Neuroactive Insecticides: Targets, Selectivity, Resistance, and Secondary Effects

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Abstract
Neuroactive insecticides are the principal means of protecting crops, people, livestock, and pets from pest insect attack and disease transmission. Currently, the four major nerve targets are acetylcholinesterase for organophosphates and methylcarbamates, the nicotinic acetylcholine receptor for neonicotinoids, the γ-aminobutyric acid receptor/chloride channel for polychlorocyclohexanes and fiproles, and the voltage-gated sodium channel for pyrethroids and dichlorodiphenyltrichloroethane. Species selectivity and acquired resistance are attributable in part to structural differences in binding subsites, receptor subunit interfaces, or transmembrane regions. Additional targets are sites in the sodium channel (indoxacarb and metaflumizone), the glutamate-gated chloride channel (avermectins), the octopamine receptor (amitraz metabolite), and the calcium-activated calcium channel (diamides). Secondary toxic effects in mammals from off-target serine hydrolase inhibition include organophosphate-induced delayed neuropathy and disruption of the cannabinoid system. Possible associations between pesticides and Parkinson’s and Alzheimer’s diseases are proposed but not established based on epidemiological observations and mechanistic considerations.
WHY NEUROACTIVE INSECTICIDES?

Insecticides are principal defenses against insect pests of crops, livestock, pets, and people. Most insecticides are nerve poisons and have been since dichlorodiphenyltrichloroethane (DDT) and various polychlorocycloalkanes (PCCAs) were introduced in the 1940s, followed by organophosphates (OPs) in the 1950s, methylcarbamates (MCs) in the 1960s, pyrethroids in the 1970s, and neonicotinoids in the 1990s (26, 138). Neurotoxicants are the major synthetic insecticides for several reasons (28). They act rapidly to stop crop damage and disease transmission. There are many sensitive sites at which even a small disruption may ultimately prove to be lethal. A lipoidal sheath protects the insect nerve from ionized toxicants but not from lipophilic insecticides. Poor detoxification mechanisms in nerves provide prolonged toxicant effects.

The nervous system has at least 11 targets or defined modes of action for neuroactive insecticides (7, 8, 23, 26, 32) ([Figure 1], A–K). Primary target site selectivity between insects and people plays an important role in insecticide safety, but secondary targets must also be considered. Selection of pest populations for target site resistance and cross-resistance is delayed or circumvented by shifting modes of action. A diversity of targets is therefore critical for insect and insecticide management (95). This review considers the targets and species selectivity of neurotoxic insecticides, the resistance mechanisms in insects, and the secondary effects in mammals, with emphasis on research by the authors. Structures for the insecticides mentioned here are given in recent manuals or reviews (8, 95, 138).

ACETYLCOLINESTERASE

Primary Target and Inhibitor Action

The toxicity of OPs and MCs to insects and mammals is attributable to inhibition of acetylcholinesterase (AChE), which is responsible for the hydrolysis of acetylcholine (ACh) at synaptic regions of cholinergic nerve endings (19, 46, 74) ([Figure 1]). AChE inhibitors cause ACh to accumulate, resulting in excessive stimulation of cholinergic receptors. The MC physostigmine from calabar beans (*Physostigma venenosum*) was used first as an ordeal poison in West Africa and then to establish the relationships between ACh neurotransmission and AChE activity and its inhibition (20). AChE inhibition by OP and MC insecticides involves phosphorylation and carbamoylation, respectively, of serine in the esteratic site ([Figures 1, 2]). AChE is inhibited by MCs as the reversible AChE–MC complex or as the methylcarbamoylated enzyme at serine that reactivates spontaneously over a short time span (75). The corresponding reaction with OPs yields the phosphorylated AChE that undergoes aging or reactivates slowly, except with an oxime reactivator (19, 46).

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**PCCA:**
polychlorocycloalkane

**Target site:** enzyme, receptor, or channel site at which specific binding initiates the physiological change

**Cross-resistance:**
target site (or metabolic) resistance conferred by a common target (or detoxification mechanism)

**Secondary effect:**
toxicity from action at a secondary target

**Ordeal poison:**
a poison given to the accused to determine guilt (dies) or innocence (lives)
1. AChE and nicotinic receptor/cation channel excitatory synapse

ACh vesicles release ACh, which binds to nicotinic receptors. This leads to the opening of ion channels, allowing Na+ and K+ to flow in and out of the cell, respectively. AChE then hydrolyzes ACh.

2. GABA receptor/Cl⁻ channel inhibitory synapse

GABA vesicles release GABA, which binds to GABA receptors, leading to the opening of Cl⁻ channels. This reduces the excitability of the postsynaptic neuron.

3. Glutamate receptor excitatory synapse

Glu vesicles release Glu, which binds to glutamate receptors, activating Na+ and Ca++ channels, leading to excitation.

4. Voltage-dependent Na⁺ channel

Voltage changes regulate the opening of Na⁺ channels, controlling action potential generation. Pyrethroids can affect these channels.

5. Ca²⁺-activated Ca²⁺ channel

Ca²⁺ enters the cell, activating muscle contraction. Ryanodine receptors mediate this process.

6. Octopamine receptor coupled to second messengers

Octopamine (OA) binds to receptors, elevating cyclic AMP, leading to neuroexcitation.

Neurotransmitters
- ACh: CH₃C(O)OCH₂CH₂N+(CH₃)₃
- GABA: NH₂CH₂CH₂CH₂C(O)OH
- Octopamine: HO-CHOHCH₂NH₂
- Glutamate: HO-CHOHCH₂NH₂

Insecticides (number of compounds)
- OP (65), MC (26), phystostigmine: AChE inhibitors
- Nicotine, neonicotinoids (7): nAChR agonists
- Nereistoxin analogs (4): nAChR blocker
- Spinosyns (2): nAChR allosteric activators
- Fiproles (2), PCCAs (2), picrotoxin: noncompetitive antagonists, blockers and convulsants
- Avermectins (4): activators, modulators
- DDT, pyrethroids, pyrethrins, veratridine (44): modulators
- Indexacarb (1): blocker
- Metaflumizone (1): blocker
- Ryanodine, diamides (2): activators
- Amitraz (1): mimics
- Octopamine (OA): Elevation of cyclic AMP
- OA = octopamine
a  AChE – chlorpyrifos oxon

b  nAChR – imidacloprid

Heteropentamer  Homopentamer

GABA_\textsubscript{A}R – fipronil

Na\textsuperscript{+} channel – deltamethrin
Selectivity and Resistance

Inhibitor specificity between the AChE of insects and mammals and between susceptible and tolerant strains of insects contributes to selective toxicity and resistance (19, 46). For example, the selectivity of methyl paraoxon for inhibiting house fly (Musca domestica) versus bovine erythrocyte AChE is greatly increased on introducing a 3-methyl substituent to yield the oxon of the fenitrothion insecticide (58). Further, diisopropyl paraoxon is a relatively poor inhibitor for honey bee (Apis mellifera) compared to house fly AChE (18). Mutations in some resistant strains confer low OP and MC inhibitor sensitivity (101, 142) and potentially altered inhibitor specificity. As yet another example, (+/-)-profenofos is effective on some insects resistant to other OPs, possibly because (+)-profenofos acts directly and (-)-profenofos after biosulfoxidation (53, 146), resulting in two toxicants with different leaving groups on AChE phosphorylation. Unlike humans, some insects (e.g., aphids and mosquitoes) have a sensitive cysteine in the acyl pocket of AChE, providing an opportunity, not yet fully realized, for target site selective sulphydryl reagent inhibitors (108, 117).

NICOTINIC RECEPTOR

Primary Target and Agonist Action

ACh is the endogenous agonist and principal excitatory transmitter for rapid neurotransmission in the insect central nervous system and the mammalian central and peripheral nervous systems (90, 130, 131, 133). ACh released from the presynaptic membrane interacts with the agonist binding site at the extracellular domain of the ACh receptor (AChR) ion channel complex (92, 131, 151) (Figure 1). The receptor then undergoes a conformational change leading to channel opening, influx of extracellular Na+, and efflux of intracellular K+. Actions resembling those of nicotine are associated with the nicotinic receptor (nAChR) and those similar to muscarine are attributed to the muscarinic receptor (mAChR), i.e., ACh has both nicotinic and muscarinic actions. The search for muscarinic antagonists and agonists selective on insects relative to mammals has thus far been unsuccessful (61). Nicotine is more toxic to mammals than to most insects, and systematic structural changes have not greatly improved its potency or safety (150). The breakthrough discovery of the highly insecticidal but photolabile nithiazine with a nitromethylene moiety and no cationic substituent (126) acting as an nAChR agonist (119) led ultimately to photostabilized compounds selective for insects relative to mammals, commercialized as imidacloprid (IMI) (70, 71) and six analogs designated neonicotinoids (67, 68, 133, 151). The guanidine or amidine unit is coplanar with the aromatic ring system and with the carboxylate of the compound, an essential feature of selectivity of this insecticide class (67). The insecticidal activity is highly dependent on the C8 substituent. Substituents that increase the stability to light and metabolism (e.g., fluorine, pyridine, quinoline) or that are less hydrophilic (e.g., ethyl, isopropyl) reduce photolability and improve insecticidal activity.

Agonist: substance that combines with a receptor to initiate a response
Antagonist: substance that combines with a receptor to oppose a response

Figure 2

Structural models of four primary targets of major neuroactive insecticides showing the proposed docking positions and some binding site residues designated as in the cited literature. (a) Chlorpyrifos oxon and AChE (mouse) showing the catalytic charge relay complex (*) of S203, E334, and H447, with specificity conferred by interactions with additional sites and pockets (not shown) (98). (b) Imidacloprid and nAChR showing structural comparison of Torpedo nAChR and Aplysia AChBP (which has no transmembrane domain or cytoplasmic end) (135) and homology model of Musca α2β1 nAChR based on AChBP, with proposed docking position of IMI and some binding site residues (137). The binding region includes 118, 174, 224, 226, 227, and 231 of the α subunit and 79, 131, 141, and 143 of the β subunit. (c) Fipronil and GABA_A (human) chloride ionophore shown as a native (α1)2(β3)2γ2 heteropentamer and a recombinant β1 homopentamer of enhanced insecticide or noncompetitive antagonist (NCA) sensitivity. The proposed β1 homopentamer channel gating amino acids are A2′, T6′, and L9′ (31), with the 6′T facing into the channel as shown in the upper cross-sections. (d) Deltamethrin and voltage-gated Na+ channel of house fly (Musca domestica) in an open conformation with residues (*) implicated in binding of M918, L925, T929, and L932 in the S4-S5 linkers, S5 helices, pore helices, and S6 helices (38, 39, 140). Abbreviations: AChBP, acetylcholine binding protein; AChE, acetylcholinesterase; BP, binding pocket; GABA, g-aminobutyric acid; GABA_A, GABA type A receptor; nAChR, nicotinic acetylcholine receptor.
and electronically conjugated with the nitro or cyano substituent, which facilitates partial negative (δ) charge flow toward the tip (135, 136). They act as agonists at multiple nAChR subtypes, with differential selectivity between insects and mammals conferred by only minor structural changes (132, 134).

Selectivity and Resistance

Electrophysiological experiments and radioligand binding studies with [3H]nicotine, [3H]epibatidine, and [3H]IMI (83, 84) establish remarkable similarities within insect nAChRs and selectivity between insect and mammalian receptors (133, 134, 135). Nicotine, epibatidine, and desnitro-IMI (referred to collectively as nicotinoids) are protonated at physiological pH and undergo cation-π interaction with tryptophan at an nAChR subsite of the α4/β2 interface in mammals (135, 136). Precise binding site interactions were established by crystallography and photoaffinity labeling studies, with ACh binding protein (AChBP) from hemolymph of the mollusk Aplysia californica and the snail Lymnaea stagnalis as models for the insect and mammalian nAChR, respectively, followed by homology modeling (90, 128, 135, 137), illustrated for Myzus in Figure 2. Binding subsite specificity plays a major role in selective toxicity for protonated nicotinoids in mammals and electronegative tip neonicotinoids in insects. The sulfoximine insecticides (i.e., sulfoxaflor) have both structural similarities to and differences from neonicotinoids consistent with their unique behavior as nicotinic agonists (145). Neonicotinoid resistance associated with a modified nAChR binding site has been observed in planthoppers, aphids, and a few other species (3, 85, 86, 90, 92).

Channel Blockers and Allosteric Activators

Cartap is a noncompetitive blocker of the nAChR (81) and spinosyns act at yet another unique site in the nAChR (73), and as such there is no cross-resistance between neonicotinoids, cartap, and spinosyns (95).

CHLORIDE CHANNEL

Primary GABA Receptor Target and Antagonist Action

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of insects and mammals and serves as the agonist for opening the pentameric transmembrane Cl− channel (6, 14, 22, 102, 105) (Figure 1). The human brain GABA type A receptor (GABA_{A}R) consists of various combinations of sixteen α subunits, seven β subunits, and four γ subunits, typically two α, two β, and one γ subunit. Insect GABARs exist as several different subtypes and form a class distinct from any vertebrate GABA_{A}Rs (14). The resistance-to-dieldrin (RDL) GABAR subtype is expressed in many insects, and RDL homomers closely mimic the pharmacology of in situ insect GABA_{A}Rs (47, 48). Picrotoxinin (PTX), the insecticide and fish poison of the fishberry plant (Anamirta cocculus), was used to probe Cl− channel functions, showing that GABA opens the channel and PTX blocks Cl− flux (102). Several billion pounds of PCCAs, including lindane, toxaphene, and the cyclodienes such as α-endosulfan, were used in crop protection before their mode of action was established (11, 22). They were joined in 1993 by the 1-phenylpyrazole fipronil, which is of lower acute toxicity to mammals. Radioligand binding and electrophysiological studies led to the recognition that the action of PCCAs and fipronil was similar to or the same as that of PTX and a series of insecticidal trioxabicyclooctanes (22, 34, 41). [3H]dihydroPTX was developed first as a
radioligand for the PTX site (91), followed by the improved bicyclophosphorothionate [35S]TBPS and two bicycloorthobenzoates, [3H]TBOB for studies with mammals and particularly [3H]EBOB for investigations with insects and mammals (33, 42, 79, 80, 107, 127). They all block GABA-induced signals and Cl− flux (62) on binding to a noncompetitive antagonist (NCA) or insecticide binding site at the receptor subunit interface (Figures 1, 2). Importantly, the human GABAAR recombinant β3-homopentamer resembles the insect receptor in sensitivity and specificity for NCAs (114, 115), prompting exhaustive site-directed mutagenesis (cysteine scanning), which pinpointed the critical active site as pore-facing residues A2′, T6′, and L9′ and led to a binding site model that fits several widely diverse classes of insecticidal NCAs (30, 31).

Selectivity and Resistance

The major GABAergic insecticides have higher potency on the native house fly or Drosophila receptor and the human β3-homopentamer than on the less sensitive mammalian native (α1)2(β3)2γ2 heteropentamer brain receptor. In house fly brain, lindane, α-endosulfan, and fipronil block the channel by binding to the critical pore residues with IC50 values of 0.5–3 nM (115). The insect versus mammalian target site specificity (105) is much greater for lindane and fipronil than for α-endosulfan (115) and is uniquely large for a highly selective isoxazoline (104).

Cross-resistance between some of the PCCAs was the first indication of a common target and defined mode of action (22). Almost all major insect pests have been selected for resistance to insecticides acting at the GABA-gated Cl− channel. In most cases, the resistance is due to a single point mutation; e.g., in cyclodiene-resistant RDL Drosophila, alanine is replaced by serine in the ion channel lining of the transmembrane 2 region, which confers insensitivity and establishes the binding site as being in the channel pore (35, 47, 48). Cross-resistance usually extends from the PCCAs to fipronil, which acts at the same or closely related sites.

Glutamate and GABA Receptor Activators

Avermectin and analogs open the Cl− channel on binding to the GABAAR at a site coupled to but distinct from the antagonist site (40, 63). However, the nematicidal and insecticidal activity of avermectin analogs is most likely due to binding to glutamate-gated Cl− channels (37, 149), and the normally low toxicity to mammals is due to the P glycoprotein drug pump acting as a part of the blood-brain barrier that restricts avermectin entry into the mammalian brain (76).

SODIUM CHANNEL

Primary Voltage-Dependent Target and Modulator Action

Voltage-gated Na+ channels open and close in response to changes in membrane potential (5). In the mammalian brain there are one alpha and two β subunits. The pore-forming α subunit consists of a single polypeptide chain with four internally homologous domains (I–IV), each having six transmembrane helices (S1–S6). The domain II S4–S5 linker, S5 and S6 helices, and domain III S6 helix interface the lipid bilayer and are therefore accessible to lipid-soluble ligands (38, 39, 123) The insect Na+ channel proteins also consist of four homologous domains, each with six transmembrane segments (122).

The pyrethrins (from pyrethrum flowers), synthetic pyrethroids, and DDT, despite different origins or structures, are considered together because of similar insecticidal mechanisms. They act on axonal neurotransmission at insect voltage-gated Na+ channel recognition sites to block Na+
transport, enhance channel inactivation, prolong the course of the Na\(^+\) current during depolarization, and induce a residual slow-acting current ("tail current") (6, 94, 122). Pyrethroid action is considered to be of two types (2, 24, 50). Pyrethrins and synthetic pyrethroids lacking an \(\alpha\)-cyano group, e.g., allethrin and permethrin, and the \(\alpha\)-cyano compound fenpropathrin induce excitation (Type I action) on binding to resting or inactivated channels, shifting the voltage dependence of activation to more negative potentials and causing a slowly activating Na\(^+\) current responsible for repetitive activity. Deltamethrin and related \(\alpha\)-cyano pyrethroids that exhibit writhing and convulsive signs in mammals (Type II action) induce profound use-dependent modification of Na\(^+\) currents, implying preferential binding to activated Na\(^+\) channel states, and recruit increasing numbers of Na\(^+\) channels into permanent open states, which results in use-dependent depolarization, inactivation of unmodified channels, and blockage of conduction. Based on these differences, consideration has been given to whether Types I and II pyrethroids should fall into the same category or be considered separately in risk analyses and residue tolerances (125).

**Selectivity and Resistance**

The higher potency of DDT and pyrethroids on insects than on mammals is proposed to be due to several features. First, the insect target is intrinsically more sensitive than that of mammals. Second, the target has a negative temperature coefficient; i.e., the insecticides are more effective at the ambient temperature of insects (e.g., 15°C–20°C) than that of people (37.5°C). Other selectivity factors are lipophilicity for selective pickup by the insect epicuticle and enzymatic detoxification. The \(1R\)-resmethrin \(\text{cis}\) and \(\text{trans}\) isomers are almost equal in insecticidal activity but greatly different in mammalian toxicity, which may be due to both target site specificity and relative detoxification rates (24, 78, 124). Fish are extremely sensitive to most pyrethroid esters but apparently less so to the pyrethroid ethers etofenprox and silarfloufen, possibly due to their low aqueous solubility (138).

When DDT first lost its effectiveness for controlling house flies, it was surprising to observe cross-resistance to the pyrethrins (16) and all synthetic pyrethroids, which was confirmed by electrophysiological studies on nerve sensitivity, suggesting a common mode of action (5, 122). Resistance to pyrethroids and DDT in *Musca* is conferred by the L1014F (kdr) mutant alone or with M918T (superkdr) in domain II of the \(\alpha\) subunit (15, 38, 140). The current binding site model rationalizes the structure-activity relationships of pyrethroids relative to DDT, Types I and II action, and the open- and closed-channel conformations (39). Some of the residues on the helices that form the putative binding contacts are not conserved between insects and vertebrates, consistent with their contribution to species specificity (103, 122).

**Other Sites**

Four other sites in insect Na\(^+\) channels are targets for insecticidal botanicals (veratridine and isobutilamides) or synthetic insecticides (oxadiazines and metaflumizone) without cross-resistance, in any case, to pyrethroids and DDT. Veratridine, an active ingredient of sabailla (*Schoenocaulon officinale*), was an early probe to study the Na\(^+\) channel, but attempts to simplify the structure and improve the insecticidal activity and safety (139) have thus far been unsuccessful. The isobutilamides or lipid amides are based on natural product prototypes (e.g., pellitorine from *Piper nigrum*) and can be quite potent but have not achieved the required balance of potency, stability, and safety to make them outstanding insecticide candidates (45). The oxadiazine indoxacarb is an effective proinsecticide that is enzymatically hydrolyzed to the active agent (77, 147). Metaflumizone is a triketone with excellent systemic aphicidal activity (118).
OTHER NERVE AND MUSCLE TARGETS

Octopamine Receptor Agonists

Octopamine and the octopamine receptor in insects, analogous to dopamine and the dopaminergic system in mammals, are coupled to second messengers to elevate cyclic AMP, leading to neuroexcitation (57) (Figure 1). Formamidines acting as octopamine mimics block the receptor and provide broad-spectrum insecticidal activity (59, 60). Chloridimeform, bioactivated by N-demethylation, was the most important insecticide of this type until its carcinogenic activity was discovered and it was replaced by amitraz (59, 64).

Homopteran Feeding Blockers

Two insecticides act as selective homopteran feeding blockers. Pymetrozine is thought to bind at an ACh site of some nAChRs that is different from that of the neonicotinoids or spinosad (95). Flonicamid blocks the A-type K+ rectifying current at the presynaptic nerve terminal neurons, leading to prolonged depolarization and uncontrolled neurotransmitter release (55).

Ryanodine Receptor Modulators

Ryanodine and 9,21-dehydroryanodine, the active ingredients of the botanical insecticide Ryania (no longer used), bind at the Ca2+-activated Ca2+ channel (109). [3H]ryanodine binding provides a convenient assay for the ryanodine receptors (RyRs) of mammalian nerve and muscle and insect muscle (82, 109, 144). The ryanodine site is similarly sensitive in insects and mammals and a large series of analogs was prepared, but high insecticidal potency combined with low mammalian toxicity was not adequately achieved (65, 66). However, the hydrolysis products ryanodol and 9,21-dehydroryanodol are selective for insects compared with mammals (144) and appear to act at a K+ channel site (141). The newest major class of insecticides is the diamides (flubendiamide, chlorantraniliprole, and cyantraniliprole), which selectively activate insect but not mammalian muscle on binding to a new and yet uncharacterized site on RyRs, leading to uncontrolled Ca2+ release (Figure 1), thereby conferring strong selectivity for insects (36, 44, 120).

Genetic Engineering

Pest insect control with baculoviruses takes several days, allowing unacceptable crop damage (10). Baculoviruses expressing selected spider and scorpion toxins act much faster but have not yet found practical applications (72).

SECONDARY EFFECTS

Serine Hydrolase Secondary Targets

OP and MC pesticides inhibit not only AChE but also many other serine hydrolases in nerve tissues, as quantified by enzymatic activity assays on specific substrates (27, 29) or activity-based protein profiling (87). The major focus in safety evaluations is on AChE inhibition, but there is also concern about OP-induced delayed neuropathy (OPIDN) and interest in behavioral effects associated with disruption of the cannabinoid system (Figure 3). OPIDN involves axonopathy and peripheral paralysis and has affected approximately 50,000 people globally in the past eight decades from compounds other than insecticides; there were also incidents with the insecticides mipafox and leptofos. The target protein was designated neuropathy target esterase (NTE) (69).
and identified as a lysophosphatidyl choline hydrolase (112, 113, 143). Diminished NTE activity in mice links OP exposure to hyperactivity (148) but not necessarily to attention deficit disorder in humans. NTE deficiency in mice is embryo lethal (93, 116, 148) but when localized to the hippocampus gives vacuolation of neuronal bodies and dendrites (1).

The cannabinoid system consists of the CB1 and CB2 G protein–coupled cannabinoid receptors in the central and peripheral nervous systems, respectively, and two endocannabinoid ligands, anandamide and 2-arachidonyl glycerol, biosynthesized as needed and degraded by the serine hydrolases fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL), respectively (Figure 3). This system is involved in appetite, pain, synaptic plasticity, mood, and the psychoactive effects of cannabis. Behavioral effects of some OPs suggested possible involvement of the cannabinoid system, and the serine hydrolases FAAH and MAGL were found to be sensitive to chlorpyrifos and profenofos in vivo in mice (25, 97). Potent OP irreversible inhibitors of MAGL and FAAH induce the full cannabinoid syndrome in mice associated with greatly elevated brain endocannabinoid levels and depleted free arachidonic acid (96). The current OP and MC insecticides as normally used do not appear to pose any cannabinoid-related toxicity problems. Selective and reversible carbamate FAAH and MAGL inhibitors have been designed with the goal of pain relief (88).
OP- and MC-induced teratogenesis by diazinon, carbaryl, and other insecticides in hen eggs involves inhibition of N-formylkynurenine hydrolysis by arylformamidase (121) and depletion of NAD levels (110, 111). The importance of this secondary OP target in mice was later evaluated by identifying the catalytic site (106) and defining the effect of gene inactivation on the kynurenine pathway metabolites (43). Another OP-sensitive serine hydrolase is KIAA1363 with an acetyl monoalkylglycerol ether substrate that also serves as a chlorpyrifos oxon hydrolase (98, 99, 100).

**Parkinson’s and Alzheimer’s Diseases**

The increasing incidence of Parkinson’s and Alzheimer’s diseases worldwide associated with aging is attributed to genetic and environmental factors, including exposure to neuroactive insecticides (49, 54). The Parkinson’s signs of tremor, rigidity, slowness of movement, and postural instability result from insufficient levels of dopamine in the substantia nigra of the midbrain leading to α-synuclein protein accumulation and formation of Lewy body inclusions. Some of the insecticides proposed to contribute to these changes and the incidence of Parkinson’s disease are the NADH oxidase inhibitors rotenone and deguelin (4, 17, 129), the PCCAs heptachlor and dieldrin (9, 12), and the pyrethroid permethrin (9, 52). Alzheimer’s disease is progressive and irreversible, leading to memory loss, language problems, unpredictable behavioral changes, and development of amyloid plaques, neurofibrillary tangles, and loss of connections between neurons in brain tissue (51, 56). Choline acetyl transferase activity is low in Alzheimer’s, resulting in low ACh levels, and other neurotransmitters (GABA and glutamate) are also possibly involved. Mild to moderate Alzheimer’s signs can be relieved by nicotine and AChE inhibitors, including the important Alzheimer’s drug Aricept® and the candidate (but withdrawn) drug metrifonate (i.e., the superseded OP insecticide trichlorfon) (13, 89), which delay ACh breakdown in the synaptic cleft, thereby enhancing cholinergic neurotransmission.

**FUTURE PROSPECTS**

**Less Emphasis on Neurotoxicants**

Almost all insecticides used in the 1940s to 1980s were neurotoxicants, but this had dropped to 79% of the total world market value by 1990. Between 1997 and 2010, there was a marked shift from OPs and MCs to neonicotinoids accompanied by a distinct trend toward a variety of non-neuroactive insecticides (Figure 4). After seven decades of continuing discoveries, there are indications that we may be coming to the end of the golden age (26) of neuroactive insecticide research. Exploring nature and sifting through chemical libraries have repeatedly ended up with insecticides dependent on the same major targets (21). This is disappointing when new targets are needed to circumvent cross-resistance mechanisms. Although insect growth regulators, including insect hormone analogs or agonists and inhibitors of chitin synthesis, can be highly potent, effective, and selective, they act slowly in stopping crop damage, thereby limiting their role in pest control. Inhibitors of Complex I and other sites in the respiratory chain, including selected thioureas, organotins, and uncouplers of oxidative phosphorylation, are limited by low selectivity between insects and mammals (23, 95).

**Insecticide Activation and Detoxification**

Neurotoxicant selectivity and resistance are attributable not only to differences in the biochemical targets considered here but also to the relative rates of P450 oxidation, hydrolysis, and conjugation
Leading to activation and detoxification in insects and mammals. Many neurotoxic insecticides are in fact proinsecticides, such as phosphorothionates and derivatized MCs, that undergo preferential bioactivation in pests to the active metabolites. The use of proinsecticides makes it possible to alter the physicochemical properties, distribution, and persistence for improved efficacy, resistance management, and safety.

**SUMMARY POINTS**

1. The four major neuroactive insecticide targets (AChE, nAChR, GABAR, and Na⁺ channels) have the following features in common: original recognition by a botanical insecticide or prototype; target site cross-resistance limiting their effectiveness; available models for insecticide binding site interactions; alternative subsites for insecticides less involved in cross-resistance phenomena.

2. Downsizing of research on insecticide chemistry and toxicology has limited the discovery of new chemotypes and biochemical targets, but this is partially compensated for by new in vitro screens and testing of massive chemical libraries.

3. Neuroactive insecticides have helped clarify the role of secondary targets in OPIDN, cannabinoid-like effects, PD, and AD.
FUTURE ISSUES

1. There is a continuing need for new chemotypes of neuroactive insecticides as the next generation of “resistance busters.”

2. An increasing diversity of non-nerve targets will help to maintain control capabilities.

3. New centers of excellence in insecticide research will meet continuing and increasing demands for safe and effective chemicals.

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LITERATURE CITED


64. IRAC: Summaries and Evaluations. 1983. Chlordimeform. IRAC Monogr. 30:61–72


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