

General Pharmacology

Advocate is a combination of moxidectin and imidacloprid in a solution containing 2.5% moxidectin and 10% imidacloprid for dogs, and 1.0% moxidectin and 10% imidacloprid for cats. It is designed for dermal application for the prevention and/or control of a range of internal and external parasites in dogs and cats. To highlight the benefits of this unique combination of active ingredients, a summary of the pharmacological properties of each active is provided in the following sections.

Imidacloprid was first commercialized in its active form by Bayer in Japan in 1986, it was developed for control of a variety of insects for both agricultural and veterinary purposes.

A novel 10% spot-on formulation of imidacloprid, Advantage®, has been available for the control of fleas on dogs and cats since 1996. Due to its rapid and highly effective activity against fleas and favorable safety profile, imidacloprid has proven to be a remarkable advancement in flea control for pets.

Imidacloprid

History

Imidacloprid was the first commercialized member of a new class of insecticides called chloronicotinyl nitroguanidines or neonicotinoids. First synthe-



Chemical Properties of Imidacloprid

Imidacloprid belongs to the neonicotinoid class of insecticides, a relatively new group of the heterocyclic nitromethylenes. The chemical structure and physicochemical properties are listed in figure 1 and table 1.

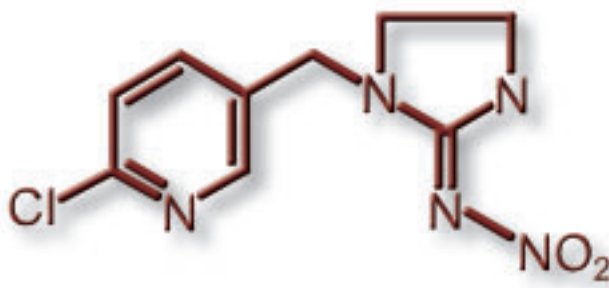


Figure 1: Imidacloprid - Chemical Structure

Mode of Action

The mechanism of action of neonicotinoids such as imidacloprid is different than that of all other classes of compounds. Imidacloprid acts as an agonist at the post-synaptic acetylcholine receptors of motor neurons in insects, such as fleas. Acetylcholine (ACh) is the most important excitatory neurotransmitter of the central nervous system of insects (Figure 2). The two different types of ACh receptors that have been identified are nicotinic (nAChR) and muscarinic (mAChR).

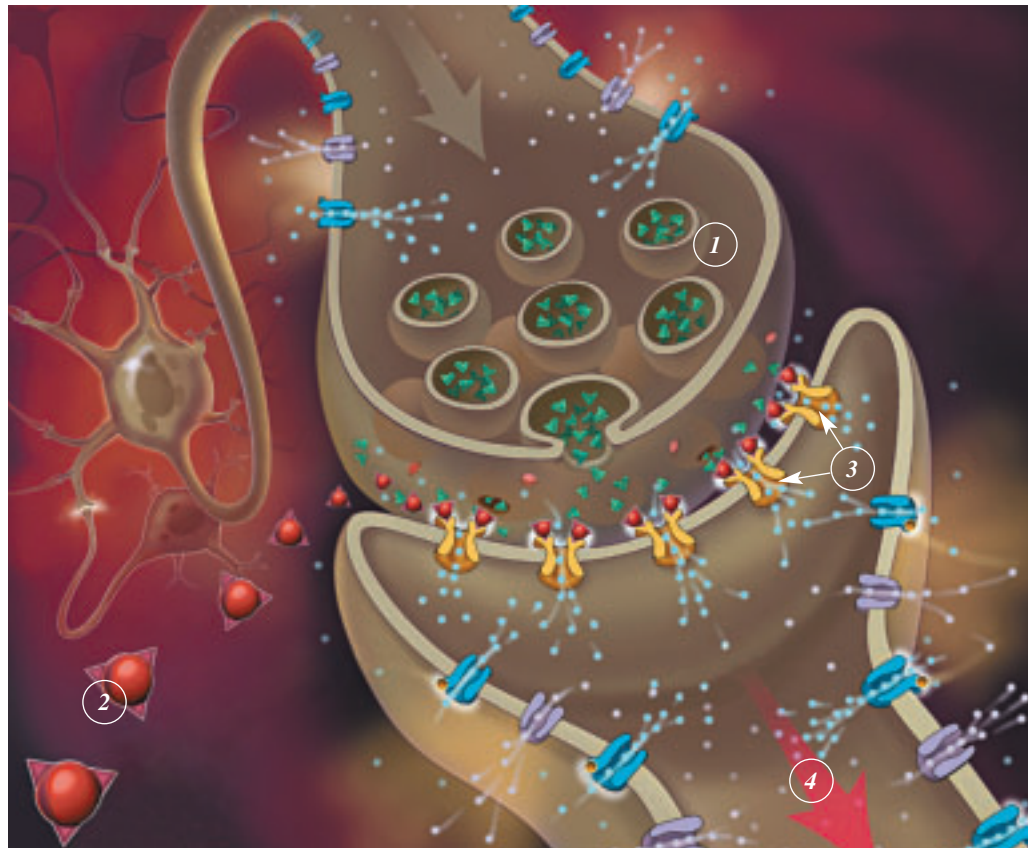
In insects, nicotinic AChRs are present in much greater concentration than muscarinic receptors – nervous tissue of insects is the richest source of nAChR in the animal kingdom. This high density of nicotinic AChRs is important as they are the molecular target of action of imida-

cloprid. By binding to these receptors, imidacloprid acts to interfere with the transmission of nerve impulses in fleas and other affected insects. The binding of the nicotinic receptors leads to opening of sodium ion channels and induces slow depolarization of the motor neurons, followed by tetanic contractions, neuromuscular destruction and death.

The activity of imidacloprid against fleas is highly rapid and potent. The lethal effects are mediated primarily by contact with and penetration of the compound through the flea intersegmental membranes, in contrast to other agents which must be ingested by the parasite to have effect.

Table 1: Physicochemical Properties of Imidacloprid

Empirical formula	C ₉ H ₁₀ ClN ₅ O ₂
Molecular weight	255.7
Physical appearance	cream coloured, crystalline powder
Melting point	144°C
Solubility at 20°C (g/1000 ml)	water: 0.61
	n-hexane: <0.1
	2-propanol: 2.3
Vapour pressure	4 x 10 ⁻¹² hPa at 20°C



1 Vesicles of acetylcholine
 2 Imidacloprid
 3 Nicotinic receptors blocked open
 4 Constant neuromuscular stimulation

Figure 2: Site of action of Imidacloprid on insect nerves

The mechanism of action of neonicotinoids, e.g. imidacloprid, is completely different from all other classes of compounds in its effect on the insect nervous system¹. This system, with its many individual nerve cells or fibres, branches out to form a complex network with numerous contacts for intensive impulse transmission. Between the individual nerve cells, which are separated from one another by the so-called synaptic cleft, a chemical substance, known as acetylcholine, one of the more prevalent neurotransmitters, transmits the impulse.

Certain types of neuronal receptors/ion channels have been demonstrated to be the major target sites of insecticides. Imidacloprid acts as an agonist on the

postsynaptic nicotinic acetylcholine receptors of motor neurons in insects. It induces slow depolarisation in cell bodies of motor neurones and is a more potent agonist than nicotine itself. This mode of action leads to tetanic muscle contractions in the insect as well as destruction of the ganglia of the head and thorax and ultimately causes the death of the insect.

Two different classes of acetylcholine receptors (AChRs) have been identified throughout the animal phyla (including insects): nicotinic (nAChR) and muscarinic (mAChR). The insect nervous system has three populations of acetylcholine receptors – those that display entirely nicotinic or muscarinic binding affinity and those which have a

mixed nicotinic/muscarinic pharmacology. Nicotinic AChRs consist of the acetylcholine agonist site and an ion-channel complex. Insect nervous tissue appears to be the richest source in the animal kingdom of neuronal nAChRs and within the insect population these vastly outnumber muscarinic receptors. Whilst the structure of nAChRs in insects and vertebrates is similar, they differ in specific detail. This is the reason for the highly selective action of imidacloprid on invertebrate receptors, while extensive toxicological and safety studies have demonstrated virtually no effect on vertebrates.

Spectrum of Activity

Based on its favorable profile in mammals, imidacloprid was developed for veterinary use and is widely used for flea control in companion animals. The target ectoparasites include various species of fleas (*Ctenocephalides spp.*), as well as biting (*Trichodectes canis*) and sucking (*Linognathus setosus*) lice². Imidacloprid is registered for use in dogs and

cats throughout the world, as well as for rabbits in some countries.

- Fleas
- Flea larvae
- Biting and sucking lice*

In addition to rapid and potent adulticidal activity against fleas present on dogs and cats, imidacloprid has been shown to have significant flea larvicidal activity, both in laboratory studies as well as in simulated home environments^{3, 4}. This is important as immature stages present in the pets' surroundings are a reservoir of reinfestation. Flea larvae in the pet's surroundings are killed after contact with a pet treated with imidacloprid. Exposure of larvae to these minute quantities of imidacloprid in the environment of treated pets results in marked reduction of developing flea populations in comparison to environments of untreated animals. In this way, it is probable that the larvicidal effects of imidacloprid are of practical significance in breaking the flea life cycle and in reducing the level of flea infestation in the domestic environment⁵.

* Note: registration for lice in Australia and New Zealand only

Table 2: Toxicological data for Imidacloprid

	Toxicological test	Dosage
Rat (unfed), oral	LD ₅₀	424 - 475 mg/kg
Mouse, oral	LD ₅₀	131 - 168 mg/kg
Rat, dermal	LD ₅₀	> 5000 mg/kg
Rat, inhalation, aerosol	LC ₅₀ , 4 hours	> 69 mg/m ³
Rat, inhalation, dust	LC ₅₀ , 4 hours	> 5323 mg/m ³
Rat (male/female), subacute	LC ₅₀	> 2400 ppm

LD₅₀ = dosage resulting in 50% mortality in treatment group. LC₅₀ = concentration producing 50% mortality in the treatment group.

Toxicology

Imidacloprid has highly selective binding affinity for insect nicotinic acetylcholine receptors, as well as the fact that insects have much higher numbers of these receptors than vertebrate species. Therefore, the compound is very well tolerated by mammals. Results of acute toxicological studies in laboratory animals (Table 2) support the low mammalian toxicity, especially when one considers the dermal route of application used in companion animals. Additionally, imidacloprid has been demonstrated to be non-carcinogenic, non-mutagenic and non-teratogenic in numerous laboratory evaluations.

References

1. Matsuda K, Buckingham SD, Kleier D, et al. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences*, 22(11):573-580, 2001.
2. Hanson I, Mencke N, Asskildt H, Ewald-Hamm D, Dorn H. Field study on the insecticidal efficacy of Advantage against natural infestations of dogs with lice. *Parasitol Res* 85:347-348, 1999.
3. Hopkins TJ, Woodley I, Gyr P. Imidacloprid topical formulation: larvicidal effect against *Ctenocephalides felis* in the surroundings of treated dogs. *Aust Vet Pract* 26:210, 1996.
4. Jacobs DE, Hutchison MJ, Stanneck D, et al. Accumulation and persistence of flea larvicidal activity in the immediate environment of cats treated with imidacloprid. *Med Vet Entomol* 15:342-345, 2001.
5. Jacobs DE, Hutchison MJ, Ewald-Hamm D. Inhibition of immature *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) development in the immediate environment of cats treated with imidacloprid. *J Med Entomol* 37:228-230.

Further Reading

- Griffin L, Krieger K, Liege P. Imidacloprid: a new compound for control of fleas and flea-initiated dermatitis. *Suppl Compend Contin Educ Pract Vet*.19(5):17-20, 1997.
- Krämer F, Mencke N. Imidacloprid. In, *Flea Biology and Control*. Springer-Verlag, Berlin, Germany, 2001, p. 63-94.
- Mehlhorn H. Mode of Action of imidacloprid and comparison with other insecticides (i.e. fipronil and selamectin) during in vivo and in vitro experiments. *Suppl Compend Contin Educ Pract Vet*. 22(4A):4-8, 2000.

Moxidectin

History

Moxidectin is a potent broad-spectrum endectocide of the macrocyclic lactone (macrolide) antimicrobial class. The macrocyclic lactones consist of two closely related chemical groups – avermectins and milbemycins. These compounds share in common a large complex macrocyclic backbone and are produced through fermentation of soil dwelling fungal organisms of the genus *Streptomyces*. The first avermectin was produced from the *Streptomyces avermitilis* organism originally isolated from a soil sample in Japan in 1976. Since that time, a number of lactones have been identified and developed with unique activity against endo- and ectoparasites – hence "endectocide" – in both humans and animals.



Figure 3: *Sarcoptes* spp.

The availability of these broadly effective and well-tolerated compounds has had a tremendous impact on internal and external parasite control in companion animals and livestock.

Moxidectin is a semisynthetic derivative of nemadectin, a fermentation product of the *Streptomyces cyaneogriseus noncyanogenus* organism. This organism was first isolated from a sample of red

sand from Australia in 1983. Moxidectin is available throughout the world in different oral, injectable and topical formulations for use in dogs, cats, horses and livestock animals.

Chemical Properties of Moxidectin

Moxidectin is a 16-member pentacyclic lactone of the milbemycin class. The milbemycins differ structurally from the avermectins, such as ivermectin, primarily in the absence of a disaccharide chain at C-13. Moxidectin has a unique structure characterized by a methoxime moiety at C-23 and an olefinic side chain at C-25 (Figure 4, Table 3).

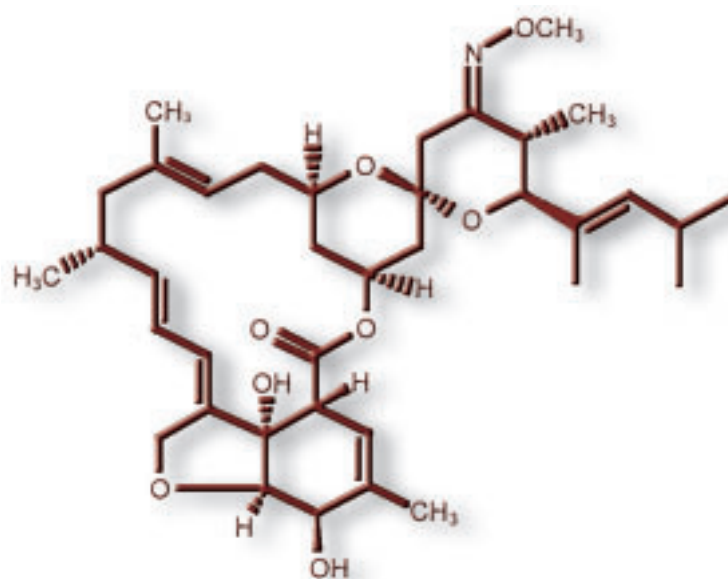


Figure 4: Moxidectin-Chemical Structure

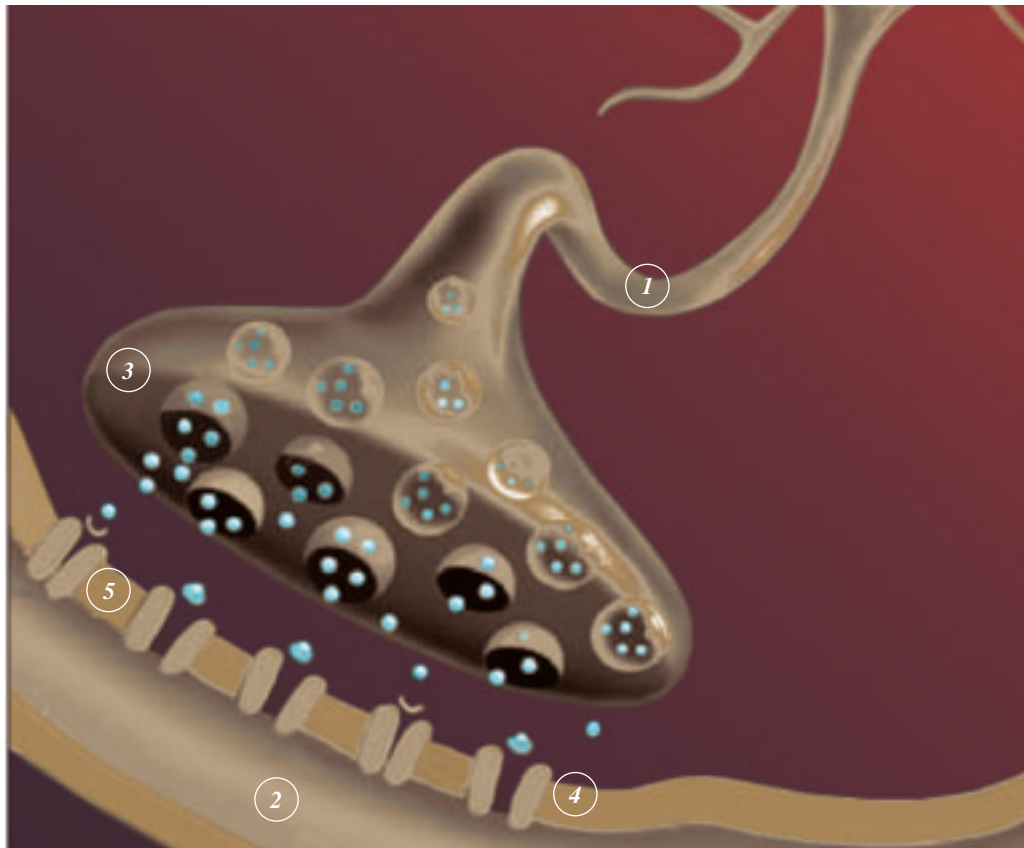
Table 3: Physiochemical Properties of Moxidectin

Molecular Formula	$C_{37}H_{53}NO_8$
Molecular Weight	639.84
Physical Appearance	White to pale yellow powder
Melting Point	145 -154 °C
Solubility (mg/1000ml)	Water: 0.51
	Organic solvents: readily

Mode of Action

Moxidectin, like other macrocyclic lactones, has a high affinity for the glutamate-gated ion channels specific to parasites. The compound binds to receptors on neuronal membranes of nematodes and muscle membranes of arthropods, causing increased permeability and influx of chloride ions. The resulting hyperpolarization of the neuronal cells prevents transmission of normal impulses and results in paralysis and death of the parasite (Figure 5).

Milbemycins such as moxidectin, and avermectins, may also have agonist activity at the gamma-amino butyric acid (GABA) receptor complexes in the peripheral nervous system of invertebrates. GABA acts as an inhibitory neurotransmitter and blocks the post-synaptic stimulation of the adjacent neuron or muscle fiber in nematodes and arthropods, respectively. In mammals, GABA receptors are restricted to sites within the central nervous system and because the blood-brain barrier prevents access to macrocyclic lactones, mammals are generally protected from any neurologic effects.



- 1 Synaptic terminal of presynaptic neuron
- 2 Postsynaptic neuron
- 3 Synaptic vesicles releasing neurotransmitter
- 4 Glutamate-gated chloride channels
- 5 Open chloride ion channel after transmitter or moxidectin exposure at binding site

Figure 5: Moxidectin site of Action

Spectrum of Activity

The broad spectrum of activity of moxidectin includes a biologically diverse range of invertebrate parasites in cats and dogs:

- Nematodes of the gastrointestinal tract
- Developmental stages of filarial nematodes (e.g. *Dirofilaria immitis*)
- Arachnids such as mites.

Historically, moxidectin has been demonstrated to be effective at relatively low dosages for a variety of parasites. It is licensed in cattle for the treatment of gastrointestinal parasites, lungworms, grubs, mites, lice and hornflies, and for the treatment of gastrointestinal parasites in sheep and horses. Moxidectin has been proven effective and used for the prevention of heartworm infection in dogs and cats. Moxidectin is also currently under

development for the treatment of human onchocerciasis (river blindness), a debilitating filarial parasitic disease endemic in parts of Africa, the Arabian peninsula and Central and South America.

Toxicology

The toxicological profile of moxidectin has been extensively evaluated during the development processes for use in various species globally, including ongoing investigations for human use. Numerous laboratory studies indicate a generous range of tolerance in mammals, especially in relation to the therapeutic doses for approved formulations. Tables 4 and 5 list some of the acute and chronic toxicity studies conducted in laboratory animals.

In addition to acute, sub-chronic and chronic exposure studies, reproductive and developmental studies were conducted in rabbits and rodents¹. No developmental effects were observed on fetuses of pregnant rabbits administered as high as 10 mg/kg/day of moxidectin, indicating no evidence of developmental toxicity. In rats, adverse effects on embryo-fetal development occurred only at dosages toxic to the dams, also supporting a lack of specific teratogenic effects from moxidectin. Mutagenicity and carcinogenicity studies indicated no mutagenic effects or oncogenic potential for moxidectin, respectively¹.

References

1. Rock DW, DeLay RL, Gliddon MJ. Chemistry, Pharmacology and Safety: moxidectin. In Vercruyse J, Rew RS (eds.): *Macrocyclic Lactones in Antiparasitic Therapy*. CABI Publishing, Wallingford, UK, 2002, p. 75-96.

Further Reading

- Lynn RC. Drugs for the Treatment of Helminth Infections. In Boothe DM (ed): *Small Animal Clinical Pharmacology and Therapeutics*. WB Saunders Company, Philadelphia, PA, 2002, p. 267-270.
- Shoop WL, Mrozik H, Fisher MH. Structure and activity of avermectins and milbemycins in animal health. *Veterinary Parasitology*, 1995, 59:139-156.
- Blagburn BL. New and emerging therapies in parasitology. *Proceedings of the 20th Annual ACVIM Forum*, 2002, p. 64-66.
- McKellar QA, Benchaoui HA. Avermectins and milbemycins. *J. Vet. Pharmacol. Therap.* 1996, 19:331-351.

Table 4: Moxidectin Acute Toxicology Studies

Species	Route of Administration	Level	
Mouse	Oral	42 - 84 mg/kg	LD ₅₀
	Intraperitoneal	86 mg/kg	LD ₅₀
	Subcutaneous	263 mg/kg	LD ₅₀
Rat	Oral	106 mg/kg	LD ₅₀
	Intraperitoneal	<640 mg/kg	LD ₅₀
	Inhalation (5 hours)	3.28 mg/l	LC ₅₀
Rabbit	Dermal Application	>2000 mg/kg	LD ₅₀

LD₅₀/LC₅₀ = Dosage/Concentration producing 50% mortality in treatment group

Table 5: Moxidectin Chronic Toxicology Studies

Species	Dosing (mg/kg/day)	NOEL (mg/kg)	
Mouse	28-day feeding	0, 6.9, 17.7, 23.2, 24.1, 32.2	6.9
	2-year feeding	0, 2.49, 5.1, 7.8, 11.8	5
Rat	28-day feeding	0, 12.2, 22.8, 26.4, 31.2	<12
	90-day feeding	0, 1.9, 3.9, 7.9, 12.2	4
	2-year feeding	0, 0.8, 3.2, 5.1, 9.8	6
Dog	28-day feeding	0, 0.5, 2, 4	0.5
	91-day feeding	0, 0.3, 0.9, 1.6	0.3
	52-week feeding	0, 0.25, 0.49, 1.12	1.1

NOEL = No Observable Effect Level

Source for tables 4 and 5: Rock DW, DeLay RL, Gliddon MJ. Chemistry, Pharmacology and Safety: Moxidectin. In Vercruyse J, Rew RS (eds.): *Macrocyclic Lactones in Antiparasitic Therapy*. CABI Publishing, Wallingford, UK, 2002, p.75-96.