, only by non-coplanar PCBs.

C. Whatever the mechanism involved with PCBs and neurtoxicological effects on development of fetal nervous systems, it is now realized that early neuron growth and synapse networking and brain pattern development is dependent on both the presence and activity of acetylcholinesterase and acetylcholine. Furthermore, it is not recognized that thyroid hormone play a role in normal brain development. Thus, any toxicants that affect AChE levels or act as agonists or antagonists at the nicotinic ACh receptors, may have an effect on later functioning of children's neurological function.