Annu. Rev. Pharmacol. Toxicol. 1992. 32:537–53

THE CHEMISTRY AND PHARMACOLOGY OF AVERMECTINS

Michael H. Fisher and Helmut Mrozik

Department of Basic Medicinal Chemistry, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

KEY WORDS: anthelmintic, endectocide, miticide, antifilarial, onchocerciasis

INTRODUCTION

The avermectins were discovered in 1975 at Merck & Co., Inc. as a group of eight closely related natural products with extraordinary anthelmintic potency (1–4). They are produced by a culture of *Streptomyces avermitilis* (MA-4680, NRRL8165) that originated from a Japanese soil sample made available by the Kitasato Institute (5). The structure of avermectin B1a, B1b (6), and the semisynthetically obtained ivermectin (7) are shown in Figure 1.

The mixture of avermectin B1a and its lower homolog B1b (Abamectin) is currently in use as agricultural miticide and insecticide (2, 8), and it also serves as starting material for the 22,23-dihydro-analog ivermectin (7). Ivermectin has been used for almost a decade as an endo- and ecto-antiparasiticide for practically all domesticated animal species (2, 9) and in humans for the treatment of certain filarial tropical diseases (2, 10).

The avermectin structures are closely related to the milbemycins, another group of 16-membered macrolide microbial products, discovered earlier by Sankyo scientists as potent miticides and insecticides on plants (11). Subsequently anthelmintic activities were also described for the milbemycins (12), as well as agricultural miticidal and insecticidal properties for the avermectins (13). More recently, several additional cultures have been identi-

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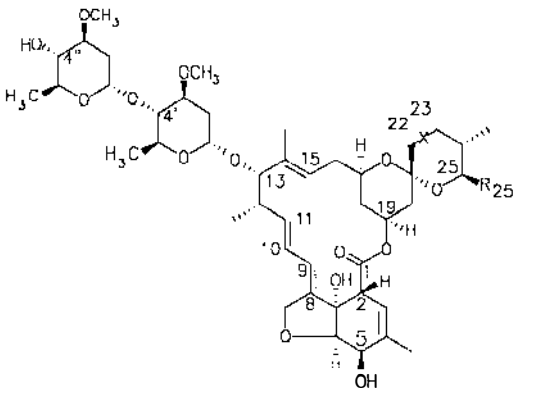


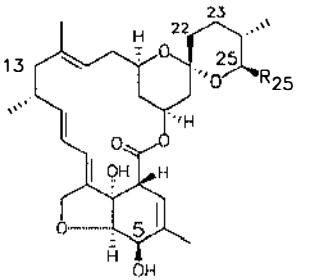
AVERMECTIN B1a : X = −CH=CH− R_{25} = $CH(CH_3)CH_2CH_3$

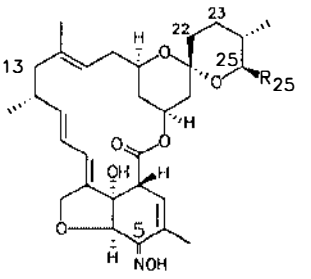
AVERMECTIN B1b : X = −CH=CH− R_{25} = $CH(CH_3)_2$

IVERMECTIN : X = $-CH_2CH_2-$ R_{25} = $CH(CH_3)CH_2CH_3$ > 80 % and $CH(CH_3)_2$ < 20 %

Figure 1 Avermectin Structures

fied that produce milbemycin-like structures (14–18). Milbemycins A3 and A4 and the semisynthetic anthelmintic milbemycin 5-oxime (19) are shown in Figure 2. The major structural difference between the milbemycins and the avermectins is the substitution of the macrolide ring of the avermectins with a disaccharide group at the 13 position. Other variations in the structures of natural avermectins and milbemycins are confined mainly to substituents at





MILBEMYCIN ALFA$_1$ (A_3) R_{25} = CH_3

MILBEMYCIN ALFA$_3$ (A_4) R_{25} = CH_2CH_3

MILBEMYCIN D R_{25} = $CH(CH_3)_2$

MILBEMYCIN OXIME R_{25} = CH_3 and CH_2CH_3

Figure 2 Milbemycin Structures

carbon atoms 4a (hydroxy, acyloxy), 5 (hydroxy, methoxy), 22 and 23 (hydroxy, double bond), and 25 (alkyl, alkenyl).

BIOSYNTHESIS

The addition of labeled precursors to the fermentation of *S. avermitilis* showed that the aglycone is derived from a head-to-tail condensation of seven acetates and five propionates (Figure 3). Also seven of the nine oxygen atoms of avermectin B2a aglycone originate from these acids (20).

The synthesis of the intermediary polyketide is initiated by a 2-methylbutyrate or an isobutyrate unit for the 2-butyl and the 2-propyl alkyl substituent of avermectins B1a and B1b, respectively. This was demonstrated by incorporation of those acids and their precursor aminoacids isoleucine and valine into C-25 and the attached alkyl sidechains (21). Addition of homologous acids to the fermentation broth leads to C-25 avermectin homologs via directed biosynthesis (22). Use of a mutant of *S. avermitilis* lacking the branched-chain 2-oxo acid dehydrogenase responsible for the decarboxylation of the isoleucine-derived 2-oxocarboxylic acid to 2-methylbutyric acid allowed the preparation of a variety of new C-25-substituted avermectin derivatives (23, 24). Similar analogs were also prepared via partial syntheses

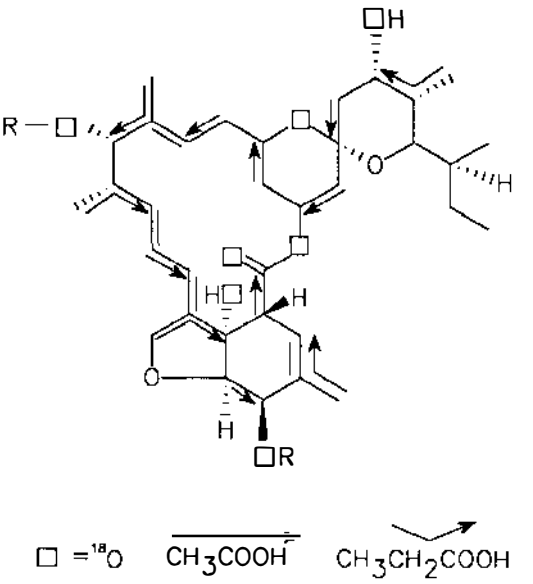


Figure 3 Biosynthesis of Avermectin B2a Aglycone

from an avermectin degradation product (25). The milbemycin biosynthesis proceeds along corresponding pathways (26).

Not surprisingly, a molecule of the complexity of the avermectins has limited stability in acid or basic media and towards oxygen and light. Nevertheless it is effective in topical formulations for animals and on agricultural crops for up to one month.

Numerous chemical modifications of avermectins and milbemycins have been reported (1, 3, 4). Of particular interest are 4''-amino-4''-deoxy avermectin derivatives with improved insecticidal and antiparasitic properties (1–4).

MODE OF ACTION STUDIES

There is an extensive body of data on the effects of avermectins on nerve signal transmission, reduction of input resistance of muscle fibers, opening of chloride channels, and on GABA-like effects. (For earlier reviews, see Refs. 2, 28). Some of the early results carried out on different species, various model systems, and at varying concentrations are as follows:

- Rapid paralysis of nematodes without hypercontraction or flaccid paralysis;
- Rapid irreversible block of inhibitory postsynaptic potential in crustacean nerve preparations at micromolar concentrations, and slower decrease of the amplitude of excitatory potentials;
- Increase in chloride ion permeability;
- Inhibition of the AVM stimulated increase in membrane conductance by GABA antagonists bicuculline and picrotoxin;
- Blockage of signal transmission from ventral interneurons to excitatory motorneurons of *Ascaris;*
- Reversible increase of chloride ion permeability of GABA sensitive fibers of the extensor tibiae muscle of the locust *Schistocerca gregaria* at nonamolar concentrations;
- Irreversible inhibition of GABA sensitive and insensitive muscle fibers of *Schistocerca gregaria* at micromolar concentrations;
- Stimulation of high-affinity binding of GABA and benzodiazepine to rat brain membranes.

Since avermectin is extremely poorly water soluble, and binds tightly to various tissues, proteins, and even glass, one can never be entirely sure of the

actual concentration of the compound at the active site of isolated organs. In animals it is bound to plasma proteins and is distributed in the blood stream throughout the body, with highest concentrations in the liver but comparatively low levels in brain, apparently because of the difficulty in crossing the blood-brain barrier. This limited distribution might be the most important factor in the differentiation of selective toxicity against invertebrates versus mammalian hosts.

Newer quantitative studies with binding affinities to rat brain, insect, and nematode *(Caenorhabditis elegans)* nerve tissue preparations suggest that the effects of avermectins on invertebrates are experienced at nonamolar and even picomolar concentrations. Specific binding sites for avermectins were identified in a crude membrane preparation obtained from the free-living, avermectin-sensitive nematode *C. elegans* with an apparent dissociation constant of 0.26 nM, and in rat brain with a 100-fold lower affinity (K_d 22 nM) (29). GABA, picrotoxin, diazepam, and several other putative neurotransmitters had no effect on this high-affinity binding, which thus suggests a separate AVM binding site (29). Anthelmintic potency and inhibition of [^{3}H]IVM binding to preparations of *C. elegans* receptor show a close correlation for different avermectin analogs (29). The membrane-bound IVM binding site of *C. elegans* was subsequently solubilized without alteration to the IVM-binding characteristics (30). Recently a ^{125}I-photoaffinity label with avermectin structure was synthesized that acted as a competitive inhibitor of ^{3}H-ivermectin binding to membranes of *C. elegans*. Upon photoactivation, three covalently linked proteins were identified from *C. elegans,* and one from head membrane preparations of *D. melanogaster* that contained specific high-affinity avermectin binding sites (31).

In one study, where the patch-clamp technique was used with excised outside-out patches of crayfish stomach muscle containing a chloride channel activated by GABA as well as glutamate and acetylcholine, it was confirmed that, at subpicomolar concentrations, AVM reversibly opens the multi-transmitter-gated chloride channels in this preparation. This model differentiates between a first conductance state activated by glutamate, quisqualic acid, ibotenic acid, and the nicotinic agonist carbachol, and a second, larger conductance state activated mainly by GABA and GABA agonist muscimol. Avermectin was never seen to activate the second conductance level like GABA. This effect is direct and not mediated via a second-messenger pathway. Upon raising the avermectin concentrations to 10 pmol or higher, an enormous irreversible increase of opening of chloride channels occurs. Both the reversible and the irreversible openings of the chloride channels are blocked by picrotoxin (32).

Additional electrophysiological studies were carried out on oocytes of *Xenopus laevis* injected with a 1–2.5-kb size class of poly (A^+) RNA from *C.*

elegans. It was shown that IVM induces an inward membrane current in a concentration-dependent manner and increases the permeability of the oocyte membrane to chloride ion. The IVM-sensitive current was blocked with picrotoxin, but GABA bicuculline, muscimol, or benzodiazepam had no effect. It was concluded that IVM opens directly a GABA-insensitive chloride channel (33).

In summary, there are probably different avermectin receptors, particularly in mammalian and invertebrate species. Avermectin binds specifically to a number of chloride channel proteins, but its binding site is distinct from that of all other known effector molecules of the chloride channel. It opens chloride channels and increases conductance. The exact sequence of events will only be determined when the avermectin binding site is isolated from a variety of species. It then might be possible to explain why avermectins are highly active against such a wide spectrum of nematodes, arthropods, and insects, but not to adult filaria worms, tapeworms, and flukes, yet are well tolerated by vertebrate hosts.

HUMAN USES OF IVERMECTIN

Despite the great success of ivermectin as an animal antiparasiticide, its use in human medicine has been less prominent, with one notable exception. It is currently the drug of choice for the prevention of the most serious effects of human infection by the filarial nematode *Onchocerca volvulus,* the causative agent of the tropical disease onchocerciasis, also known as river blindness (2, 10). The drug is a safe and long-acting microfilaricide. When applied at a single dose of 150 μg/kg it kills the immature microfilariae, but not the adult worms, and keeps the numbers of the reappearing microfilariae well below pretreatment levels for up to one year. Adverse reactions are mild, and no statistically significant difference in the rate of abnormalities were found in babies born to women inadvertently treated during pregnancy. Although ivermectin does not provide a permanent cure by killing the adult worms, the incidence of onchocercal eye disease is significantly reduced. As an additional bonus, treatment of whole communities by the World Health Organization significantly reduced the transmission of the disease by limiting the source of infection (34).

The effects of ivermectin against the related parasite *Wuchereria bancrofti,* the nematode responsible for the lymphatic filariasis also known as elephantiasis, is still under investigation. In a recent study in Haiti, an initial dose of 20 μg/kg followed five days later by a single dose of 200–400 μg/kg cleared the microfilaremia to under 3% for one year (35). Ivermectin, however, appears to be not fully effective in removing microfilariae of *Brugia malayi*.

The use of ivermectin for gastrointestinal nematode infections of humans, however, has not been pursued due to relatively low anthelmintic potency

against human hookworms. This is particularly surprising and disappointing since dog hookworms are highly susceptible to low doses of ivermectin. A recent study (36) suggests a possible use in human strongyloidiasis, which remains a significant problem among the intestinal helminth infestations. Cure rates of patients infected with strongyloidiasis after single 200 μg/kg ivermectin doses were as high as 94%, and 100% after two repeat doses. At the same time, a single 200 μg/kg dose in human patients was also 100% effective for ascariasis, 78% for enterobiasis, and 88% for trichuriasis, but responses against the hookworms were not significant.

USES OF AVERMECTINS IN ANIMALS

The efficacy of ivermectin against a very wide range of endo- and ectoparasites of domesticated and wild animals has been demonstrated. The high potency allows for a number of efficient formulations such as oral drench, subcutaneous injection, or topical transdermal delivery. It is used extensively for cattle, sheep, swine, and horses, and as a prophyllactic treatment for dog heartworms. Anthelmintic activity extends to most gastrointestinal and systemic nematodes but not to tapeworms or flukes. Unlike conventional anthelmintic treatments, the same dose rate of 0.2 to 0.5 mg/kg of ivermectin is also highly effective against many economically important ectoparasites. In cattle, for instance, control of grubs, lice, mites, certain ticks, and flies is achieved through the use of different formulations of IVM. Blood-sucking parasites or those intimately attached to the host respond better to systemic treatment, whereas superficially attached species are better controlled by topical applications. Despite wide use only rare occurrences of resistance development against Haemonchus spp. and Trichstrongylus spp. of sheep have been observed. Strategically timed repeat application of ivermectin or sustained release formulations are required for the extended control of most endo- and ectoparasites. Although ivermectin is generally well tolerated, certain dogs of the collie breed have been found supersensitive to otherwise therapeutic doses of 0.1 to 0.2 mg/kg and its use is contraindicated in that breed. The drug has been successfully used under carefully supervised conditions in many species of exotic zoo animals.

CROP PROTECTION

ABAMECTIN Avermectin B_1 is the most effective of the avermectin family of natural products against agriculturally important insects and mites. It has been commercialized for agricultural use as a foliar spray, under the nonproprietary name abamectin. A summary of its biological activity is shown in Table 1 (8).

Table 1 Activity of abamectin B_1 against mites and insects

Species	Common Name	LC_{90} (ppm)
Mite (Contact effect against adult mites)		
Phyllocoptruta oleivora	Citrus rust mite	0.02
Tetranychus urticae	Two-spotted spider mite	0.03
Tetranychus turkestani	Strawberry mite	0.08
Panonychus ulmi	European red mite	0.04
Panonychus citri	Citrus red mite	0.24
Polyphagotarsonemus latus	Broad mite	0.03
Insect (Foliar residue bioassay)		LC_{90} (ppm)
Leptinotarsa decemlineata	Colorado potato beetle	0.03
Manduca secta	Tomato hornworm	0.02
Epilachna varivestes	Mexican bean beetle	0.20
Acyrthosiphon pisum	Pea aphid	0.40
Trichoplusia ni	Cabbage looper	1.0
Heliothis zea	Corn earworm	1.5
Spodoptera eridania	Southern armyworm	6.0
Keiferia lycopersicella	Tomato pinworm	0.031

Emamectin (MK-244) Inspection of the biodata shown in Table 1 reveals that whereas abamectin is extremely effective against mites, it is much less so against insects, especially lepidoptera larvae. Nevertheless, two important uses are for the control of liriomyza and psylla species. An extensive program

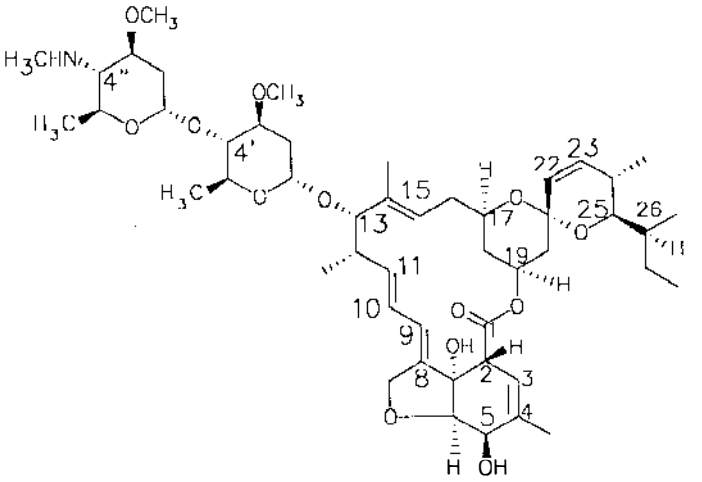


Figure 4 MK-244 EMAMECTIN

Table 2 Foliar ingestion activity of 4''-epi-methylamino-4''-deoxyavermectin B_1 against insect larvae and adult spider mites and aphids

Species	Common Name	LC_{90}(ppm) at 96 hr
Manduca sexta (L.)	Tobacco hornworm	0.003
Trichoplusia ni (Huebner)	Cabbage looper	0.014
Spodoptera exigua (Huebner)	Beet armyworm	0.005
Spodoptera frugiperda (J. E. Smith)	Fall armyworm	0.01
Leptinotarsa decemlineata (Say)	Colorado potato beetle	0.032
Epilachna varivestis (Mulsant)	Mexican bean beetle	0.20
Tetranychus urticae (Koch)	Two-spotted spider mite	0.29
Aphis fabae (Scopoli)	Bean aphid	19.9

of synthetic chemistry and biological testing was initiated to find avermectin derivatives with improved insecticidal activity. 4''-Deoxy-4''-epimethylamino avermectin B_1 was found to be the most active insecticidal compound in the series, showing up to 1600-fold improvement in activity over abamectin against some insect species. It has been assigned the code name MK-244 and the nonproprietary name emamectin.

A summary of the foliar ingestion activity of MK-244 against a variety of insect larvae and adult spider mites and aphids is shown in Table 2 (37).

TOXICOLOGY

IVERMECTIN Ivermectin is widely used as an endectocide in both food animals and companion animals. It has also been tested extensively in human onchocerciasis and is now considered to be the drug of choice. Toxicological studies in animals were designed to assess risk to the meat-eating public, to the target animals, to workers handling the drug, and to humans receiving the drug directly.

Genotoxicity In vitro studies were conducted in a number of tests for potential genotoxicity, including the Ames test in *Salmonella typhimurium,* a mouse lymphoma assay looking for forward mutations at the thymidine kinase locus, and unscheduled DNA synthesis using normal human embryonic lung fibroblast cells. In each of these tests ivermectin showed no genotoxicity.

Acute toxicity Ivermectin is considerably more toxic to rodents than to other species. A summary of LD_{50} values using various routes of administration is shown in Table 3 (38).

Subchronic toxicity Subchronic studies were carried out in rats, beagles, and rhesus monkeys. A summary of results of these studies is shown in Table 4 (38).

Table 3 Acute toxicity of ivermectin

Species	Route of Administration	LD_{50}(mg/kg)
Mouse	Oral	25
Mouse	Intraperitoneal	30
Rat	Oral	50
Rat	Intraperitoneal	55
Rat (infant)	Oral	2 to 3
Rat	Dermal	>660
Rabbit	Dermal	406
Dog	Oral	~80
Rhesus monkey	Oral	>24

Developmental and reproductive toxicity Assessment of potential development and reproductive toxicity has become an important component of the safety of new drugs. A summary of the results of oral daily administration of ivermectin to mice rats and rabbits during the major period of organogenesis is shown in Table 5 (38).

It is noteworthy that all abnormalities detected were produced at levels close to or at those producing severe maternotoxicity, including death of

Table 4 Summary of results of subchronic oral toxicity studies with ivermectin

Species	Duration	Dose levels (mg/kg/day)	Results
Rats	3 months	0.4, 0.8, 1.6	Splenic enlargement and reactive hyperplasia suggestive of intravascular hemolysis at 0.8 mg/kg/day and above. NEL[a] = 0.4 mg/kg/day
Dogs	3 months	0.5, 1.0, 2.0	Mydriasis at 1.0 mg/kg day and above. Tremors, ataxia, and anorexia at 2.0 mg/kg/day. NEL > 1.2 mg/kg/day
Rhesus monkey *(Macaca mulatta)*	2 weeks	0.3, 0.6, 1.2	No treatment-related effects up to 1.2 mg/kg/day. NEL > 1.2 mg/kg/day
Neonatal rhesus monkeys *(Macaca mulatta)*	2 weeks	0.04, 0.1	No treatment-related effects up to 0.1 mg/kg/day. NEL > 0.1 mg/kg/day.

[a] NEL: No-effect level

Table 5 Summary of results of developmental and reproductive toxicity studies with ivermectin

Species	Dose levels (mg/kg/)	Observations	NEL[a] (mg/kg)	
Mouse	0.1, 0.2, 0.4, 0.8	Mortality, tremors convulsions, coma at 0.2, 0.4 and 0.8 mg/kg; cleft palate at 0.4 and 0.8 mg/kg	Maternotoxicity developmental toxicity	0.1 0.2
Rat	2.5, 5.0, 10.0	Sedation, cleft palate at 10 mg/kg	Maternotoxicity developmental toxicity	5.0
Rabbit	1.5, 3.0, 6.0	Sedation and decreased body weight at 6 mg/kg; decreased fetal weights, increased number of deaths, cleft palate, and clubbed forepaws at 3.0 and 6.0 mg/kg	Maternotoxicity developmental toxicity	3.0 1.5
Rat Multigeneration	0.05, 0.1, 0.2, 0.4	Neonatal mortality at 0.4 mg/kg/day	Neonatal and developmental toxicity	0.2

[a] NEL: No-effect level

pregnant females. The two effects are linked, hence there is no selective embyrotoxicity. This view is reinforced by the widespread use of ivermectin in a variety of pregnant animals without incidence of developmental toxicity.

Abamectin Abamectin differs from ivermectin only in that it contains a 22,23-olefin whereas ivermectin is saturated at these positions. It is, therefore, not surprising that both toxicologies are similar, although ivermectin is somewhat less toxic, as shown in Table 6 (38).

Neonatal rats are clearly more sensitive than adults, presumably due to their underdeveloped blood-brain barrier.

Because of its manner of use, abamectin was also studied for long-term

Table 6 Comparative acute toxicity of abamectin and ivermectin

Type of study	Species	Abamectin (mg/kg/day)	Ivermectin (mg/kg/day)
Oral LD_{50}	CFl mouse	13.6–23.8	24.6–40
Oral LD_{50}	CRCD rat	10.6–11.3	42.8–52.8
Oral LD_{50}	CRCD Neonatal rat	1.52	2.3
Acute toxicity	Monkeys	MEL = 2 mg/kg	MEL = 2 mg/kg

MEL: Minimum Effect Level

toxicity and carcinogenicity. When given daily to rats at oral doses of 0.75, 1.5, and 2.0 mg/kg for 105 weeks there was no statistical increase in tumor incidence or histopathological changes. A similar 94-week study in CD-1 mice at doses of 2, 4, and 8 mg/kg/day in the diet showed no treatment-related changes and no oncogenic effects.

Because of potential dermal toxicity dermal penetration studies of abamectin emulsifiable concentrate, the dilute emulsifiable concentrate and suspended powder were conducted in monkeys. Dermal penetration was found to be 0.17–0.55% of the applied dose. This finding indicates that the hazard to farm workers is significantly reduced.

SUMMARY OF TOXICOLOGY

A variety of studies in vitro and in vivo have indicated a wide margin of safety for both ivermectin and abamectin. Furthermore, ivermectin has been used extensively in humans at doses up to 0.2 mg/kg p.o. without any adverse drug-related effects. Even an acute overdose in a child exposed to 8 mg/kg p.o. produced clinical signs that reversed rapidly (38).

Target animals Ivermectin is principally used in cattle, horses, pigs, sheep, goats, and dogs. In all those species there is a wide margin of safety between the therapeutic dose and the lowest dose showing clinical signs. No adverse effect on breeding animals has been detected. As noted above, a subset of dogs of the collie breed are unusually sensitive (39). Whereas a dose of 5 mg/kg p.o. in beagles causes mydriasis and tremors, dogs within this collie subset show similar signs at 0.15–0.2 mg/kg. The reason is not clearly understood although preliminary studies indicate higher levels of ivermectin in the brains of affected dogs. It should be noted, however, that the only formulation for dogs is a tablet or chewable pellet to be given monthly at a dose rate of 0.006 mg/kg for prevention of canine heartworm. This represents a wide margin of safety even for sensitive collies, which tolerate 0.05 mg/kg without effect.

METABOLISM

Ivermectin For any food or animal drug a radiolabeled compound is needed to address total residues remaining in edible tissues at appropriate times after dosing. A chemical assay is also necessary, accurate to low ppb levels, to allow estimates of drug residues that may remain in edible tissues. Radiolabeled ivermectin can be synthesized either by managanese dioxide oxidation of the C5-alcohol to a ketone followed by stereo-selective reduction

Table 7 Total radioactive residue levels (ppb) in tissues and fluids from cattle dosed subcutaneously with 22,23-[^{3}H]ivermectin at 0.3 mg/kg body weight

Days post dose	7	14	21	28
Abomasum	44	17	10	1
Adrenals	29	6	7	2
Bile	273	54	22	1
Bone marrow	92	21	23	9
Brain	4	1	0	0
Cecum	33	9	3	0
Colon	44	11	9	0
Fat	270	83	69	29
Heart	41	8	3	0
Small Intestine	22	5	6	1
Kidney	68	6	7	2
Liver	782	55	68	11
Lung	66	12	4	1
Lymph gland	41	13	20	6
Muscle	23	2	4	0
Pancreas	83	16	9	1
Plasma	45	11	6	3
Rumen	34	10	10	2
Rumen fluid	7	1	0	0
Spleen	38	8	5	3
Thymus	64	21	9	1
Thyroid	58	16	8	6
Tongue	27	4	4	0
Injection site	70	28	33	39

with sodium borotritide, or by catalytic tritiation of the C22,23-olefin using Wilkinson's catalyst.

Tissue residues Studies were carried out on cattle, swine, sheep, and rats dosed once at 0.3 or 0.4 mg/kg subcutaneously, intraruminally, or orally. A representative experiment in cattle is shown in Table 7 (40).

Ivermectin is extremely lipophilic, is almost totally excreted in the feces, and concentrates in adipose tissue such as fat and liver regardless of the route of administration. The parent drug is by far the most abundant species in all animals whereas 24-hydroxymethyl ivermectin and 3''-O-desmethyl ivermectin are found as minor metabolites. The low residues and comparative safety of ivermectin in animals and humans indicate no human health hazard from residues in meat.

Abamectin Degradation of a pesticide in the environment plays an important role in the safety assessment of agricultural products. The types of studies run include photolysis by sunlight in the solid state or in water, hydrolysis, oxidation, and metabolism by soil organisms.

Sunlight Abamectin is rapidly degraded when exposed to sunlight in water, as a thin film on glass, leaves, or on soil particles, with a half-life of less than 12 hr. Only one product, the delta 8,9 Z isomer of avermectin B1a, was fully characterized. Several other photooxidation degradates missing the characteristic UV at 245 nM and containing up to 6 atoms of oxygen were recognized but not identified.

Soil metabolism Soil metabolism of abamectin was studied in sandy loam, clay, and sand. The half-life ranged from 20–47 days and 13 degradates were identified. The major product was the 8a-hydroxy derivative that had also been synthesized by chemical oxidation. The other products were not identified. Interestingly, no metabolism took place in sterile soil, which indicates that soil organisms are responsible for degradation (41).

Environmental Aspects

Studies on the environmental fate of a compound take into account how it is introduced into the environment, how much, whether or not it accumulates, degradation and the extent to which it becomes available to living organisms. An overriding consideration is the intrinsic toxicity of the compound.

Ivermectin Ivermectin enters the environment by excretion from animals dosed with the drug. Due to its highly lipophilic nature, more than 98% is found in the feces, regardless of the method of dosing. The major component by far is unchanged parent drug.

The highest ivermectin levels in soil would be expected to arise from feedlot manure from ivermectin-treated cattle plowed into fields following normal farming practice. Calculations from worst-case assumptions project a level of 0.2 ppb in soil (42).

The physical properties of ivermectin, insolubility in water (5 ppm), high octanol water partition coefficient (1651), low vapor pressure (10^{-9}) and tight binding to soil result in minimal leaching into ground water. One could argue that its strong affinity for soil and lack of mobility would result in soil accumulation. However, degradation measurements in soil/feces mixtures gave half-lives of 90–240 days in winter, 7–14 days in summer, and 3 hr in sunlight on a glass surface. Ivermectin/feces mixtures had no effect on soil respiration at levels 150-fold greater than the calculated exposure and its no-effect level to earthworms is 12 ppm.

Fresh water organisms are very sensitive to ivermectin having LC_{50} values

for *Daphnia magna* 0.025 ppb, rainbow trout 3 ppb, bluegill sunfish 4.8 ppb. However, because of its tight soil binding the ivermectin concentration in runoff and groundwater is well below the concentrations necessary to have an effect on these organisms (42). Other studies have shown that it is not very toxic to wild birds (43) or mammals (44).

The possible detrimental effects of ivermectin on the decomposition of cattle dung have been widely discussed. It has been reported that in an experimental trial, feces from untreated cattle were decomposed after 40 days whereas feces from ivermectin-treated cattle were solid and intact after 100 days (45). It is true that ivermectin in feces has an effect on some important dung fauna, such as the larval stages of some dung beetles and diptera. However, under a natural setting, dung beetles fly from pat to pat and disintegration is further accelerated by trampling, wind, rain, and other factors. A trial using pastured animals found no difference between the rate of decomposition of feces from ivermectin-treated and untreated animals after 9 weeks (46). Furthermore, the infrequency of treatment reduces any potential environmental impact.

Abamectin The major use of abamectin is in agriculture, as a foliar spray on crops, at a maximum rate of 0.025 lb/acre 3 times/season or 0.02 lb up to 10 times/season. A minor use is control of fire ants at a use rate of 0.00011 lb/acre. The environment is therefore exposed to the compound directly, as are workers who apply the compound and harvest treated crops. The environmental risk is fundamentally a comparison of the levels known to be toxic to nontarget organisms to the expected exposure levels. Comprehensive descriptions of environmental and worker safety have recently been published (44).

Detailed studies on its environmental fate in soil and water and its effects on terrestrial and aquatic organisms indicate that it can be used in an environmentally safe manner. Abamectin photolyzes rapidly in water and in soil and as thin films. Abamectin is metabolized in soil to less toxic metabolites. Furthermore, its tight binding to soil both prevents run off into rivers and lakes and also reduces its bioavailability.

Literature Cited

1. Fisher, M. H., Mrozik, H. 1984. The avermectin family of macrolide-like antibiotics. In *Macrolide Antibiotics,* ed. S. Omura, pp. 553–606. New York: Academic
2. Campbell, W. C., ed. 1989. *Ivermectin and Abamectin,* Berlin/Heidelberg: Springer-Verlag. 363 pp.
3. Blizzard, T., Fisher, M. H., Mrozik, H., Shih, T. L. 1990. Avermectins and milbemycins. In *Recent Progress in the Chemical Synthesis of Antibiotics,* ed. G. Lukacs, pp. 65–102. Berlin/Heidelberg: Springer-Verlag
4. Davies, H. G., Green, R. H. 1986. Avermectins and milbemycins. *Nat. Prod. Rep.* 3:87–121
5. Burg, R. W., Miller, B. M., Baker, E. E., Birnbaum, J., Currie, S. A., et al. 1979. Avermectins, a new family of po-

tent antihelminthic agents: producing organism and fermentation. *Antimicrob. Agents Chemother.* 15:361–67
6. Albers-Schonberg, G., Arison, B. H., Chabala, J. C., Douglas, A. W., Eskola, P., et al. 1981. Avermectins: Structure determination. *J. Am. Chem. Soc.* 103:4216–21
7. Chabala, J. C., Mrozik, H., Tolman, R. L., Eskola, P., Lusi A., et al. 1980. Ivermectin, a new broad-spectrum antiparasitic agent. *J. Med. Chem.* 23:1134–36
8. Dybas, R. A., Green, A. S. J. 1984. Avermectins: their chemistry and pesticidal activity. *Proc. Br. Crop Prot. Conf. Brighton,* 9B-3:947–54
9. Campbell, W. C. 1985. Ivermectin: an update. *Parasitol. Today* 1:10–16
10. Taylor, H. R., Greene, B. M. 1989. The status of ivermectin in the treatment of onchocerciasis. *Am. J. Trop. Hyg.* 41: 460–66
11. Takiguchi, Y., Mishima, H., Okuda, M., Terao, M., Aoki, A., Fukuda, R. 1980. Milbemycins, a new family of macrolide antibiotics: fermentation, isolation and physico-chemical properties. *J. Antibiot.* 33:1120–27
12. Putter, I. 1979. *US Patent No.* 4,144,352
13. Putter, I., MacConnell, J. G., Preiser, F. A., Haidri, A. A., Ristich, S. S., Dybas, R. A. 1981. Avermectins: Novel insecticides, acaricides and nematicides from a soil microorganism. *Experientia* 37:963–64
14. Carter, G. T., Nietsche, J. A., Borders, D. B. 1987. Structure determination of LL-F28249-$\alpha,\beta,\delta,\lambda$, potent antiparasitic macrolides from *Streptomyces cyaneogriseus* SSP noncyanogenus. *J. Chem. Soc. Chem. Commun.,* pp. 402–4
15. Ramsay, M. V. J., Roberts, S. M., Russell, J. C., Shingler, A. H., Slawin, A. M. Z., et al. 1987. Novel antiparasitic agents derived by modification of a new natural product series. *Tetrahedron Lett.* 28:5353
16. Hood, J. D., Banks, R. M., Brewer, M. D., Fish, J. P., Manger, B. R., Poulton, M. E. 1989. A novel series of milbemycin antibiotics from *Streptomyces* strain E225. *J. Antibiot.* 42:1593–98
17. Baker, G. H., Dorgan, R. J. J., Everett, J. R., Hood, J. D., Poulton, M. E. 1990. Isolation, characterization, structure elucidation and solution conformations. *J. Antibiot.* 43:1069–76
18. Pfizer Europ. Patent Appl. EPO 334,484
19. Bowman, D. D., Lin, D. S., Johnson, R. C., Hepler, D. I. 1991. Effects of milbemycin oxime on adult *Ancylostoma caninum* and *Uncinaria stenocephala* in dogs with experimentally induced infections. *Am. J. Vet. Res.* 52:64–67
20. Cane, D. E., Liang, T. C., Kaplan, L., Nallin, M. K., Schulman, M. D., et al. 1983. Biosynthetic origin of the carbon skeleton and oxygen atoms of the avermectins. *J. Am. Chem. Soc.* 105: 4110
21. Chen, S. T., Arison, B. H., Gullo, V. P., Inamine, E. 1983. Further studies on the biosynthesis of the avermectins. *Am. Chem. Soc.* Abstr.
22. Chen, S. T., Inamine, E. S., Hensens, O. D., Zink, D., Ostlind, D. A. 1989. Directed biosynthesis of avermectins. *Arch. Biochem. Biophys.* 269:544–47
23. McArthur, W. C., Wernau, J. 1991. Branched-chain fatty acid requirement for avermectin production by a mutant of *Streptomyces avermitilis* lacking branched-chain 2-oxo acid dehydrogenase activity. *J. Antibiot.* 44:349–56
24. Dutton, C. J., Gibson, S. P., Goudie, A. C., Holdom, K. S., Paccy, M. S., et al. 1991. Novel avermectins produced by mutational biosynthesis. *J. Antibiot.* 44:357–65
25. Shih, T. L., Mrozik, H., Holmes, M. A., Fisher, M. H. 1990. A Wittig approach to novel C_{24} and C_{25}-substituted avermectins. *Tetrahedron Lett.* 31:3529–32
26. Ono, M., Mishima, M., Takiguchi, Y., Teroa, M. 1983. Milbemycins, a new family of macrolide antibiotics. Studies on the biosynthesis of milbemycins alpha 2, alpha 4 and D using ^{13}C labeled precursors. *J. Antibiot.* 36:991–1000
27. Halley, B. A., Jacob, T. A., Lu, A. Y. H. 1989. The environmental impact of the use of ivermectin environmental effects and fate. *Chemosphere* 18:1543–63
28. Wright, D. J. 1986. Biological activity and mode of action of avermectins. In *Neuropharmacology and Pesticide Action,* ed. M. G. Ford, G. G. Lunt, R. C. Reay, P. N. R. Usherwood, pp. 174–202. UK: Harwood
29. Schaeffer, J. M., Haines, H. W. 1989. Avermectin binding in *Caenorhabditis elegans*. A two-state model for the avermectin binding site. *Biochem. Pharmacol.* 38:2329–38
30. Cully, D. F., Paress, P. S. 1991. Solubilization and characterization of a high affinity ivermectin binding site from *Caenorhabditis elegans*. *Mol. Pharmacol.* 40:326–32
31. Rohrer, S. P., Meinke, P. T., Hayes, E. C., Mrozik, H., Schaeffer, J. M., 1991. Photoaffinity labeling of avermectin

binding sites from *Caenorhabditis elegans* and *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA*. In press
32. Zufall, F., Franke, C., Hatt, H. 1989. The insecticide avermectin B-1A activates a chloride channel in crayfish muscle membrane. *J. Exp. Biol.* 142:191–205
33. Arena, J. P., Liu, K. K., Paress, P. S., Cully, D. F. 1991. Avermectin-sensitive chloride currents induced by *Caenorhabditis elegans* RNA in *Xenopus oocytes*. *Mol. Pharmacol.* 40:368–74
34. Taylor, H. R., Pacque, M., Munoz, B., Greene, B. M. 1990. Impact of mass treatment of onchocerciasis with ivermectin on the transmission of infection. *Science* 250:116–18
35. Richards Jr., F. O., Eberhard, M. L., Bryan, R. T., McNeely, D. F., Lammie, P. J., et al. 1991. Comparison of high dose ivermectin and diethylcarbamazine for activity against bancroftian filariasis in Haiti. *Am. J. Trop. Med. Hyg.* 44:3–10
36. Naquira, C., Jimenez, G., Guerra, J. G., Bernal, R., Nalin, D. R., et al. 1989. Ivermectin for human strongyloidiasis and other intestinal helminths. *Am. J. Trop. Med. Hyg.* 40:304–9
37. Dybas, R. A., Hilton, N. J., Babu, J. R., Preiser, F. A., Colce, G. J. 1989. Novel second-generation avermectin insecticides and miticides for crop protection. In *Novel Microbial Products for Medicine and Agriculture*, ed. A. L. Demain, G. A. Somkuti, J. C. Hunter-Cevera, H. W. Rossmoore, pp. 203–12. New York: Elsevier
38. Lankas, G. R., Gordon, L. R. 1989. Toxicology. See Ref. 2, pp. 89–112
39. Campbell, W. C., Blair, L. S., Seward, R. L. 1983. Ivermectin vs. heartworm. *Proc. Heartworm Symp. Orlando, FL*, pp. 146–49
40. Chiu, S. H. L., Lu, A. Y. H. 1989. Metabolism and tissue residues. See Ref. 2, pp. 131–43
41. Wislocki, P. G., Grosso, L. S., Dybas, R. A. 1989. Environmental aspects of abamectin use in crop protection. See Ref. 2, pp. 182–200
42. Halley, B. A., Nessel, R. J., Lu, A. Y. H., Roncalli, R. A. 1989. The environmental safety of ivermectin: An overview. *Chemosphere* 18:1415–72
43. Kelso, P. C. 1984. Ivermectin cures scaly mite infestation. *Vet. Med.* 79:446
44. Nessel, R. J., Jacob, T. A., Robertson, R. T. 1983. The human and environmental safety aspects of ivermectin. In *Recent Developments in the Control of Animal Parasites*, ed. W. H. D. Leaning, O. H. Siegmund, C. M. Fraser, pp. 98–100. Rahway, NJ:MSD-AGVET
45. Wall, R., Strong, L. 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. *Nature* 327:418–21
46. McKeand, J., Bairden, K., Ibarra-Silva, A. M. 1988. The degradation of bovine fecal pats containing ivermectin. *Vet. Rec.* 122:587–88