Neurotoxicological Effects And The Mode of Action of Pyrethroid Insecticides*

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ABSTRACT

Neuroexcitatory symptoms of acute poisoning of vertebrates by pyrethroids are related to the ability of these insecticides to modify electrical activity in various parts of the nervous system.

Repetitive nerve activity, particularly in the sensory nervous system, membrane depolarization, and enhanced neurotransmitter release, eventually followed by block of excitation, result from a prolongation of the sodium current during membrane excitation. This effect is caused by a stereoselective and structure-related interaction with voltage-dependent sodium channels, the primary target site of the pyrethroids.

Near-lethal doses of pyrethroids cause sparse axonal damage that is reversed in surviving animals. After prolonged exposure to lower doses of pyrethroids axonal damage is not observed.

Occupational exposure to pyrethroids frequently leads to paresthesia and respiratory irritation, which are probably due to repetitive firing of sensory nerve endings. Massive exposure may lead to severe human poisoning symptoms, which are generally treated well by symptomatic and supportive measures.

I. INTRODUCTION

The pyrethroids constitute a major class of highly active synthetic insecticides derived from the natural pyrethrins. Since the early 1970s, when the first photostable pyrethroids were discovered,[1,2] numerous new pyrethroids have been synthesized, some of which seem only remotely related to the parent compounds.[3] Structural formulas of pyrethrin I, the most active natural compound, and of some important pyrethroids are depicted in Figure 1.

Pyrethroids are much more effective against a wide spectrum of economically important pests than the organochlorine, organophosphate, and carbamate insecticides. Yet they have a low oral toxicity to mammals and, in general, their insect (topical) to mammal (oral) toxicity ratio is much higher than that of the other major classes of insecticides.[4] The favorable properties have promoted the widespread application of pyrethroids in virtually all sectors of insect control. In the past decenium, the major chemical companies have been marketing pyrethroids that have since gained an important position on the world market of insecticides, replacing many of the earlier compounds.

In all species thus far investigated pyrethroids induce toxic signs that are characteristic of a strong excitatory action on the nervous system. Toxic doses of pyrehthroids generally cause hypersensitivity to sensory stimuli, and a number of compounds may induce tingling sensations in the skin. In mammals two distinct toxic syndromes have been described.[5] The T-syndrome is induced by pyrethrins and noncyano pyrethroids, e.g., permethrin, and this syndrome is named after the prominent symptom of whole-body tremors. The CS-syndrome, induced by deltamethrin and most other cyano pyrethroids, is characterized by choreoathetosis and salivation. Some pyrethroids produce tremors and salivation, classified as the intermediate TS-syndrome.

The promising features and the potential for widespread application of the pyrethroids have strongly stimulated the thorough examination of their toxic effects and their mode of action. As a consequence, the information on the mode of action of pyrethroids available before introduction on the market was more detailed than for any other class of insecticide. Although the molecular aspects of pyrethroid action are not yet fully understood, detailed electrophysiological investigations strongly suggest that the voltage-dependent sodium channel in the nerve membrane is the common target in both insects and mammals, including man.

Although pyrethroids have been used intensively over a number of years, few serious problems have been reported. Cases of human poisoning appear restricted to overt accidental or intentional overexposure. Nevertheless, the pyrethroids, which turn into highly neuroactive substances when the nervous system is directly accessible, cannot be regarded as being harmless. This review critically evaluates the neurotoxicological effects of pyrethroids on the vertebrate nervous system, with special attention on adverse effects in man. Besides data from the open scientific literature, information made available by the chemical industry on a number of pyrethroids has been included.[6-10] It will attempt to relate the various neurotoxic symptoms to basic effects of pyrethroids at the subcellular and molecular level.

For detailed information on chemical structure, insecticidal

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action, practical applications, metabolism, and degradation of pyrethroids* the reader is referred to a number of recent reviews.[3,4,11-15]

II. BASIC EFFECTS ON THE NERVOUS SYSTEM

The first scientific demonstration that natural pyrethrins, the predecessors of the synthetic pyrethroid insecticides, modify nervous system activity was published in 1909 by Fujitani, who isolated "pyrethron", a viscous yellow substance, from pyrethrum flowers cultivated in Japan. His results from experiments on intact frogs, frog heart and muscle preparations, and on a variety of other species, e.g., amoebae, insects, fish, and mammals, clearly indicated that a relatively unstable ester in the pyrethron extract caused excitation and paralysis due to effects on the neuromuscular and nervous system.[16] However, it was not until the middle of this century that the effects of the mixture of natural pyrethrins on nerve activity in various arthropod preparations were described.[17-22] Early information on the cellular mechanism of action of allethrin was obtained from intracellular recordings of the action potential in cockroach giant axons.[23,24] Details on structure-related effects of pyrethroids on the electrical activity of several preparations of the desert locust were first obtained less than 2 decades ago.[25] Although invertebrate studies have made major contributions to the development of the current knowledge on the mode of action of pyrethroids, the present description of the basic neurotoxic effects on the nervous system is confined to vertebrates. A brief comparison to the mode of action in invertebrates is presented in a separate section. For extensive data on neurotoxic effects of pyrethroids on the invertebrate nervous system and the relation to insecticide mode of action several recent reviews are available.[11-15]

A. Vertebrate Peripheral Nerve Activity

In recent years, ample experimental evidence has been presented indicating that in vertebrates, like in invertebrates, the pyrethroids act primarily on the nervous system.[26,27] The principal action of pyrethroids in the peripheral nervous system is to induce pronounced repetitive activity.[28] In particular sense organs produce trains of nerve impulses instead of single nerve impulses after exposure to pyrethroids, either *in vitro* or *in vivo* (Figure 2).[29-31]

The potential of a series of pyrethroids to induce repetitive activity in various parts of the frog peripheral nervous system has been investigated in detail. The range of compounds tested includes noncyano pyrethroids (allethrin, resmethrin, phenothrin, permethrin, des-cyano-deltamethrin, fenfluthrin, and the chlorovinyl analog of tetramethrin) and cyano pyrethroids (cyphenothrin, cypermethrin, fenpropathrin, fenvalerate, and deltamethrin). Pure active isomers were used when available. Noninsecticidal (1*S*)- and (α*R*)-cyano-substituted isomers have proven to be inactive up to the level of possible contamination with active ingredients (ca. 1%).

In the frog lateral-line sense organ, all insecticidally active pyrethroids induce repetitive activity. This sense organ is located superficially in the skin and serves as a water motion detector. Spontaneous electrical activity of single sensory fibers of the nerve innervating the lateral-line can be recorded in an isolated piece of skin.[32] The intensity of the repetitive activity, i.e., the duration of nerve impulse trains recorded from the lateral-line sense organ in the presence of pyrethroids, varies greatly with pyrethroid structure (Figure 3).[33] Noncyano pyrethroids induce short nerve impulse trains, which contain no more than a few dozen of repetitive nerve impulses. On the other hand, the cyano pyrethroids cause long-lasting trains that contain hundreds or even thousands of repetitive nerve impulses. On several occasions, repetitive discharges lasting over 30 s have been recorded. In addition, the duration of nerve impulse trains induced by noncyano as well as cyano pyrethroids in the lateral-line sense organ increases dramatically as the temperature is lowered (Figure 3B,C). This effect is readily reversed by raising the temperature.

The same repetitive activity is produced with both *in vitro* and *in vivo* exposure. Time to onset of repetitive activity varies greatly for different pyrethroids at micromolar concentrations. With fenfluthrin the latency to onset of repetitive activity decreases from 1 to 1.5 h at 0.1 μ*M* to approximately 15 min at concentrations ≥ 1 μ*M*. Some compounds, in particular fenvalerate, are very slow acting so that *in vivo* exposure for prolonged periods (up to 23 h) is the only way to obtain repetitive activity in the lateral-line sense organ. It is to be noted that the organochlorine insecticide DDT induces repetitive activity in the lateral-line sense organ of the frog very similar to that of the noncyano pyrethroids, but after prolonged *in vivo* exposure only (i.e., 18 h at 3 ppm).[29] In view of results obtained with locust and leech ganglia *in vitro*,[34] it is conceivable that partitioning of the pyrethroids between the water phase and the frog skin is an important factor in the determination of the time to onset of repetitive activity.

In peripheral nerves of the frog noncyano pyrethroids also cause repetitive firing. This repetitive activity has been observed in sensory nerve fibers and in motor nerve terminals, but not in the more proximal parts of motor nerve fibers.[35,36] In contrast, cyano pyrethroids do not induce repetitive activity in peripheral nerves. However, they may cause a frequency-dependent, reversible depression of the nerve impulse in sensory nerve fibers.[37] Noncyano as well as cyano pyrethroids in concentrations beyond the micromolar range may cause a reduction of the nerve impulse amplitude in frog peripheral nerves and of the frequency of spontaneous firing in the lateral-line

*A complementary assessment of the neurotoxicity of pyrethroids, focused on toxicokinetic and metabolic aspects by Dr. W. N. Aldridge is cosubmitted for publication in *CRC Reviews in Toxicology*.

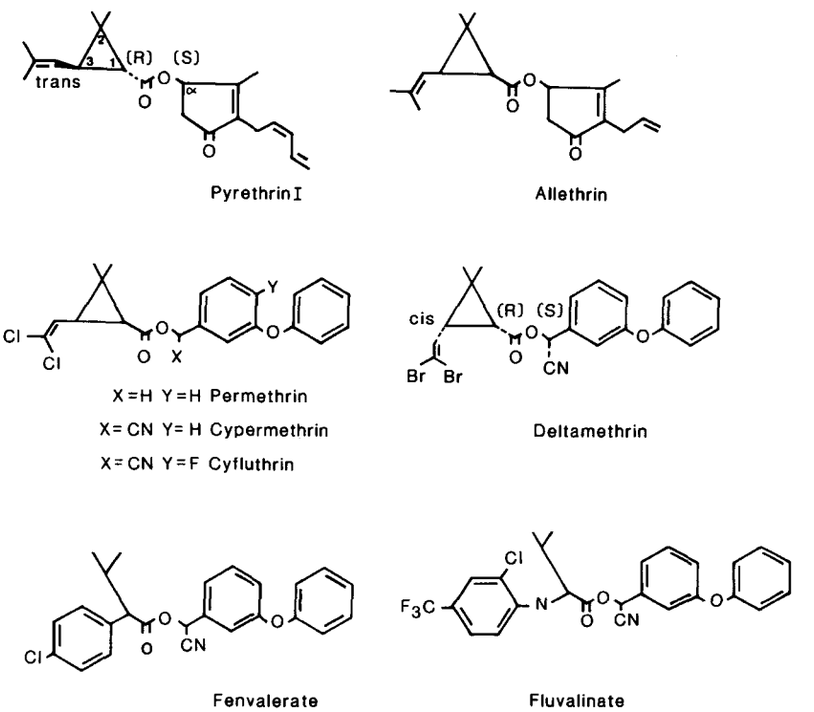

FIGURE 1. Chemical structure of the natural pyrethrin I that served as a model for the first synthetic pyrethroid, allethrin. Within the chemically diverse class of pyrethroids halogenated phenoxybenzyl analogs in particular have developed into economically important insecticides that are widely used at present. Several key structures of noncyano and cyano pyrethroids are depicted.

sense organ without causing repetitive activity.[33,38] This blocking effect is distinct from the frequency-dependent suppression by cyano pyrethroids mentioned above. In frog presynaptic motor nerve endings, all noncyano pyrethroids as well as several cyano pyrethroids induce repetitive activity. Conversely, in the frog postsynaptic muscle fiber membrane the cyano pyrethroids and some noncyano compounds cause repetitive activity.[39,40]

From these results, summarized in Table 1, it can be concluded that all pyrethroids induce repetitive activity in sense organs. Depending on pyrethroid structure, repetitive activity may also occur in frog sensory nerves, motor nerve endings, and muscle fibers.

Generally, *in vitro* effects of pyrethroids are poorly reversible, because of the highly lipophilic nature of these insecticides. It should be pointed out, however, that from the poor reversibility of the effects *in vitro* it cannot be inferred that pyrethroids cause irreversible effects *in vivo*. For example, deltamethrin is not metabolized *in vitro* by rat brain homogenate, but the clearance of intravenously administered pyrethroids from rat blood and tissues is rapid and animals that have survived near-lethal doses of pyrethroids appear quite normal.[41,42]

In the mammalian peripheral nervous system the effects of pyrethroids on electrical activity are similar to those described for the frog. Cis-methrin induces repetitive activity in tactile and hair follicle skin sense organs of the rabbit.[43] Furthermore, the variation in the effects of noncyano and cyano pyrethroids on the electrical activity of rat peripheral nerve and muscle[44-46] is very similar to that in isolated frog preparations treated with pyrethroids. The effects described in the mammalian peripheral nervous system were induced by intravenous administration of near-LD_{50} doses of pyrethroids. Electrophysiological effects of lower doses of pyrethroids on the mammalian peripheral nervous system have not been reported.

B. Voltage-Dependent Sodium Channels

The basis for nerve impulse generation and conduction lies in the selective ionic permeability of the excitable membrane combined with the concentration gradients for sodium and potassium ions: high sodium outside and high potassium inside of the membrane. The resting membrane is electrically polarized by these concentration gradients, which are maintained by the Na/K pump, and the inside of the cell is 60 to 80 mV negative with respect to the outside. A nerve impulse is brought about by a rapid, temporary increase in the permeability of the

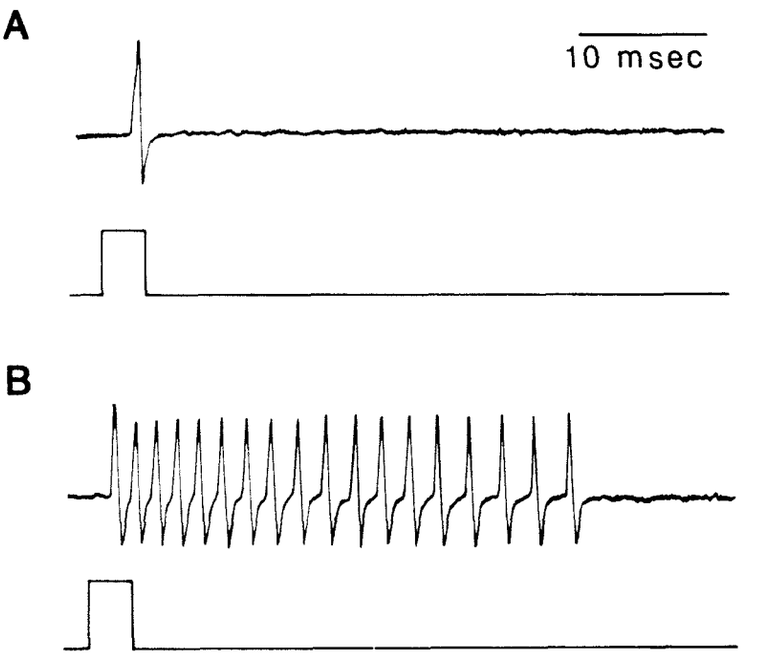

FIGURE 2. Responses of a cutaneous touch receptor in the skin of the frog *Xenopus laevis*. (A) Nerve impulse evoked in a single sensory nerve fiber by a brief mechanical stimulus (lower trace) in the control situation. (B) Instead of a single nerve impulse a train of repetitive nerve impulses is evoked by the same stimulus after 30 min of *in vitro* exposure of the skin to 10 μM of the pyrethroid allethrin. (From Akkermans, L. M. A., van den Bercken, J., and Versluijs-Helder, M., *Pestic. Biochem. Physiol.*, 5, 451, 1975. With permission.)

membrane to sodium ions, resulting in a transient inward sodium current, followed by an increase in the potassium permeability, resulting in an outward potassium current. The ionic currents cause a transient, local reversal of the membrane potential from negative to positive and a nerve impulse occurs that is conducted along the nerve fiber.[47]

The changes in nerve membrane ionic permeability are mediated by discrete molecular structures, called ionic channels. Ionic channels are formed by complex, integral membrane proteins embedded in the lipid matrix of the membrane. Voltage-dependent sodium and potassium channels undergo conformational changes under the influence of the electric field across the membrane to become selectively permeable to either sodium or potassium ions, respectively.[48-50]

The changes in nerve membrane permeability and hence the kinetic properties of ionic channels can be studied in detail by the voltage clamp technique. With this technique it is possible to control the membrane potential and to simultaneously record the ionic currents through the membrane.[47] In recent years, the voltage clamp technique has been elaborated to enable recording of the minute ionic currents through individual ionic channels by means of the single channel patch clamp technique.[51]

Voltage clamp studies on vertebrate nerve cell preparations have shown that the voltage-dependent conformational changes of sodium channels in excitable membranes are greatly affected by pyrethroids. Pyrethroids cause sodium channels to stay open much longer than normal, resulting in a prolongation of the transient sodium current associated with membrane depolarization and a marked, slowly decaying sodium tail current after termination of the depolarization.[52-56] The prolonged sodium tail current induced by the pyrethroids is directly responsible for the repetitive activity described above.

The action of pyrethroids can be explained in terms of the classic Hodgkin-Huxley model for the molecular operation of the voltage-dependent sodium channel. According to this model, the opening and closing of the sodium channel are governed by two largely independent gates, which are oppositely dependent on membrane potential.[47] In the normal situation depolarization of the membrane causes transient opening of sodium channels, due to the rapid opening of the activation gate and the slower closing of the inactivation gate. It has been shown that the major effect of pyrethroids is to delay the closing of the sodium channel activation gate.[54] Upon repolarization of the membrane, this effect prevents the rapid closing of

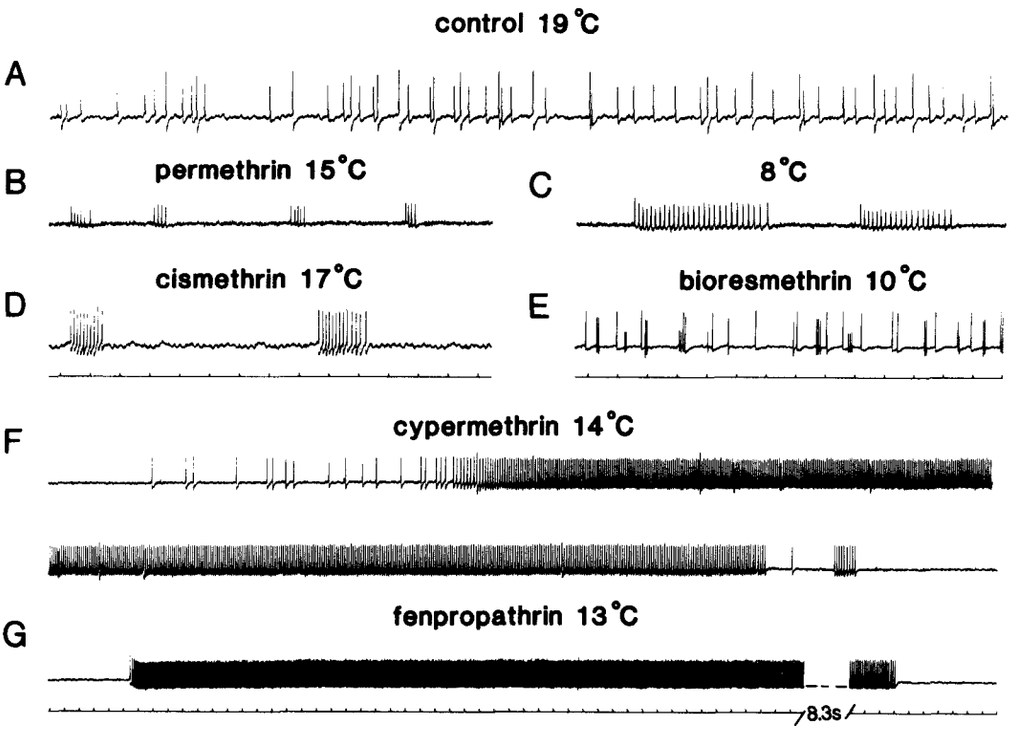

FIGURE 3. Effects of various pyrethroids on the spontaneous afferent activity of the lateral-line sense organ in the skin of the frog, *Xenopus laevis, in vitro*. In the control situation (A) single nerve impulses spontaneously occur at irregular intervals. The noncyano pyrethroids (B to E) cause short nerve impulse trains in contrast to the cyano pyrethroids (F, G) after which long-lasting repetitive discharges are observed. Cooling of the skin preparation causes an increase of the duration of pyrethroid-induced nerve impulse trains as demonstrated for permethrin (B, C). The conservation of pyrethroid was always 5 μM; time calibration 100 ms/div, upper scales refer to A to E and lower scale to F and G. (From Vijverberg, H. P. M., Ruigt, G. S. F., and van den Bercken, J., *Pestic. Biochem. Physiol.*, 18, 315, 1982. With permission.)

sodium channels, and as a result a prolonged sodium tail current occurs (Figure 4). The duration of the pyrethroid-induced sodium tail current, i.e., the time constant of tail current decay, is directly related to the pyrethroid structure. In frog myelinated nerve fibers, time constants of decay of the prolonged sodium tail current at 15°C range from 6 ms for *trans*-phenothrin to almost 2 s for deltamethrin. Table 2 shows a continuous range of time constants of decay of the tail currents induced by the various pyrethroids. Each compound can be characterized by the time constant of the prolonged sodium tail current. The time constants are decreased by raising the temperature and increased by cooling.[57,58] The further prolongation of sodium current by pyrethroids at a lowered temperature accounts for the prolongation of repetitive nerve impulse trains as shown in Figures 3B and 3C.

The effects of pyrethroids on the gating of single sodium channels in frog spinal ganglion neurons and in cultured mouse neuroblastoma cells have also been investigated.[59-61] These studies have shown that the open time of single sodium channels is greatly prolonged after treatment with pyrethroids (Figure 5), which supports the results on the prolongation of the membrane sodium current at the molecular level. In addition, the results from single channel studies indicate that pyrethroids stabilize the open as well as other states of the sodium channel.[56,62]

In mouse neuroblastoma cells and in mouse and fish brain synaptosomes, pyrethroids enhance the toxin-induced sodium influx through voltage-dependent sodium channels.[63-66] Sodium channel toxins and other agents that augment intracellular sodium concentration also stimulate the production of second messengers. Through activation of specific protein kinases, this may lead to altered protein phosphorylation levels.[67] In guinea pig synaptoneurosomes pyrethroids stimulate the breakdown of phosphoinositides into the second messengers inositol triphosphate and diacylglycerides. The larger effects of the cyano pyrethroids deltamethrin and fenvalerate are partially antagonized by the sodium channel blocker tetrodotoxin (TTX). The smaller effects of the noncyano pyrethroids allethrin, resmethrin, and permethrin appear independent of sodium influx through the voltage-dependent sodium channel.[68] DDT and deltameth-

Table 1
Ability of Various Pyrethroids to Induce Repetitive Activity in Various Parts of the Frog Peripheral Nervous System

Compound	Configuration		Repetitive activity: Sciatic nerve	Motor nerve ending	Muscle fiber	Sense organ
Noncyano pyrethroids						
Allethrin	Mixture		+	+	−	+
Resmethrin	(1*R*), *trans*		+	+	−	+
	(1*R*), *cis*		+	+	−	+
Phenothrin	(1*R*), *cis*			+	−	+
Permethrin[a]	(1*R*), *trans*		+	+	−	+
	(1*R*), *cis*		+	+	+	+
RU23603	(1*R*), *cis*			+	+	
Fenfluthrin[a]	(1*R*), *cis*		+	+	+	+
NAK1963	Mixture			+	+	
Cyano pyrethroids						
Fenpropathrin		(α*RS*)	−	+		+
Cyphenothrin	(1*R*), *trans*,	(α*S*)		+	+	+
Cyphenothrin	(1*R*), *cis*,	(α*S*)		+	+	+
Fenvalerate[a]	(2*RS*),	(α*S*)	±	+	+	+
S-5655	Mixture			−	+	
Cypermethrin	(1*R*), *cis*,	(α*S*)	−	−	+	+
Deltamethrin	(1*R*), *cis*,	(α*S*)	−	−	+	+

[a] (1*S*), *cis*- and (1*S*), *trans*-permethrin; (1*S*), *trans*-fenfluthrin, and (2*R*), (α*R*)- and (2*RS*), (α*R*)-fenvalerate are inactive.
RU23603: "des-cyano"-deltamethrin
NAK1963: *N*-(3,4,5,6-tetrahydrophthalimido) -methyl-3-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropanecarboxylate.
S-5655: α-ethynyl-3-phenoxybenzyl-2-(4-chorophenyl)-3-methylbutyrate.

From Vijverberg, H. P. M., de Weille, J. R., Ruigt, G. S. F. , and van den Bercken, J., *Neuropharmacology and Pesticide Action*, Ford, M. G., Lunt, G. G., Reay, R. C., and Usherood, P. N. R., Eds., Ellis Horwood, Chichester, England, 1986, 267. With permission.

rin, but not the inactive (1*S*)-isomer of deltamethrin, enhance cyclic AMP-dependent phosphorylation of the α-subunit of the sodium channel protein in rat brain synaptosomes.[69] The consequences of phosphorylation for sodium channel kinetics are presently unknown.

Additional evidence for the interaction of pyrethroids with sodium channels of the central nervous system is obtained from the result that in rat brain synaptoneurosomes cypermethrin isomers allosterically enhance the *in vitro* binding of a tritiated derivative of batrachotoxin, a radioligand selective for voltage-dependent sodium channels, in a stereoselective manner. This stereoselective effect on sodium channels correlates with the differential insecticidal potency of the pyrethroid isomers.[70,71]

The action of pyrethroids on sodium channels results in variable effects on the electrical activity of the peripheral nervous system. Repetitive activity as well as frequency-depen-

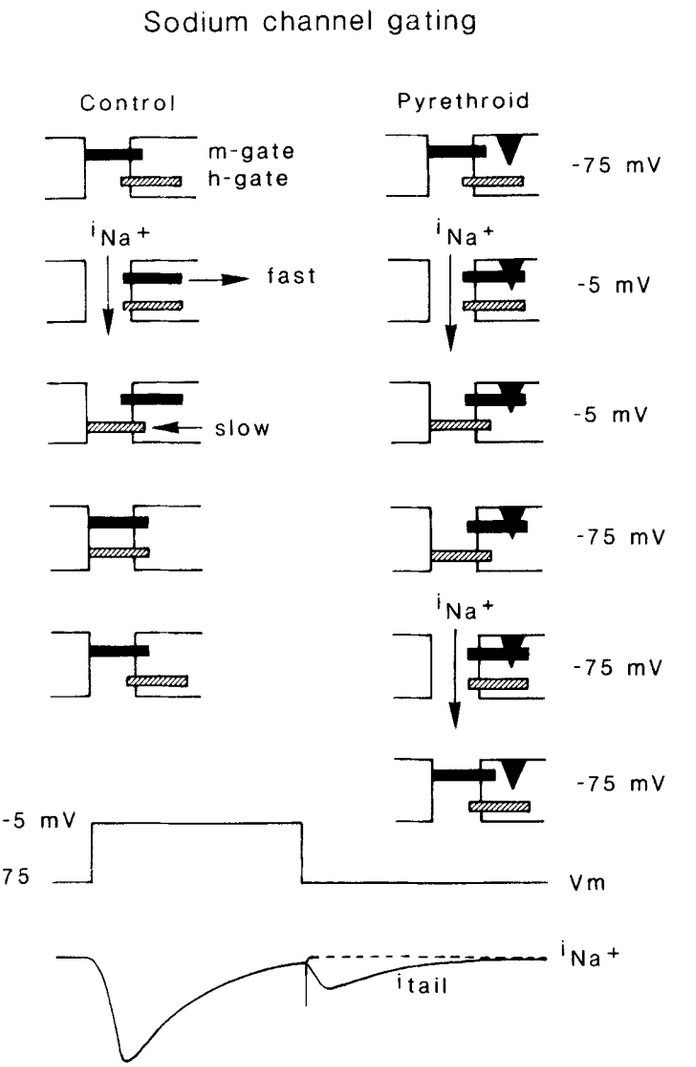

FIGURE 4. Schematic sequence of simplified gating events during the opening and closing of a voltage-dependent sodium channel according to Hodgkin and Huxley[47] in the control situation (left column) and the major modification by pyrethroid insecticides (right column). Figures at the right indicate membrane potential. The pyrethroid (symbolized by an arrowhead) delays the closing of the activation gate (m-gate) and thereby prevents the rapid closing of sodium channels that normally occurs when a membrane depolarization is terminated. Below the scheme are depicted the membrane depolarization (V_m) and the superimposed control and pyrethroid-modified sodium currents (i_{Na}), which are composed of large numbers of fluctuating microscopic contributions from single sodium channels. After termination of depolarization the control sodium current rapidly declines to zero level (dashed line), whereas a slowly decaying sodium tail current remains after exposure to the pyrethroid. The closing of modified sodium channels constitutes the rate limiting step for sodium tail current decay. (Modified after Van den Bercken, J. and Vijverberg, H. P. M., *Insect Neurobiology and Pesticide Action (Neurotox 79)*, Society of Chemical Industry, London, 1980, 79; Adapted from Vijverberg, H. P. M. and van den Bercken, J., *Neuropathol. Appl. Neurobiol.*, 8, 421, 1982. With permission.)

Table 2
Time Constants of Sodium Tail Currents Induced by Various Pyrethroids in Frog Nerve Fibers and in Cultured Mouse Neuroblastoma Cells Compared to Poisoning Syndromes Repored in Mammals and Insects

Compound	Configuration	τ_{tail} (ms) Frog[a]	τ_{tail} (ms) Mouse[b]	Syndrome Rat[c]	Syndrome Cockroach[d]
Noncyano pyrethroids					
Phenothrin	(1*R*), *trans*	6		N	I
Permethrin	(1*R*), *trans*	7		N	I
Allethrin	Mixture	10			I
Phenothrin	(1*R*), *cis*	13	4	N	I
Cismethrin[a]	(1*R*), *cis*	21		T	I
Permethrin	(1*R*), *cis*	28		T	I
Fenfluthrin	(1*R*), *cis*	105	14—30		
NAK1963	Mixture	150			
S-5655	Mixture	150		T(S)	
Cyano pyrethroids					
Cyphenothrin	(1*R*), *trans*, (α*S*)	290		T	II
Cyphenothrin	(1*R*), *cis*, (α*S*)	385	140	CS	II
Fenvalerate	(2*RS*), (α*S*)	545		CS	II
Fenvalerate	(2*S*), (α*S*)	600		CS	II
Cypermethrin	(1*R*), *cis*, (α*S*)	1115		CS	II
Deltamethrin	(1*R*), *cis*, (α*S*)	1770	440—800	CS	II

[a] Measured in frog myelinated nerve fibres at 15°C.
[b] Measured in mouse neuroblastoma cells at 18°C.
[c] Data from Reference 5: intravenous application; N = no symptoms; T = tremor; S = salivation; C = choreoathetosis.
[d] Data from Reference 138: topical application; I = incoordination; II = convulsions.

NAK1963; S-5655: see Table 1.

From Vijverberg, H. P. M., de Weille, J. R., Ruigt, G. S. F. , and van den Bercken, J., *Neuropharmacology and Pesticide Action*, Ford, M. G., Lunt, G. G., Reay, R. C., and Usherood, P. N. R., Eds., Ellis Horwood, Chichester, England, 1986, 267. With permission.

dent depression in frog nerves are much more pronounced in sensory fibers than in motor fibers. This can be explained on the basis of fundamental differences in the gating kinetics of sodium channels in sensory and motor nerve fibers of the frog.[72,73] Variations in the intensity of repetitive activity in sense organs with temperature as well as with pyrethroid structure both parallel observed differences in the prolongation of the current through pyrethroid-modified sodium channels. Although the structure-activity relationships for the occurrence of repetitive firing in nerve and muscle are quite different, the ability of pyrethroids to induce repetitive activity in these parts of the peripheral nervous system also appears to be correlated to the degree of sodium current prolongation. These results indicate that the stimulatory and blocking effects of pyrethroids on electrical activity originate from the same basic action on sodium channels.[33,37,58] Whether repetitive activity, a depolarizing afterpotential, or membrane depolarization associated with block of excitation occur in the presence of a pyrethroid will depend on the amplitude and the time course of the prolonged sodium current component and on additional features of the various excitable tissues. The combination of variables involved may well explain the differences in repetitive activity in various parts of the frog peripheral nervous system as well as variation of the effects of pyrethroids between species. It is also conceivable that the large quantitative differences between the basic effects of cyano and noncyano pyrethroids on voltage-dependent sodium channels are responsible for the distinct toxic symptoms in intact animals (Table 2).[40]

Another indication that the alterations of sodium channel function are closely associated with the toxicity of pyrethroids comes from temperature effects. The toxicity of pyrethroids to insects and cold-blooded vertebrates shows a negative temperature coefficient and lowering of the temperature causes the appearance of toxic signs.[74-78] The latter effect is rapidly reversible[74] and parallels the further prolongation of the pyrethroid-induced sodium tail current on cooling *in vitro*.[40,57] In homeothermic animals the effect of changes in environmental temperature appears more complex as reflected by the negative temperature coefficient of oral but not of intravenous toxicity of cismethrin to the rat.[79]

In conclusion, pyrethroids prolong the open time of voltage-dependent sodium channels, resulting in a prolongation of the inward sodium current during excitation. This effect not only accounts for the repetitive activity in sense organs, but also for membrane depolarization, suppression of the amplitude of the nerve impulse, and block of excitation that may occur in various parts of the nervous system.

C. Other Voltage-Dependent Channels

Low concentrations of pyrethroids, which considerably affect sodium channels, do not significantly alter the functioning of the potassium channels. In invertebrate nerve preparations a suppressive effect on the potassium as well as on the sodium current has been observed only at high concentrations of pyrethroids.[80-83] A less specific effect of pyrethroids, similar to anesthetic effects produced by various nonionic esters and ketones,[84] is the most likely cause of ionic channel block. The neurotoxicological relevance of potassium channel block by pyrethroids is questionable, as in voltage clamped crayfish giant axons it has been shown that the sodium current is greatly prolonged by 10 μ*M* fenvalerate, whereas the potassium current remains virtually unaffected at this concentration.[85] Moreover, in mammalian myelinated nerve fibers the nerve impulse is independent of potassium channels that are absent in the mammalian node of Ranvier.[86]

The pyrethroid tetramethrin has been reported to partially block one of two types of voltage-dependent calcium current in mouse neuroblastoma cells.[87] This result has not been con-

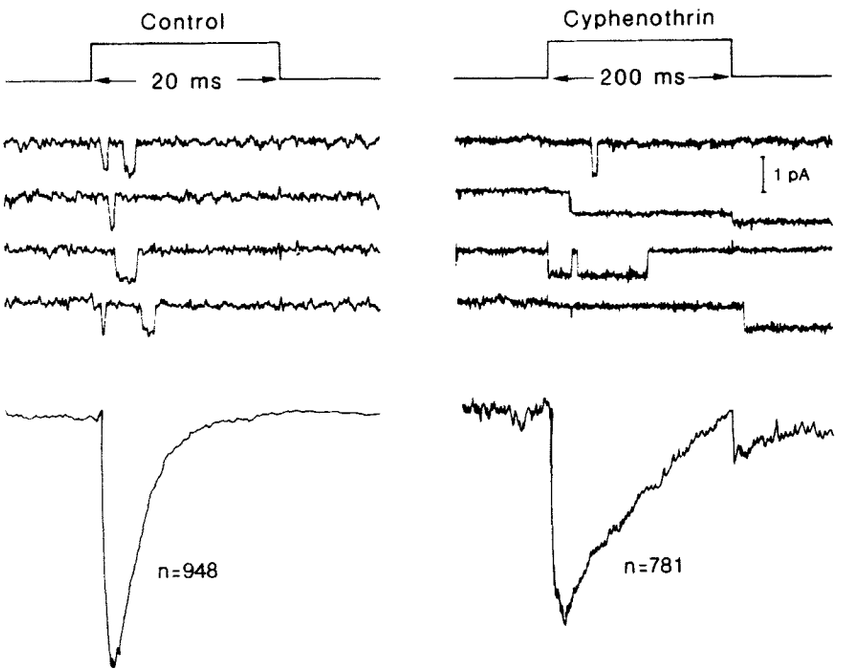

FIGURE 5. Opening and closing of individual sodium channels in excised patches of the membrane of cultured moust neuroblastoma cells. Downward deflections of the current traces are discrete openings of individual ion channels. In the presence of 10 μM (1*R*), *cis*-cyphenothrin the open time of sodium channels is greatly prolonged and channel openings are also observed after termination of membrane depolarization. Summation of large numbers (n) of single channel records result in a rapid, transient sodium current in the control situation and a prolonged sodium current followed by a sodium tail current in the presence of the pyrethroid. (Data adapted from de Weille, J. R., The Modification of Nerve Membrane Sodium Channels by Pyrethroids, Ph.D. thesis, University of Utrecht, 1986.)

firmed in experiments with fenfluthrin, permethrin, and cypermethrin.[88] The toxicological significance of the effect of tetramethrin on calcium channels remains unclear, since toxic signs produced by tetramethrin in the frog are not dissimilar from those of the other noncyano pyrethroids.[78]

D. Receptor-Operated Channels

Several studies have suggested that pyrethroids also affect ionic channels that are coupled to neurotransmitter receptors. The opening and closing of these channels depend on the occupation of the receptors by agonist molecules.

High concentrations of cyano pyrethroids cause a stereoselective partial inhibition of the binding of radiolabeled bicyclophosphorus esters to the γ-aminobutyric acid (GABA) receptor-ionic channel complex.[89,90] However, pyrethroids fail to produce a functional modification of the postsynaptic GABAergic neurotransmission in crayfish as well as in rat neurones, even at concentrations that cause pronounced repetitive nerve activity.[91-93] The GABA-induced chloride current in voltage-clamped rat dorsal root ganglion neurones remained completely unaffected in the presence of deltamethrin.[93] The same pyrethroid, but not cismethrin, increased the resting membrane resistance in rat diaphragm muscle fibers as well as in the vagus nerve, apparently by reducing a component of chloride conductance.[94] Experiments in conscious rats do not support the hypothesis that cyano pyrethroids exert GABA-antagonistic effects. Instead, deltamethrin and fenvalerate, but not cismethrin, enhance GABA-ergic recurrent inhibition in the dentate gyrus of the hippocampus, supposedly by a presynaptic effect on interneurones.[95] Similar results were obtained with deltamethrin in urethane-anesthetized rats.[96] However, in the latter study the noncyano pyrethroids allethrin and cismethrin were also reported to enhance interneuronal inhibition.

Results from studies of cholinergic and glutamatergic neurotransmission suggest that pyrethroid effects are induced presynaptically and do not involve postsynaptically located neurotransmitter receptor-operated ionic channels.[35,36,97] Effects of pyrethroids on the ligand-binding characteristics of the acetylcholine (ACh) receptor-ionic channel complex have led to the suggestion that these insecticides delay the closing or desensitization of the ionic channels coupled to the nicotinic ACh receptor.[98,99] Recent voltage clamp studies have shown that relatively high concentrations of pyrethroids on the postsynaptic ACh receptor-ionic channel complex in cultured mouse

neuroblastoma cells reduce the amplitude without affecting the kinetics of the ACh response. This effect on the ACh-induced inward current appears to be nonspecific, as it is produced by both insecticidal and noninsecticidal isomers. Similar effects were observed on the serotonin-induced response in neuroblastoma cells, which is mediated by an independent population of 5-HT_3 receptor-operated ionic channels.[100]

The available evidence indicates that the effects of pyrethroids on receptor-operated ionic channels are mainly indirect and do not contribute significantly to the excitatory symptoms associated with pyrethroid exposure.

E. Neurotransmitter Release

In insects, deltamethrin causes massive neurotransmitter release with depletion of synaptic vesicles in the motor nerve terminal due to presynaptic membrane depolarization.[101,102] In rat brain, the ACh content is significantly reduced 2 h after an oral dose of 50 mg/kg deltamethrin.[103] Other studies have demonstrated that in particular cyano pyrethroids enhance the release of radiolabeled GABA and norepinephrine (NE) from mammalian brain synaptosomes.[104-106] Both the pyrethroid-induced GABA release and the NE release induced by some of the cyano pyrethroids are inhibited by TTX, which selectively blocks normal as well as pyrethroid-modified sodium channels.[104,107] At a concentration of 10 μM several noncyano pyrethroids are ineffective, whereas delta-methrin is most effective in enhancing NE release from depolarized rat brain synaptosomes. The release caused by cypermethrin and fenvalerate correlates well with the degree to which the sodium current is prolonged by these pyrethroids relative to deltamethrin.[106] These results are consistent with the finding that catecholamines in plasma of the rat are increased during cismethrin-induced tremors to a level comparable to that attained during sustained exercise, whereas during deltamethrin-induced choreoathetosis plasma levels are indicative of massive catecholamine release.[108] Doses of deltamethrin that induce burrowing and pawing in the rat also cause a significant increase of plasma catecholamine levels and doses three times lower still cause a significant elevation of plasma corticosterone levels.[109] Little is known about the origin of the increased plasma neurotransmitter levels, but sympathetic nerves constitute a deltamethrin-sensitive, peripheral source of NE release.[110] The release of tritiated ACh and dopamine from slices of the rabbit striatum is enhanced by the insecticidal isomers of fenvalerate, but not by the noninsecticidal isomers. This effect is also inhibited by TTX. Conversely, fenvalerate does not affect ACh and dopamine release in rabbit hippocampal slices, suggesting regional differences in sensitivity to cyano pyrethroids.[111] The finding that pyrethroids enhance the binding of tritiated batrachotoxin to voltage-dependent sodium channels in various regions of the rat brain to a different degree[112] lends support to the same hypothesis.

Cismethrin, cypermethrin, and deltamethrin cause an increase in the level of cyclic GMP, but not of cyclic AMP, in rat brain.[103,113] This effect, which occurs particularly in the cerebellum, appeared correlated with the duration of motor symptoms and is regarded as secondary to the development of toxic signs.[114,115] The stimulatory effect of cypermethrin is not found in cerebellar slices, suggesting that it originates outside the cerebellum.[113] In general, the cyclic GMP level in excitable cells is enhanced by an increase of the intracellular calcium concentration, which can be achieved by depolarizing agents as well as by electrical stimulation.[116] Possible consequences of changes in second messenger levels for protein phosphorylation have been discussed in Section II.B.

It is concluded that enhanced neurotransmitter release in the presence of pyrethroids is secondary to sodium channel modification. Available evidence also suggests that effects of pyrethroids on the electrical activity of different types of central neurones vary similar to the effects in the peripheral nervous system.

F. Cardiovascular Effects

Pyrethroids induce cardiovascular effects in mammals. In the conscious rat intraperitoneal injection of 40 mg/kg deltamethrin causes a small decrease in arterial blood pressure prior to the onset of choreoathetosis.[117] In the anesthetized dog an intravenous LD_{50} dose of delta-methrin causes a rapid fall in arterial blood pressure associated with bradycardia, followed by an increase in blood pressure and tachycardia.[118] Intravenous deltamethrin, but not cismethrin, causes an increase in mean arterial pressure and differential pressure in the pithed rat. It was concluded that this effect is due to an increase of peripheral catecholamine release and a direct positive inotropic effect on the heart, both mediated by a peripheral site of action.[119,120] Cardiovascular effects of pyrethroids in conscious, anesthetized, and pithed animals are probably influenced by differences in the route of application of the pyrethroids as well as in sympathetic and vagal reflex compensation in the various physiological states of animals in the investigations referred to.[121] However, in isolated guinea pig atrial muscle both the catecholamine-mediated and the direct effect of deltamethrin are inhibited by TTX. Therefore, the most likely cause of these effects is prolongation of the presynaptic as well as the postsynaptic sodium current.[122] At present, there is no evidence for other effects of pyrethroids on the cardiovascular system.

G. Effects on ATPases

Activities of ATPases of vertebrates and also of invertebrates are inhibited by high concentrations of pyrethroids to a variable extent depending on the compounds, animal species, and tissues studied.[123-128] In contrast, pyrethroids stimulated purified Ca/Mg ATPase from rabbit muscle sarcoplasmic reticulum reconstituted in an artificial phospholipid bilayer. *Cis*-permethrin, deltamethrin, and the insecticidally active (1*R*), (α*S*) isomer as well as the nontoxic (1*S*), (α*R*) isomer of *cis*-cyhalothrin

all stimulate Ca/Mg ATPase activity to approximately the same extent. In these experiments it was shown that pyrethroids do not affect the fluidity of the lipid bilayers.[129] In yet another study it was shown that deltamethrin affects neither partially purified Na/K ATPase from rat brain nor that from guinea pig heart.[122] It is unknown whether pyrethroids interact directly with ATPases or indirectly affect ATPase activity. Because of the variability and the poor stereoselectivity the toxicological significance of these effects is questionable.

H. Comparative Effects in Invertebrates

Although the toxicity of pyrethroids varies widely between species, effects on the nervous system are remarkably constant. Sodium currents in invertebrate and vertebrate nerve preparations are modified by noncyano and cyano pyrethroids in a qualitatively very similar manner. Voltage clamp experiments on squid,[130,131] crayfish,[132,133] and cockroach axons[134,135] have also demonstrated that the cyano pyrethroids prolong the sodium current to a much greater extent than the noncyano pyrethroids. In the invertebrate nerve membrane the sodium current prolongation appears to be more pronounced than in frog myelinated nerve fibers and in neuroblastoma cells. Noncyano pyrethroids cause repetitive firing in the peripheral as well as in the central nervous system of invertebrates, whereas cyano pyrethroids cause membrane depolarization due to a persistent inward sodium current.[136,137] In insect sensory nerves the depolarizing action of cyano pyrethroids leads to nerve conduction block that may or may not be preceded by a transient period of repetitive activity.[135,138] In invertebrate nerve preparations membrane depolarization and excitation block are less pronounced and higher concentrations of pyrethroid are generally required to obtain these effects. The similarity between the effects of (noncyano) pyrethroids and DDT on the sodium channel of vertebrates[30,54] also extends to invertebrates,[132] and insects showing a particular form of DDT resistance based on nerve insensitivity (*kdr*), are also resistant to pyrethroids.[139-142]

I. Brain and Spinal Cord

The quite different poisoning syndromes initially observed after intravenous application of noncyano and cyano pyrethroids to the rat led to the hypothesis that the two subclasses of pyrethroid cause distinct peripheral and central nervous system effects.[5] Subsequently, it has been demonstrated that the two distinct types of pyrethroid-induced motor signs in the rat originate at the level of the spinal cord.[120,143] A similar spinal origin has been described for motor signs induced in mammals by DDT,[144,145] which prolongs the sodium current in the same way as the noncyano pyrethroids and to a comparable extent.[54] Detailed electrophysiological investigations into the effects of noncyano and cyano pyrethroids on spinal cord electrical activity of rat, rabbit, and cat have revealed an increased excitability of spinal interneurones, resembling that produced in peripheral nerves.[43,146,147] The additional finding that *cis*-permethrin, but not deltamethrin, greatly facilitates polysynaptic discharges suggests that it is this facilitation that causes the greater hyperexcitability produced by noncyano pyrethroids *in vivo*.[147] Similar increased interneuronal firing is supposed to be the cause of the enhancement by pyrethroids of recurrent inhibition in the dentate gyrus of rat hippocampus.[95,96]

EEG recordings of rats given high doses of cismethrin and deltamethrin revealed that only the cyano pyrethroid causes generalized epileptiform discharges, which can be triggered by sensory stimulation, e.g., sound. At the same time the amplitude of primary somatosensory and auditory-evoked potentials is reduced. The noncyano pyrethroid causes little change of the EEG and of the amplitude of the evoked potentials despite severe toxic signs, but abnormal late components appear in the auditory-evoked potential in parallel with an increased startle response.[117,148,149] Apparently, pyrethroids may enhance excitation as well as inhibition in different regions of the central nervous system.

J. Neurobehavioral Effects

Neurobehavioral investigation of xenobiotics is emerging from the need to characterize sublethal neurotoxic effects at a more integrated level. Permethrin and deltamethrin cause a dose-dependent, reversible decrease in the rate of lever pressing during operant behavior at doses well below the LD_{50} without overt toxic signs. The more toxic *cis* isomer of permethrin is more effective in this respect than *trans*-permethrin.[150,151]

In the rat noncyano pyrethroids as well as DDT enhance the acoustic startle reflex, but the latency of the startle reflex remains unaffected. These effects are produced in the absence of overt toxic signs. In contrast, cyano pyrethroids produce variable effects on the startle reflex as well as on its latency in addition to a suspected direct effect on muscle. A rapid onset and recovery of these effects is generally observed.[152-156] The differences between the behavioral effects of noncyano and cyano pyrethroids might be related to their differential effects on the sensory nervous system (e.g., auditory-evoked potentials) and on muscle, which are mentioned in previous sections. Treated animals all show transiently decreased motor activity in a set of "figure eight" mazes.[152,153] However, the older literature reports that low doses of DDT cause significant disinhibitory effects leading to increased exploratory behavior in mice.[157]

The practical value of the observed neurobehavioral effects for the evaluation of the neurotoxic potential of pyrethroids is difficult to estimate at present. Although it appears possible to grossly distinguish between the effects of low doses of cyano and noncyano pyrethroids, the results of this relatively new approach remain to be validated by extensive data on behavioral effects of different classes of neurotoxic compounds.

III. PERIPHERAL SENSORY PHENOMENA

A. Occupational Exposure

Forestry workers exposed to wettable powders of permethrin (*trans* : *cis* = 75 : 25) and fenvalerate may experience itching and burning sensations, paresthesia, blisters, nasal hypersecretion, sneezing, coughing, dyspnoea, and eye irritation. These symptoms occurred at a frequency of approximately 10% in a group of 139 unprotected persons using only gloves occasionally. The fraction of people without symptoms was 27% for fenvalerate and 37% for permethrin. In other persons, similarly exposed to an organic solvent emulsion of permethrin (*trans* : *cis* = 60 : 40), the frequency of symptoms appeared much lower. With the latter formulation, 67% of the workers were completely free of symptoms. Transient burning and tingling skin sensations in the face of hands, experienced after work with fenvalerate, disappeared overnight.[158,159]

Examination of 199 workers engaged for 0.5 to 4.5 months in dividing and packaging of fenvalerate (20% emulsion), deltamethrin (2.5% emulsion), and cypermethrin (10% emulsion) has revealed abnormal facial sensations in 60%, sneezing and increased nasal secretion in 32%, as well as dizziness and red miliary papules each in 14% of the subjects as the main symptoms. No clinical signs of acute pyrethroid poisoning have been observed and functional tests showed no aberrations in blood, heart, lungs, liver, kidneys, and nervous system. Despite lower air concentrations of the pyrethroids in summer all symptoms, except sneezing and nasal secretion, were more pronounced during this season. The sensory symptoms developed with a latency of approximately 30 min and generally did not last longer than 24 h.[160]

In another group of 16 agricultural workers exposed in the field to fenvalerate (Pydrin), all subjects experienced burning and tingling sensations and 4 of them subsequent numbness. The symptoms were exacerbated by perspiration, sun, heat, and exposure to water, and generally disappeared overnight. Neither edema nor vesiculation were apparent, indicating that pyrethroids do not evoke a primary inflammatory reaction.[161]

Supplementary information comes from the industrial research data.[6-10] All companies report that people handling the active substances, in particular the cyano pyrethroids, often experience transient local burning or tingling sensations and also itching and numbness of the facial skin, mainly in the periorbital area, but also of other sites of direct skin exposure (e.g., arms and legs). In all cases the skin effects were found to be reversible, generally within hours, but in some instances lasted for up to 48 h or in the extreme up to 7 d. Large differences exist in individual susceptibility to these effects of the pyrethroids. On one occasion individuals, who have shown extreme sensitivity, are advised to stop working with pyrethroids.[9] The skin symptoms can be reduced or eliminated by preventive measures, such as gloves and face masks.

In addition to producing skin sensations deltamethrin may be irritating to mucous membranes. This effect is considered to be partially caused or enhanced by the solvent in the EC formulation.[9] Cyfluthrin also causes irritant effects in the mouth and throat area in addition to skin effects.[6] Fluvalinate causes mild to severe respiratory irritation, manifested by coughing, throat irritation, and sneezing more frequently than itching and burning skin sensations. Most cases have been reported after exposure to spray particles of the flowable formulation of this pyrethroid. The skin sensations caused by flavulinate may be accompanied by rashes, and itching may occur to the point that scratching causes scars.[9] Respiratory irritation has not been reported to alphamethrin, cypermethrin, and permethrin.[8,10] Inhalation exposure to permethrin has never been found to produce respiratory symptoms in man.[8]

A single case of chronic unpleasant taste has been reported after exposure to technical fluvalinate vapor from a contaminated oven maintained at 53°C. This symptom disappeared immediately after the oven was cleaned.[9]

The data above indicate that flowable formulations of flavulinate as well as EC formulations and wettable powders of other cyano pyrethroids may produce rather severe skin and respiratory irritation in man. Apparently, the rate of absorption of the active ingredient by the epidermis and mucous membranes depends greatly on the formulation. The physicochemical properties of some of the vehicles used may also greatly affect the toxicity of pyrethroids[162] as well as the minimum concentration of pyrethroids needed to induce skin sensory effects in rodents (see below).

In conclusion, the most frequently reported symptom of human occupational exposure consists of paresthesia of the skin after handling of cyano pyrethroids. We consider it most likely that these burning and itching skin sensations are the result of repetitive firing of sensory nerve endings. The respiratory irritation as induced by flavulinate flowable formulation and by other pyrethroids in EC formulation might well have a similar origin.

The skin sensations and other peripheral sensory phenomena in man should, in our opinion, be considered as a warning signal for overexposure, indicating that adequate preventive measures should be taken. As long as pyrethroids are prevented from entering the circulatory system directly, these local symptoms have proven to be transient and are most unlikely to progress into the more general neurotoxic syndrome in the absence of further exposure.

B. Human and Animal Experiments

1. Human Studies

In a double blind study fenvalerate (81 $\mu g/cm^2$ in ethyl alcohol) applied to a 4 cm^2 area of one earlobe of 36 human volunteers was shown to induce significant paresthesia. The other earlobe was treated with the vehicle only and served as a control.[163] In a subsequent study four formulation grade

pyrethroid insecticides were diluted in distilled water and applied to the earlobe (130 μg/cm^2) of six human volunteers. Permethrin caused the least and flucythrinate the most pronounced paresthesia, whereas the two intermediate compounds fenvalerate and cypermethrin gave almost equal cutaneous sensations.[164] Vitamin E oil, vitamin E acetate, and mineral oil equally reduced the pyrethroid-induced paresthesia.[164,165] Although a solvent/leaching effect may be involved, the mechanism for the relief of paresthesia has not been verified.

In the experimental treatment of scabies the results of single applications of permethrin to the entire human skin have been reported. A 1% (w/w) permethrin solution in liquid paraffin was used by 95 patients in Zimbabwe. The amount applied ranged from 3 ml for babies and children to 7 ml for adults. The scabicidal efficacy of this treatment appeared equal to that of a control treatment of 134 patients with lindane in liquid paraffin at doses of 60 and 140 mg, respectively.[166] More recent studies with 5% permethrin (*trans* : *cis* = 75 : 25) in a thixotropic cream base gave 90 to 100% cure rates in two groups of 23 and 10 patients 1 month following single application.[167,168] In the latter study, which was restricted to adults, the dermal application of 1.0 to 1.5 g permethrin appeared without adverse side effects.[168] In contrast, wettable powders of the same racemic mixture of permethrin are irritative in occupational exposure,[159] which again emphasizes the importance of formulation in this respect.

2. Animal Experiments

Direct application of controlled doses of pyrethroids to the guinea pig skin revealed that extremely low doses of fenvalerate (Pydrin 2.4 EC), to the equivalent of 30 μg of the active ingredient on a 35 cm^2 area of skin, can cause sensory stimulation. Activity was quantified by counting the number of times the animals responded by licking, rubbing, scratching, or biting the test flank when compared with the contralateral control flank. At much higher dose levels permethrin caused significantly less stimulation than flucythrinate, cypermethrin, and fenvalerate. By 5 h after application the animal responses had returned to normal. At the highest doses of pyrethroids the sensory stimulation was reduced.[169] Virtually identical results were obtained in another study of time and concentration dependence of the effects of permethrin, cypermethrin, and deltamethrin on guinea pig skin. Optimum stimulation was obtained after applying 0.1 ml of 1% (w/v) solutions of deltamethrin (*cis*) and cypermethrin (*trans* : *cis* = 45 : 55) in dimethylformamide to a 30 mm^2 area of skin. Threshold effects were observed at 0.01% (i.e., 10 μg of active ingredient). However, up to 10% solutions of permethrin (*trans* : *cis* = 54 : 36) were ineffective. Effects were maximal approximately 1 h after application and disappeared within 24 h. This study also showed that dermal application of veratrine, a mixture of alkaloids containing toxins that prolong the open time of voltage-dependent sodium channels very similar to the cyano pyrethroids, causes the same qualitative behavioral response as cypermethrin and deltamethrin, but of a lesser intensity.[170]

From a behavioral study it has been suggested that deltamethrin produces taste aversion in rats, but this effect was confounded by procedural variables and has not been substantiated statistically.[150] Fenfluthrin, applied to the rat taste receptor in concentrations up to 10 μ*M*, did not affect the salt response recorded from the chorda tympani. In the same preparation, inhibitors of epithelial sodium transport caused a reduction of the salt response.[171]

Dermal application of fluvalinate and other cyano pyrethroids to rats, rabbits, and guinea pigs under (semi)-occlusive patches, sometimes covered with plastic wraps, even to the abraded skin in amounts sufficiently high to produce toxic signs, has shown that these compounds are mildly to moderately irritating to the skin. However, the EC formulation of fenvalerate appeared severely irritating.[9]

Rats, repeatedly dosed with fluvalinate in ground feed (e.g., 30 mg/kg/d), showed severe skin lesions described as "ulcerative dermatitis".[9] Similar dermal lesions were observed in rodents during long-term dietary studies, not only with fluvalinate, but also with the cyano pyrethroids flucythrinate (120 ppm), fenvalerate and cypermethrin. If dermal exposure was scrupulously avoided, toxic oral doses of fluvalinate did not lead to dermal lesions. Fluvalinate applied dermally to rats at concentrations higher than 0.15% (0.4 mg/kg) in acetone without occlusive patches or wrapping induces frequent, vigorous scratching, sometimes with chewing or biting of the skin at and near the site of application. Animals showed severe ulcerative skin lesions, as a result of self-inflicted wounds and subsequent infection of the skin. The concentration to evoke these effects strongly depends on the vehicle used. Minimum effective concentrations amounted to 0.3% in acetone and 10% in olive oil. Furthermore, when the pyrethroid was injected subcutaneously, neither scratching nor dermal lesions occurred, provided that the compound did not leak to the surface of the skin.[9] From these results it is clear that fluvalinate and other cyano pyrethroids cause intense itching of the skin in rodents, probably by stimulation of nerve endings in the epidermis. It has also been reported that dogs lick their lips following consumption of a diet containing 270 ppm alphamethrin, a concentration sufficiently high to produce overt toxic signs. This effect was not observed at lower concentrations.[10]

Rats exposed to a nominal aerosol concentration of 0.59 mg/l air of fluvalinate showed signs of respiratory (and eye) irritation. Coughing and sneezing occurred within 5 min and disappeared within 1 hr after exposure.[9] Recently a study has been performed in which rats were exposed for 4 weeks (5

d/week; 6 h/d) to aerosols of clyfluthrin. Upon exposure to nominal concentrations of 3 and 30 mg/m^3 air of cyfluthrin (analytical concentrations: 0.4 and 6.0 mg/m^3 air, respectively) the animals showed a transient reflex bradypnoe, which remained constant throughout the testing period. This result suggests that the sensitivity of the sensory structures involved remains unaffected, i.e., that adaptation does not occur.[6]

The data available on peripheral sensory phenomena are inconclusive with respect to the important question whether repeated occurrence of skin and respiratory irritation caused by pyrethroids may eventually result in adaptation or irreversible changes of the sensory system. The literature indicates that adequate animal experiments can be designed to investigate adverse skin and respiratory effects of pyrethroids and their formulations. In particular the guinea pig flank model[169,170] seems suitable to study possible long-term effects of pyrethroids on the sensory system *in vivo*.

IV. AXONAL DAMAGE

A. Neurophysiological Findings

Electrophysiological studies of acute and subchronic effects of pyrethroids in the rat revealed that the excitability of the tail nerve was enhanced following supramaximal electrical stimulation. The period of enhanced excitability following the nerve impulse was much longer after deltamethrin than after cismethrin. Deltamethrin at 50 to 200 ppm in the diet for 1 week produced excitability changes similar to those produced by a single intravenous injection of 0.5 to 1.5 mg/kg. The chronic effects tended to decrease over a period of 8 weeks. Cumulative effects of feeding deltamethrin were not observed and nerve responses were back within normal limits 24 to 48 h after return to a normal diet.[172,173] Rats treated orally with tefluthrin (5 mg/kg/d, 5 d/week, for 8 weeks) showed no functional signs of peripheral neuropathy. Motor and sensory nerve conduction velocities were identical to control values.[174]

Neurological examination of 23 workers exposed during manufacturing and testing various synthetic pyrethroids revealed no abnormal neurological signs, and the electrophysiological function of arm and leg nerves was within the normal range. Nineteen persons in this group had experienced at least one episode of skin sensations and 6 persons at least five of these episodes.[175] Medical monitoring of virtually unprotected spray operators in the Ivory Coast over a 17-month period, including a 3-month spraying season, revealed a decrease of peripheral sensory and motor conduction velocity and a decrease in cornea reflex latency time, one of the trigeminal nerve function parameters measured. These changes could also be attributed to a seasonal variation, and it was concluded that there was no evidence that the observed changes were related to pyrethroid exposure, which was monitored by the urinary excretion of the major metabolite.[176]

Electrophysiological studies have shown that even near-lethal doses of cypermethrin do not affect maximal motor conduction velocities of the sciatic and tail nerve of rats.[10]

B. Morphological and Biochemical Findings

Orally applied deltamethrin (10 mg/kg/d for 15 d) causes histopathological changes in some nerve fibers of the sciatic nerve of the rat.[177] Sporadic lesions resembling Wallerian degeneration have been observed in sciatic and posterior tibial nerves of rats treated with near-lethal dietary doses of cypermethrin, *cis*-cypermethrin, fenpropathrin, and permethrin. Permethrin was the least effective in producing nerve injury, whereas after *cis*-cypermethrin about 10% of the fibers were affected in some animals. Single ($0.5-2$ times LD_{50}) dose experiments with cypermethrin, fenvalerate, and permethrin also revealed nerve lesions in a small fraction of the animals. The lesions were not produced by doses below the lethal range.[178] In rats fed 1000 ppm cypermethrin for 1 or 2 years,[178] as well as in rats fed 100 ppm and mice fed 1250 ppm of fenvalerate for up to 2 years,[179,180] no compound-related neuropathological effects were found. In a 6-month study, in which up to 1000 ppm of fenvalerate was fed to dogs, slight to moderate axonal dystrophy in the spinal cord and in peripheral nerves was observed sporadically, scattered through most groups, including control animals. No treatment-related pattern could be ascertained, and these effects were judged unrelated to the test material.[181] Slight peripheral nerve damage was detected in rats and mice 10 d after a single oral dose of $\geq$180 and $\geq$56 mg/kg fenvalerate, respectively. The incidence and severity of the nerve lesions were dose related. However, even at lethal doses (up to 1000 mg/kg in rats and 240 mg/kg in mice) nerve lesions could not be detected in a significant number of animals.[182]

Because of the difficulties in detecting possible nerve damage produced by pyrethroids histopathologically, biochemical methods for the detection of this type of neurotoxicity have also been applied.[183] The activity of the lysosomal enzymes β-glucoronidase and β-galactosidase was used as a measure of peripheral nerve damage. Significant increases in both enzyme activities occurred 2 to 3 weeks following a 7-d treatment of rats with daily sublethal doses (approximately 1/4 of the acute LD_{50}) of permethrin, cypermethrin, and deltamethrin, but not of resmethrin. These pyrethroid-induced enzyme changes were qualitatively similar to those observed in Wallerian degeneration, but were an order of magnitude less than those induced by acrylamide and methylmercury, which were used as reference compounds for their known delayed neurotoxic effects. In addition, it was concluded that no direct correlation exists between the time course of the neuromuscular dysfunction caused by the pyrethroids and the

neurobiochemical changes. Based on the results of this biochemical study it was suggested that the pyrethroids have at least two distinct actions—a short-term pharmacological effect and, at near-lethal doses, a more chronic neurotoxic effect that results in sparse axonal nerve damage.[183] By analogy two distinct types of neurological effects of pyrethroids were postulated:[182] an acute, reversible muscular weakness due to the pharmacological effect and a more chronic neuropathological effect at high doses manifested by sparse axonal damage, which appeared not to be associated with functional or clinical signs.

Industrial research data clearly show that pyrethroids have the potential of producing axonal damage in peripheral nerves of rodents. The compounds tested include alphamethrin,[10] cyfluthrin,[6] cypermethrin,[8,10] deltamethrin,[7] fluvalinate,[9] and permethrin.[8,10] Nerve lesions have been demonstrated histopathologically in rats, mice, and hamsters receiving high, lethal, or near-lethal oral doses of pyrethroids. The lesions have been observed mainly in peripheral nerves, notably in the sciatic nerve and occasionally in the spinal cord and the brain. In all cases these nerve lesions were found in a fraction of the treated animals only. After prolonged administration of lower doses, that caused overt toxic signs, peripheral nerve axonal damage has not been detected. In rats given 40 to 60 mg/kg/d cyfluthrin for 5 months, 1000 ppm cypermethrin for 1 year, 50 mg/kg/d fluvalinate for 3 months, 2500 ppm permethrin for 2 years, or 10 mg/kg/d deltamethrin for 3 months, no significant differences were found in the incidence of abnormal nerve fibers in the control and the test groups.

The time course of development of and recovery from nerve damage, as measured by lysosomal enzyme activities, were investigated in rats given high doses of alphamethrin or cypermethrin over a period of 4 weeks (total dose 562.5 and 2550 mg/kg, respectively). Maximum enzyme activities in the sciatic and posterior tibial nerves were found after 5 weeks for both compounds and the activities had returned to control values by 12 weeks. In another study with rats oral doses of 40 and 80 mg/kg cyfluthrin for 14 d caused neuropathological changes in sciatic nerve fibers. The incidence decreased as the observation period progressed, and effects were no longer apparent at the end of a 3-month recovery period.

Dogs and hens appeared to be less sensitive to pyrethroid-induced axonopathy than rodents. In dogs fed with a diet containing 600 ppm cyfluthrin for 6 months, histopathological examinations of the central and peripheral nervous system did not reveal any deviations from the physiological norm. In another study with dogs fed 1500 and 600 ppm cypermethrin for 3 and 24 months, respectively, no compound-related lesions of the sciatic nerve were found. No microscopic changes were observed in central or peripheral nerve tissues in a 90-d dog study with doses of cypermethrin up to 1500 ppm.

The hen, the standard test animal for the delayed neurotoxic effects produced by organophosphates, appears to be very insensitive to pyrethroids. Oral doses of 5000 mg/kg cyfluthrin, 1000 mg/kg/d for 5 d, or 10,000 mg/kg cypermethrin or 9000 mg/kg permethrin failed to cause clinical signs of poisoning in hens, nor were there any neuropathological changes.

V. ACUTE POISONING AND POSSIBLE ANTIDOTES

A. Animal Experiments

Pyrethroids can be divided into two classes according to signs of poisoning in the rat.[5] With few exceptions, this subdivision corresponds to the presence or absence of an α-cyano group on the 3-phenoxybenzyl alcohol moiety.

Noncyano pyrethroids initially cause aggressive sparring behavior and increased sensitivity to external stimuli. This is followed by a fine tremor, gradually becoming more severe until the animal finally becomes prostrate with coarse whole-body tremor. This sequence has been designated T-syndrome.

A quite different sequence, the CS-syndrome, is observed with most of the cyano pyrethroids. After initial pawing and burrowing behavior the rat shows profuse salivation, coarse whole-body tremor, increased startle response, and abnormal locomotion involving the hind limbs. The coarse tremor progresses into a sinuous writhing of the whole body (choreoathetosis) that gradually becomes more violent. Death is sometimes preceded by clonic seizures. Some cyano pyrethroids (e.g., (1*R*), *trans*-cyphenothrin, its difluorovinyl analog and fenpropathrin) as well as the α-ethynyl analog of fenvalerate cause an intermediate syndrome, including toxic signs from either T- and CS-syndrome.[5,45] The toxic signs usually start to develop within a few minutes after intravenous injection, and surviving animals recover after several hours.[5] For a number of pyrethroids, details on dose and time dependence of the development of toxic signs and the relative contributions of the various neurotoxicological effects to the poisoning syndrome in rodents have been published.[44,46,114,143,147,149,170,173] The reader is also referred to various sections of this review. In general, poisoning symptoms correlate with the degree of sodium current prolongation in the following order: T-syndrome < intermediate syndrome < CS-syndrome (see Table 2).[39,40,46]

Several types of compounds have been reported to affect the experimentally induced neuroexcitatory effects of pyrethroid insecticides in laboratory animals.

In the frog, pretreated with diazepam and with the benzodiazepine Ro-3636 increased the intracerebroventricular LD_{50} values of cyano pyrethroids, 23- to 50-fold, more than those of the noncyano pyrethroids. Nine other benzodiazepines and pyrazolpyridines were ineffective, while phenobarbital was moderately active with a protection factor of 2 to 5. Diazepam

was found most effective in the frog when applied at the time of appearance of the first toxic signs.[78]

In the rat the benzodiazepines diazepam and clonazepam, as well as the anticonvulsant sodium valproate, were reported to be ineffective in preventing pyrethroid-induced motor symptoms. The doses of these anticonvulsants were sufficiently large to prevent pentylenetetrazol-induced seizures.[184] Phenytoin and baclofen were also found to be ineffective in reducing motor symptoms in the rat induced by intravenous cismethrin and deltamethrin.[120] Tremor and hyperresponsiveness produced by oral permethrin in the rat were reduced by pretreatment with phenytoin.[154] However, phenytoin failed to affect the lowering of the threshold of pentylenetetrazol-induced seizures produced by intraperitoneal deltamethrin and permethrin.[185]

In the mouse intraperitoneal application of diazepam significantly reduced the percentage of animals that showed a loss of righting reflex after a subsequent intravenous ED_{50} dose of permethrin. Agonists and antagonists of norepinephrine, dopamine, acetylcholine, and serotonin receptors were found either to be ineffective or to potentiate the effect of a much smaller dose of permethrin.[186] Pretreatment of mice with diazepam intraperitoneally has been reported to cause a delay in the onset of action of deltamethrin and fenvalerate, but not of allethrin and permethrin. The LD_{50} values of deltamethrin and *cis*-permethrin were both increased after diazepam by a factor of 6 and 9, respectively. Phenobarbital was less effective and also less specific with respect to noncyano and cyano pyrethroids in these experiments.[187]

In the cat diazepam reduced the normal firing rate of spinal interneurones by approximately 50%, but failed to antagonize both the large *cis*-permethrin- and the smaller deltamethrin-induced increased in the interneuronal firing rate.[147] Studies on the therapeutic effect of diazepam, clometiazole, and atropine in dogs showed that the combination of these drugs was effective in alleviating central and motor symptoms associated with poisoning by intravenous deltamethrin up to LD_{100} doses. The LD_{50} value of deltamethrin, however, was only marginally increased by the combination of drugs.[7,188]

The results show that the effects of anticonvulsants in experimental animal poisoning vary considerably with animal species. Only in some cases have successful prophylaxis been obtained. Therefore, anticonvulsants should not be regarded as specific antidotes for pyrethroid poisoning.[189]

More consistent results have been obtained with the central muscle relaxant mephenesin and two of its analogs. In intact conscious rats poisoned by up to twice LD_{50} intravenous doses of cismethrin and delta-methrin repeated intravenous injections or continuous infusion of mephenesin act prophylactic as well as therapeutic.[120,190] In the rat motor symptoms and fatalities produced by the pyrethroids were prevented by mephenesin treatment. Similar results were obtained with methocarbamol, a longer acting and less toxic mephenesin analog. Repeated intraperitoneal administration of this drug at the time of tremor or hyperexcitability reversed and prevented further toxic signs produced by lethal oral doses ($>LD_{50}$) of fenvalerate, cypermethrin, fenpropathrin, and permethrin. Mortality was reduced from approximately 70 to 0% except for permethrin-poisoned rats in which motor symptoms were incompletely suppressed by the amount of methocarbamol used (initial dose 400 mg/kg i.p. and repeated doses 200 mg/kg i.p.) and in which 10% mortality remained after methocarbamol. Recovery of the animals over a period of 17 to 30 h required one to seven treatments with methocarbamol.[191] These studies indicate that poisoning with the noncyano pyrethroids, higher doses of the therapeutic drug are required, possibly because of the more intense spinal excitation associated with the T-syndrome produced by noncyano pyrethroids.[147] Therapeutic effects of mephenesin-carbamate in intravenous deltamethrin poisoning in the rat were very similar to those of methocarbamol. The LD_{50} of deltamethrin was three times higher in rats symptomatically treated with repeated intravenous doses of 100 mg/kg mephenesin-carbamate as compared to control animals.[192]

The combinations urethane/atropine and phenprobamate/atropine suppressed deltamethrin poisoning symptoms and increased the LD_{50} by a factor of 3 to 4. Hind leg paralysis, which was often observed after 12 h in deltamethrin poisoned rats treated with urethane, recovered to normal after 24 to 36 h.[188,192] On the other hand, meprobamate, a *bis*-carbamate ester with sedative and hypnotic action, was reported to be ineffective in modifying motor symptoms associated with pyrethroid poisoning in the rat.[120]

Atropine appeared to be effective in treating cholinergic site effects of pyrethroid poisoning in the rat, i.e., salivation, bradycardia, and, to a limited extent, chewing, pawing, and choreoathetosis.[117] Atropine has been used for the same purpose in most other studies quoted in this section with equally good results, and it was judged indispensable for the treatment of salivation and bronchial hypersecretion in animals poisoned by deltamethrin.[188]

B. Human Experience

Little information is available on the efficacy of the various experimental therapies in the treatment of human accidental pyrethroid poisoning.

Until 1988 a single fatal case of human poisoning had been reported in the literature. A male person 45 years of age, who had accidentally ingested more than 0.7 g cypermethrin 10% (Cymbush), rapidly developed convulsions, passed into a coma, and died 3 h later. The death of this patient was ascribed to respiratory paralysis. Details on the emergency treatment have not been reported.[193]

Recently a review has appeared on 573 cases of acute pyrethroid poisoning, including 229 occupational and 344 accidental cases, reported in the Chinese literature during the period 1983 to 1988.[194] Occupational poisoning was due to inappropriate handling, while accidental poisoning was mostly due to inges-

tion. Cases involved mainly deltamethrin (325), fenvalerate (196), and cypermethrin (45). The initial symptoms with occupational intoxication were burning, itching, or tingling sensation of the face or dizziness that usually developed 4 to 6 h after exposure. The skin symptoms could appear early after several minutes of spraying and disappeared after several hours to 1 d. In more serious cases they were followed by systemic symptoms as late as 48 h after exposure. After ingestion the initial symptoms were mainly digestive, such as epigastric pain, nausea, and vomiting, and developed within 10 min to 1 h. Skin symptoms were not significant in patients with ingestive poisoning.

The systemic symptoms include dizziness, headache, nausea, anorexia, and fatigue. In more serious cases coarse muscular fasciculations, associated with repetitive discharges in the EMG, developed in large muscles of the extremities. A number of cases (51) showed disturbance of consciousness, and some patients who ingested large doses (200 to 500 ml) of pyrethroid EC developed coma within 15 to 20 min. Thirty-five cases had convulsive attacks, but only five of them were due to occupational exposure. The convulsions lasted for 30 s to 2 min and manifested as flexion of the upper limbs and extension of lower limbs with opisthotonos and unconsciousness during attacks. Seizures occurred at a frequency of up to 10 to 30 per d in the first week of intoxication and subsequently decreased gradually to recover completely within 2 to 3 weeks.

All patients were treated with symptomatic and supportive therapies. In 189 cases treatment included atropine, which reduced salivation and pulmonary edema in a few severe cases. Deltamethrin-induced seizures were not well controlled by diazepam and baclofen in one severe case of accidental poisoning. In another case of occupational deltamethrin poisoning phenobarbital, chlorpromazine, and phenytoin were ineffective.[195] Both patients recovered in a period of 3 weeks and were in good health at the follow up after 1 year.

The vast majority of patients recovered within 1 to 6 d, although the hospitalization period of some seriously affected patients with convulsions was longer, the longest being 55 d. In the follow up of 15 cases no longstanding or residual symptoms were found.

The total number of deaths was seven, including one patient misdiagnosed as having acute organophosphate poisoning who died of atropine intoxication, one acutely intoxicated with fenvalerate/dimethoate, two cases of occupational acute deltamethrin poisoning, and three of ingestive acute fenvalerate poisoning. According to the author the prognosis of occupational acute pyrethroid poisoning is generally good and is always better than acute organophosphate poisoning, even in seriously affected patients.[194]

VI. SUMMARY

Although pyrethroid insecticides have been introduced on a large scale fairly recently, extensive data have been published on their mode of action, and valuable information is available on potential side effects in man.

The basic mechanism of action of the pyrethroids on the vertebrate nervous system has been investigated in detail. All available evidence clearly indicates that the primary neurotoxic target site of this chemically diverse class of insecticides is confined to the voltage-dependent sodium channels in excitable membranes. The stereoselective interaction of pyrethroids with a fraction of the sodium channels results in a prolongation of the inward sodium current during excitation, as pyrethroid-modified sodium channels stay open much longer than normal. The prolonged sodium current induced by the pyrethroids results in pronounced repetitive activity, notably in sense organs, but — depending on pyrethroid structure — also in sensory nerve fibers, motor nerve terminals, and skeletal muscle fibers. Besides repetitive firing, membrane depolarization resulting in enhanced neurotransmitter release and eventually block of excitation may also occur.

Studies on sense organs in the vertebrate skin have shown that the cyano pyrethroids evoke more intense repetitive activity than the noncyano pyrethroids. This is accounted for by large, quantitative differences in the prolongation of the sodium current by cyano and noncyano pyrethroids. For a range of pyrethroids the symptoms observed in experimental animal poisoning correlate well with the extent to which the sodium current is prolonged.

Postsynaptic neurotransmitter responses are unaffected by concentrations of pyrethroids that cause marked sodium channel modification. At high concentrations insecticidal as well as noninsecticidal pyrethroid isomers cause a nonspecific suppressive effect on the postsynaptic neurotransmitter response.

Cardiovascular effects of pyrethroids can be attributed to modification of presynaptic as well as postsynaptic sodium channels.

Paresthesia and other peripheral sensory phenomena, e.g., respiratory irritation, are repeatedly experienced in man after occupational exposure to cyano pyrethroids in particular. These symptoms, which are most likely caused by repetitive firing of sensory nerve endings, should be considered a warning of overexposure, indicating that adequate preventive measures should be taken. The quality as well as the intensity of the peripheral sensory phenomena depends not only on pyrethroid structure, but also varies with the formulation and with environmental factors.

The question whether repeated occurrence of peripheral repetitive firing may eventually lead to injury of sensory nerve endings or central sensory adaptation remains unanswered. The guinea pig flank provides an adequate model to quantify the cutaneous sensations of pyrethroids. This model may be particularly useful to compare the degree of skin sensory irritation caused by different formulations of pyrethroids and could also be of value to investigate possible chronic effects.

Lethal and near-lethal doses of pyrethroids cause sparse ax-

onal damage in a fraction of the exposed animals, which is reversed after cessation of exposure. Threshold concentrations from acute and chronic studies are available. After prolonged chronic exposure to lower doses of pyrethroids axonal damage has not been observed. It has been suggested that the histopathological changes are unrelated to the basic neuroexcitatory action of pyrethroids. The hen sciatic nerve is not suitable for studying pyrethroid-induced nerve damage, as — in contrast to organophosphates — birds are highly insensitive to pyrethroids.

The limited information available on neurobehavioral effects of pyrethroids is difficult to evaluate, as the significance of such data for toxicological risk assessment is still under debate.

Established anticonvulsants are only moderately effective in the treatment of acute pyrethroid poisoning in animals and man. The results of animal experiments indicate that mephenesin and related compounds, combined with atropine to suppress cholinergic side effects, are the more promising antidotes presently known.

Although severe acute human poisoning with pyrethroids in the Western Hemisphere seems very unlikely, recent experience in the People's Republic of China shows that these insecticides should certainly not be considered harmless. However, with adequate therapeutic treatment, the prognosis of acute pyrethroid poisoning is generally good.

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