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Review

Insecticide Targets: Learning to Keep Up with Resistance and Changing Concepts of Safety

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Pest insect control is dependent on about 200 insecticides that work by relatively few mechanisms. The targets they disrupt are mostly involved in the nervous system, respiratory chain, growth and development, or the gut. The major nerve targets are: acetylcholinesterase for the organophosphates and methylcarbamates; the nicotinic acetylcholine receptor for the neonicotinoids; the γ-aminobutyric acid receptor for several chlorinated hydrocarbons and fipronil; the voltage-gated sodium channel for DDT and pyrethroids. Selection of resistant strains often confers cross-resistance to some or all other insecticides working at the same site. The toxicological properties of different compounds acting on the same target are increasingly considered together, summating the risk even though the compounds are of quite diverse chemical types. Continuing attention is also being given to secondary targets not involved in the primary mechanism of toxicity but instead in side effects that must be considered in the overall safety evaluation. Research on insecticide targets is important in learning to keep up with resistance and changing concepts and policies on safety. These relationships are illustrated by recent studies in the Environmental Chemistry and Toxicology Laboratory of the University of California at Berkeley.

Key words: *esterase and amidase inhibitors, nicotinic acetylcholine receptor, γ-aminobutyric acid receptor, respiratory inhibitors.*

Insecticide Targets

Importance of insecticides. Agricultural chemicals are required to produce our food and protect us against pest infestations and disease. As an example, a major epidemic of typhus transmitted by the human body louse was stopped in Naples, Italy in 1944 with the use of an insecticide (Fig. 1). This was the first time in history that a major insect-transmitted disease epidemic was quickly brought under control. On this basis Paul Müller was awarded the Nobel Prize for the discovery of DDT. This forever changed our approach to pest insect control. We are dependent on having the right pest control chemical in the right place at the right time.

Safety depends on selective toxicity. Selective toxicity is the key to safe and effective pest control agents. The mechanism is usually either target-site or metabolic specificity, differing between mammals and insects. ECTL research often uses mice and houseflies to study the mechanisms. An illustration of selective toxicity between these species is shown in Fig. 2.

Insecticide targets. Insect pest control of the past half century relied heavily on four major nerve targets, i.e. AChE and the nAChR in the cholinergic nervous system, the GABA receptor and the voltage-gated sodium channel

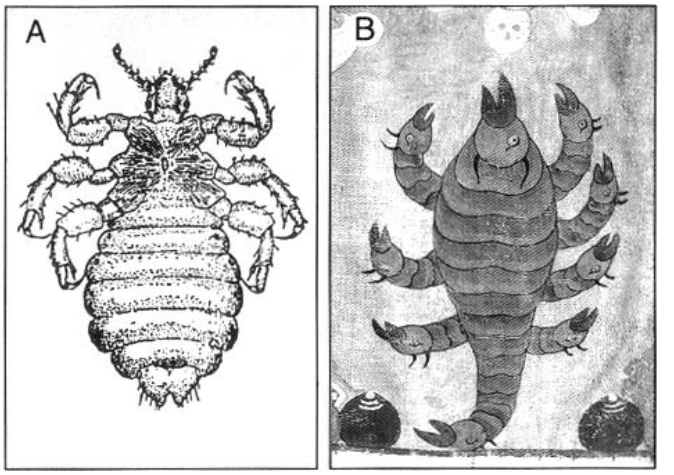

Fig. 1. Illustrations of two pests. (A) The human body louse *Pediculus humanus* transmits the bacterial pathogen *Rickettsia prowezekii*, which causes the disease epidemic typhus. (B) Small creature from a centuries-old Tibetan tangka painted without the aid of a microscope and possibly associated with a disease epidemic such as typhus. No method of control was available.

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Abbreviations: Ach, acetylcholine; AchE, acetylcholinesterase; BuChE, butyrylcholinesterase; Bt, *Bacillus thuringiensis*; ChE, Cholinesterase; EBOB, ethynylbicycloorthobenzoate; ECTL, environmental chemistry and toxicology laboratory; ETP, electron transport particles; FMN, flavin mononucleotide; GABA, γ-aminobutyric acid; MCs, methylcarbamates; nAChR, nicotinic acetylcholine receptor; Ops, organophosphates; P450, cytochrome P450; TDP, (trifluoromethyl)diazirinylpyridaben.

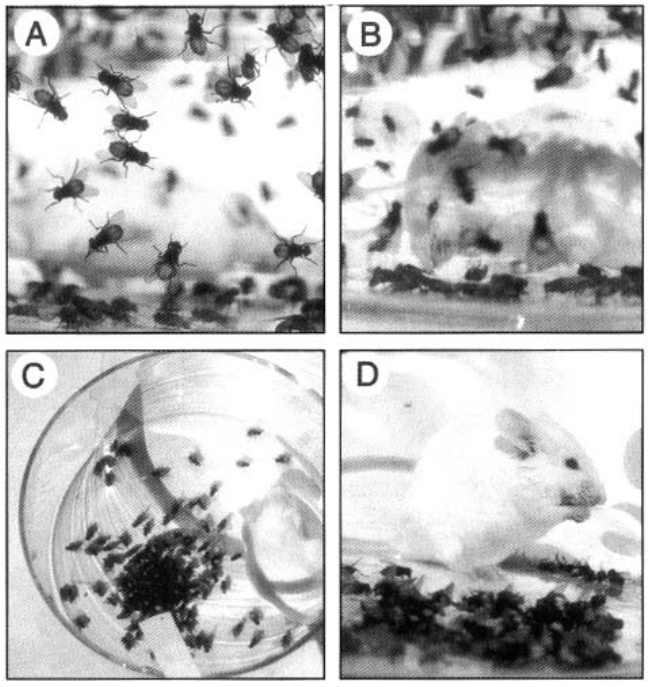

Fig. 2. Example of selective toxicity. One mouse and more than 100 houseflies are placed in a dish with a glass plate cover to create a small world or environment. (A) The world from the standpoint of the flies. (B) The world from the standpoint of the mouse, which becomes very disturbed and then nearly frantic with flies walking all around. (C) The flies are controlled by placing a treated filter paper strip under the glass cover to release dichlorvos (a volatile and selective insecticide). Within a few minutes all of the flies are killed and the mouse is not harmed. (D) After an additional few minutes the mouse picks up each individual fly and very daintily eats them one by one until they are all gone. In this idealized world, the insecticide removed the annoyance, killed the pest, and provided a food supply.

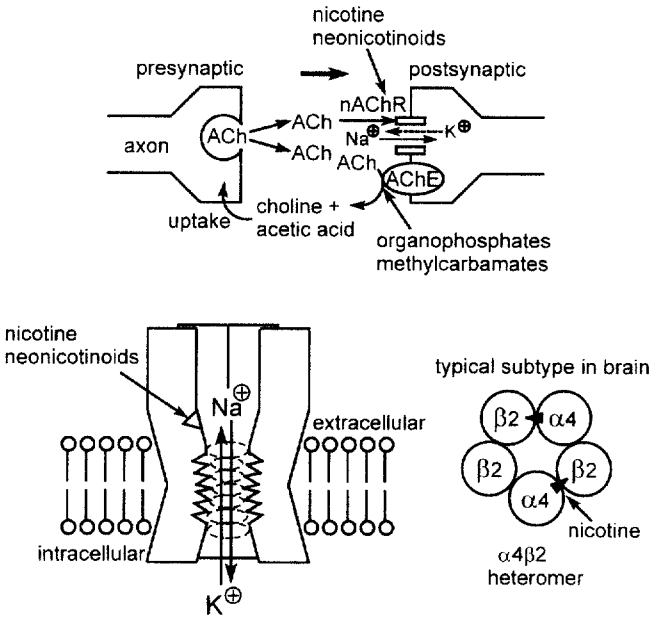

Fig. 3. Neurotransmission through the cholinergic synapse. ACh is released presynaptically and destroyed on rapid hydrolysis by AChE postsynaptically. This is the site of inhibition by OPs and MCs. The nAChR is an ACh-gated ion channel complex responsible for rapid synaptic transmission. The binding site for nicotine in the mammalian neuronal nAChR (α4β2 heteromer) is at the α4-β2 interface blocking Na^+ and K^- transport. The neonicotinoids act in an analogous manner in insects.

(Table 1). Three factors have reduced the effectiveness or challenged the safety for compounds working at these targets. The first was resistance followed by cross-resistance to other compounds working at the same target. Then, the risk cup concept was introduced, summating the combined residues for compounds acting at a common target. Thirdly, increased scrutiny of toxicology studies for secondary targets has also been influential. These combined factors made a critical need for new targets to "start over again" and industry has responded with compounds working on other nerve targets, respiration and growth and development. The largest impact has been the ability to use protein toxicants in genetically-modified crops for insect control, in large part the *Bacillus thuringiensis* (Bt) δ-endotoxin complex (Table 1). This review considers recent contributions from the ECTL on insecticide targets and future insecticide development.

Esterase and Amidase Inhibitors

History and status. AChE has for many decades been the most important single target for insecticide action. This enzyme hydrolyzes acetylcholine (ACh) released as the chemical transmitter for synaptic transmission (Fig. 3). Inhibition by OP and MC insecticides leads to ACh accumulation thereby disrupting nerve function. AChE is dialkylphosphorylated at a serine residue within the esteratic site (Fig. 4). The dialkylphosphorylated AChE then ages" on loss of an alkyl group and the ionized monoalkylphosphorylated AChE is resistant to reactivation both directly and induced by pralidoxime (Fig. 4). The OPs and MCs were mostly introduced in the 1940s, 50s and 60s with few new compounds since then. The common AChE target has led to declining effectiveness because of cross-resistance from selection for an insensitive AChE, often involving a single amino acid change in the active site. Summating the risks from residues of all OPs (or even with MCs) has led to further restriction in development and use of compounds acting at this target.

Phosphocholinesterases. A new aspect of the formation of phosphocholinesterase was recently observed in ECTL studies with phosphoryl chloride ($POCl_3$) (an intermediate in synthesis of some OP insecticides) and ethephon (a plant growth regulator) (Fig. 4). $POCl_3$ (following rapid hydrolytic activation to phosphorodichloridic acid) inhibits AChE and ethephon inhibits plasma butyrylcholinesterase (BuChE), in each case apparently by phosphorylating the active site serine. These phosphorylation reactions play a role in the

Table 1. Chronology of insecticide targets.

Target	Importance worldwide	Common Site		Other Sites
		Natural Product	Synthetic	
nerve[a]				
AChE	++++	physostigmine (<1927)	parathion (1946), aldicarb (1965)	
nAChR	+++	nicotine (<1690)	imidacloprid (1990)	cartap (1967)
Cl^- channel	++	picrotoxin (<1875)	lindane (1942), α-endosulfan(1956) fipronil (1992)	avermectins (1981)
Na^+ channel	++++	pyrethrum (<1820)	DDT (1939), deltamethrin (1974)	indoxacarb (1997)
respiration[b]				
NADH:Q oxidoreductase	+	rotenone (1848)	fenazaquin (1991), pyridaben (1988)	chlorfenapyr (1992)
uncouplers	+		DNOC (1892)	
growth and development				
juvenile hormone receptor	++	juvenile hormone (1940-1967)	methoprene (1973)	
ecdysone receptor	+	ecdysone	tebufenozide (1986)	nikkomycin (1985)[c]
chitin	++		diflubenzuron (1972), buprofezin (1981)	
gut	++++	Bt-δ-endotoxin	GM crops (1994)	

[a]Spinosyn (1995) may act on the nAChR and pymetrozine (1994) on nerves associated with feeding.
[b]Diafenthiuron (1988) inhibits oxidative phosphorylation.
[c]Experimental natural product acting as inhibitor of chitin synthetase.

toxicity of $POCl_3$ to insects and mammals and the ability of ethephon to synergize drugs detoxified by BuChE.

Safety conferred by metabolic activation and detoxification. Dimethoate and acephate are typical of most OP insecticides in undergoing metabolic activation and detoxification. Dimethoate is oxidized in insects by cytochrome P450 (P450) to the oxon (omethoate) that is a potent inhibitor of insect AChE (Fig. 5). The mammal is more effective at hydrolyzing the amide prior to oxidative activation, providing a high level of selectivity. The insect activates by oxidation and the mammal detoxifies by hydrolysis. Acephate is one of the most important OP insecticides with outstanding systemic activity for sucking insects and relatively low toxicity to mammals. Acephate undergoes two bioactivation steps, amidase cleavage to methamidophos and presumably sulfoxidation to the actual esterase inhibitor (Fig. 6). This is an efficient overall process in insects which are very sensitive. In mammals, however,

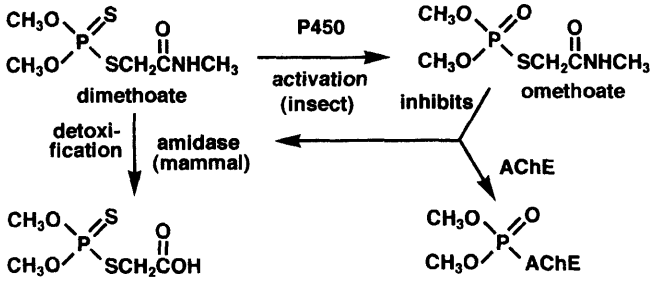

Fig. 5. Dimethoate as an example of selectivity from oxidative activation in insects and hydrolytic detoxification in mammals.

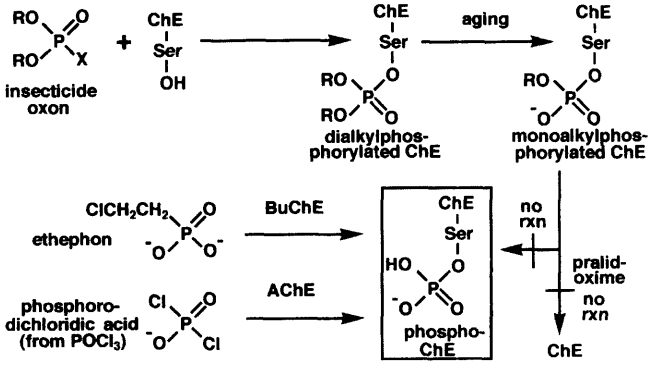

Fig. 4. Formation of di- and monoalkylphosphorylated cholinesterases from reaction with insecticide oxons and phosphocholinesterases from ethephon and $POCl_3$.

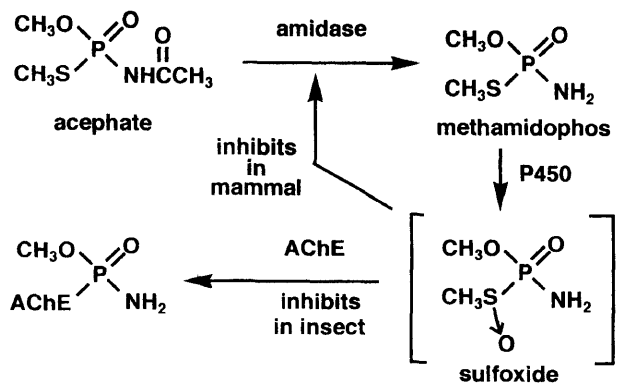

Fig. 6. Acephate as an example of selectivity from product inhibition of the activating amidase in mammals.

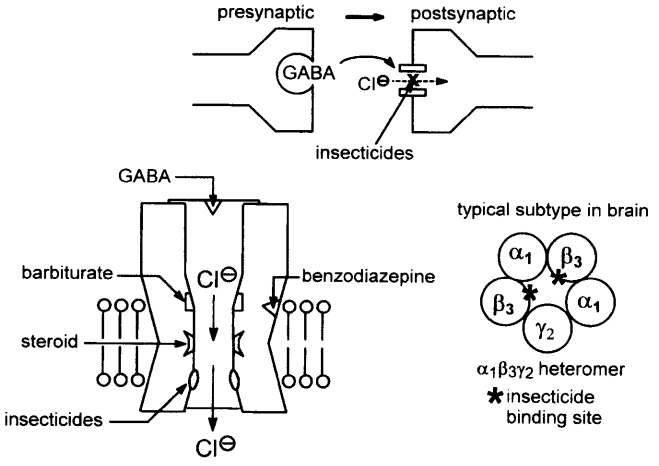

Fig. 8. Neurotransmission through the GABA-mediated inhibitory synapse and the GABA receptor-chloride ionophore complex. GABA is released presynaptically and acts at the postsynaptic GABA receptor. Channel function is altered by compounds binding to the indicated GABA, barbiturate, benzodiazepine, steroid and insecticide sites. For the mammalian receptor, binding at the insecticide site on a β3 subunit blocks the channel and at an avermectin site (not shown) opens the channel.

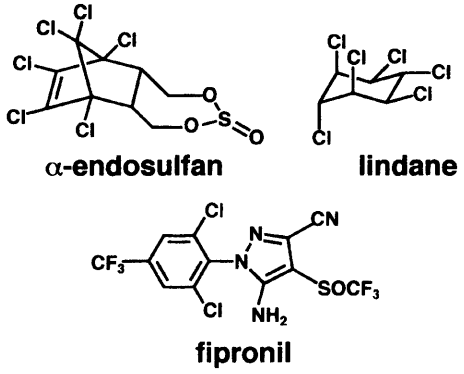

Fig. 9. GABAergic insecticides.

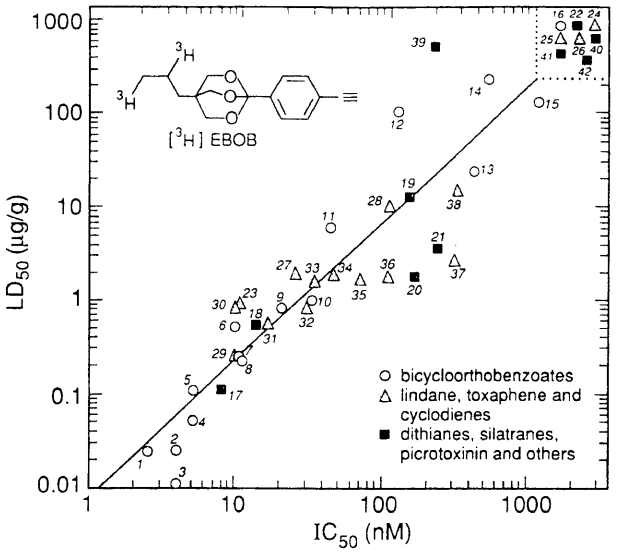

Fig. 10. Potency of diverse classes of insecticides in blocking the chloride channel correlates with toxicity. IC_{50} values for [^{3}H]EBOB binding assays with housefly head membranes and LD_{50} values for houseflies pretreated with a synergist. The radioligand structure is shown.

Table 3. Selectivity differences for α-endosulfan, lindane and fipronil at the housefly, human β3 and human native GABA receptors

	α-Endosulfan	Lindane	Fipronil
receptor IC_{50} (nM)			
housefly	10	11	6.3
human β3[a]	0.47	0.90	2.4
human native	7.3	306	2470
selectivity ratio (human/housefly)			
β3	0.05	0.08	0.38
human native[b]	0.7	28	392

[a]remarkable sensitivity of human β3 homooligomer
[b]remarkable selectivity of human native receptor

and the central and peripheral nervous systems of insects (Fig. 8). GABA opens the channel as do the avermectins as activators. Several major insecticides are potent chloride channel blockers. Many billions of pounds of hexachlorocyclohexanes (e.g. lindane), cyclodienes (e.g. endosulfan) and toxaphene had been used before the target was identified as the GABA receptor-chloride channel (Fig. 9). Fipronil was later shown to act by the same mechanism. These targets are normally studied by electrophysiological techniques and by radioligand binding using [^{3}H]ethynylbicycloorthobenzoate ([^{3}H]EBOB).

Target for diverse classes of insecticides. The binding assay with [^{3}H]EBOB in brain membranes is a direct measure of the GABA receptor. Diverse classes of insecticides act at the channel. This was established by comparing potency at the housefly receptor with insecticidal activity (using a synergist to measure intrinsic toxicity by suppressing detoxification). A high correlation between potency at the receptor and toxicity (Fig. 10) indicates that binding to the receptor is the cause of toxicity and that many classes of compounds act at the same site in the same way, i.e. bicycloorthobenzoates, lindane, toxaphene, cyclodienes, dithianes, picrotoxinin and others (plus fipronil shown in a separate study).

Safety from target site selectivity. The [^{3}H]EBOB assay measures the amount of open GABA-gated chloride channel equally well in housefly and human brain membranes. The binding sites can therefore be compared with the various classes of chloride channel blockers for possible selective action (Table 3). High selectivity is observed with lindane and fipronil but not with α-endosulfan. The subunits can be examined individually and in combination to determine the localization of the target site and the effect of subunit assembly on [^{3}H]EBOB binding and displacement by unlabeled insecticides (Fig. 8). The binding site in mammals is on the β2 or β3 subunit (of the

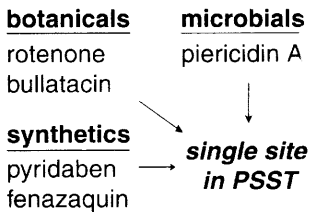

Fig. 15. Common binding site for respiratory inhibitors of diverse structures and origins.

inner mitochondrial membrane. The required specificity was achieved by photoaffinity labelling in which the ligand was designed for potency, photoreactivity and relevance and finally labelled at high specific activity. The photoaffinity probe was allowed to bind at its high-affinity site where, on exposure to UV light, it covalently derivatized the site (e.g. subunit) for characterization. (Trifluoromethyl)diazirinyl-[^{3}H]pyridaben ([^{3}H]TDP) designed as a potent and photoreactive derivative of pyridaben proved to be an outstanding photoaffinity probe for complex I (Fig. 14). Binding at the 23-kDa protein involved a high-affinity site correlating with inhibition of NADH oxidase activity. The high-affinity site was identified as PSST (Fig. 14) based on molecular mass and immunoprecipitation with subunit specific antibodies. Fig. 12 gives the proposed positioning of the insecticide binding site in complex I. PSST is suggested to couple electron transfer from iron-sulfur cluster N2 to Q_{10}.

Target for diverse classes of insecticides. By competing the other insecticides with [^{3}H]TDP it was established that many compounds of diverse structures bind to the same site in PSST. This is illustrated for two botanicals, one microbial and two synthetic miticides in Fig. 15.

Conclusion

Continued success in safely controlling pest insects depends on new agents, new targets and increased fundamental knowledge on the mechanisms of insecticide action. Many areas of biology and chemistry contribute to this goal and in turn new insecticides serve as probes to better define life processes. It is essential to thoroughly understand these targets and manage the pesticides to minimize the impact of resistance and conserve these finite resources for pest control.

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References

Appropriate literature citations can be obtained by computer search for the past 5 years of papers reported by John E. Casida and/or Gary B. Quistad and their laboratory colleagues Motohiro Tomizawa, Franz Schuler and Gurpreet Ratra.