Environmental Impact Analysis Report

IVOMEC® (ivermectin) 0.08% Oral Solution for Sheep

A. February 11, 1985

B. Merck Sharp & Dohme Research Laboratories
Merck & Co., Inc.

C. P.O. Box 2000
Rahway, New Jersey 07065

D. Environmental Information

1. Describe the Proposed Action

(a) Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., has filed a New Animal Drug Application for IVOMEC® (ivermectin) 0.08% Oral Solution for Sheep to be administered to sheep and lambs for the treatment and control of the following internal parasites:

Gastrointestinal roundworms: Stomach Worm or Wire Worm (*Haemonchus contortus*, *H. placei*), Brown Stomach Worm (*Ostertagia circumcincta*), Stomach Hair Worm or Bankrupt Worm (*Trichostrongylus axei*, *T. colubriformis*), Cooperids (*Cooperia oncophora*, *C. curticei*), Nodular Worm (*Oesophagostomum columbianum*, *O. venulosum*), Thread-necked Strongyle (*Nematodirus spathiger*, *N. battus*), Intestinal Threadworm (*Strongyloides papillosus*).

1. Describe the Proposed Action

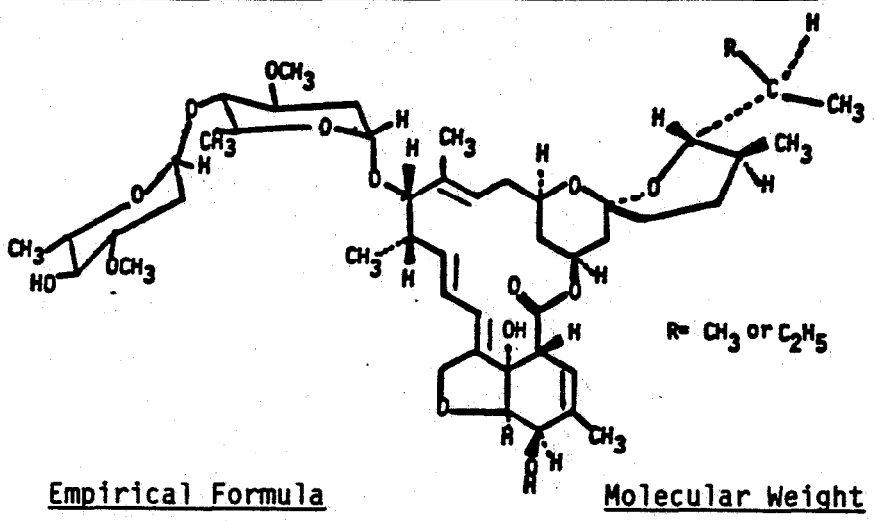
(a) Large-mouthed Bowel Worm (Chabertia ovina), Whipworm (Trichuris ovis); Lungworm (Dictyocaulus filaria); and all larval stages of nasal bot (Oestrus ovis).

It is intended to be administered to sheep at a dose rate of 0.2 mg ivermectin per 2.2 pounds of body weight (1 kg). The anticipated highest retreatment rate is 4 times per year and the drug withdrawal period prior to slaughter is eleven days.

Treatment may be repeated at intervals of not less than 11 days; however, rate of parasite reinfestation, prudent husbandry practices and economic considerations would dictate that most sheep would be treated four times or less during the life of the animal. Sheep kept for breeding purposes might be treated once or twice yearly throughout their lives.

1. Describe the Proposed Action

(b) Physical and Chemical Properties Are as Follows:



Empirical Formula	Molecular Weight
(R = C_2H_5) $C_{48}H_{74}O_{14}$	875.10
(R = CH_3) $C_{47}H_{72}O_{14}$	861.07

Ivermectin is produced by fermentation and subsequent chemical hydrogenation and is a mixture of two closely related homologues belonging to a class of compounds known as avermectins. The chemical names of the two homologues are: 22,23-dihydroavermectin B_{1a} ($R=C_2H_5$) and 25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl) avermectin B_{1a} ($R=CH_3$). The latter is also known as 22, 23-dihydroavermectin B_{1b}.

1. Describe the Proposed Action

(b) Physical and Chemical Properties (Cont'd)

Ivermectin contains at least 80% of the compound in which R in the above structure is the ethyl group and less than 20% of the compound in which R is the methyl group. It is a white to yellowish white crystalline powder and has an ill-defined melting point of about 150°C. The material is optically active and has a specific rotation $[\alpha]_D^{25°C}$, ~-19° (C=0.5, CH_3OH). The ultraviolet absorption spectrum in methanol is characterized by maxima at 237, 245 and 253 nm with A1%1cm values of about 349, 382 and 248, respectively. Ivermectin is very insoluble in water, the concentration of a saturated aqueous solution being 5 ppm. Ivermectin is freely soluble in methanol, chloroform, p-dioxane, dimethylformamide and ethyl acetate; soluble in 95% ethanol, diethyl ether, methylene chloride, acetone, and aromatic hydrocarbons; and very slightly soluble in aliphatic hydrocarbons. The infrared and nuclear magnetic absorption spectra are consistent with proposed structures.

Ivermectin has been shown to be stable for at least

1. Describe the Proposed Action

(b) Physical and Chemical Properties (Cont'd)

six months when stored under ambient conditions. In a solution, ivermectin is photolabile. This suggests that sunlight could photochemically degrade ivermectin residues in the environment.

Ivermectin contains at least 95% of the two compounds shown above as determined by UV absorption and liquid chromatography.

Based on radioactivity measurements, the octanol coefficient for ivermectin is 1651; i.e.,

$$K_D \text{ of } \frac{\text{octanol}}{\text{pH 7 buffer (or water)}} = 1651$$

This indicates a strong affinity of ivermectin for lipid systems, but the residue data contained in the New Animal Drug Application show a rapid depletion of drug and metabolites from animal fat.

1. Describe the Proposed Action

(c) Pharmacology

Ivermectin inactivates nematodes, arachnids and insects. Its action on the nematodes is by inhibiting signal transmission from the ventral cord interneurons to the excitatory motor neurons. It acts by stimulating the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) from presynaptic nerve terminals as well as by potentiating GABA binding to the postsynaptic receptors. The ivermectin-treated nematodes lose central command to move. Ivermectin acts on the arthropods by inhibiting signal transmission at the neuromuscular junctions via the same mechanism of amplifying GABA action. The treated arthropods become paralyzed.

Ivermectin and the avermectins are not effective against flukes and tapeworms, in which GABA is not found as a neurotransmitter. Ivermectin has much less effect on mammals since the principal peripheral neurotransmitter in mammals, acetylcholine, is unaffected by ivermectin. Ivermectin does not readily penetrate the

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

central nervous system of mammals where GABA functions as a neurotransmitter.

In a laboratory screen, a mixture of at least 80% avermectin B_{1a} and not more than 20% avermectin B_{1b}, was given by gavage to rodents harboring 3-week-old Fasciola hepatica infections. Five control rodents had 1 to 3 worms at necropsy four days after treatment, while two animals dosed at 2.5 mg/kg of the avermectins had 2 and 3 worms each. In a similar screen using the tapeworm Hymenolepis diminuta, laboratory rodents harboring 14-day-old worms were given placebo, commercial yomesan at 37.5 mg/kg as a positive control or ivermectin at 1 mg/kg. Necropsy 6 days after treatment indicated 3 to 5 worms in each of the four animals receiving the placebo, zero worms in the three animals receiving yomesan, and 2, 4 and 6 worms in the three animals receiving ivermectin.

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

Also in field trials, ivermectin, at 50 to 400 mcg/kg had no effect against the tapeworms Dipylidium caninum and Taenia spp in dogs.[1]* Similarly, in anthelmintic tests in equids, ivermectin was shown to be ineffective against natural tapeworm infections.[2]

Ivermectin administered orally to sheep at levels up to 4 mg/kg body weight (20 times the recommended use level) resulted in coughing and tachypnea but no evidence of toxicosis or serious adverse reaction. Ivermectin is also well tolerated in humans in single doses up to 200 mcg/kg body weight.

Ivermectin is unrelated structurally to any of the present available parasiticides. Because of this and its unique mode of action not shared by any other parasiticides, cross-resistance is not expected to occur.

*Literature references on page 163.

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

Ivermectin has mild anticonvulsant activity in the pharmacometric screen of central nervous system effects in the mouse. The LD_{50} 24 hours after drug administration was estimated to be less than 10 mg/kg.

Ivermectin was virtually inactive against electroshock and bicuculline-induced convulsions one hour following treatment. However, anticonvulsant activity of ivermectin increased markedly in both assays when measured 4 hours after treatment.

At a dose of 0.5 mg/kg IV, ivermectin had no significant effect on blood pressure or heart rate of anesthetized dogs, nor did it modify blood pressure or heart rate responses to autonomic drugs in a standard assay. The B_{1a} component of ivermectin enhanced the ^{3}H-diazepam binding in rat brain P_2 membranes by 32% at a concentration of 1 mcM.

Ivermectin, at an intragastric dose of 0.5 mg/kg, did not affect evoked or basal gastric secretion in dogs with a chronic gastric fistula.

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

Ivermectin, at 1 and 2 mg/ml (parts per thousand), did not inhibit the growth of 9 bacterial or 5 fungal strains. The bacterial strains were Staphylococcus aureus, Streptococcus pyogenes, Bordetella bronchseptica, Klebsiella pneumoniae, Aerobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa (two cultures), and Proteus mirabilis. The fungal strains were Alternaria, Fusarium, Cephalosporium, Pullularia pullulans, and Aspergillus niger. The solvent for the ivermectin, DMSO, was present at a level of 1%. This level of DMSO also had no effect on the growth of the bacterial and fungal cultures. Ivermectin was also tested in the antibacterial agent screen at 1 and 2 mg/ml against 5 strains each of Escherichia coli and Salmonella typhimurium of animal origin (calf and pig animal sources). The solvent, DMSO, was again present at a level of 1%. Neither the ivermectin nor the DMSO had any inhibitory effect towards growth of any of the test organisms at these levels.

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

Ivermectin displays no substantial activity against protozoa. In an in vitro assay using Trichomonas foetus, the major isomer of ivermectin, H_2B_{1a}, displayed some activity in reducing T. foetus growth, but only at 100 mcg/ml in a stock solution. Concentrations of H_2B_{1a} at 0.2 to 50 mcg/ml were not effective in the 40-hour assay, whereas the 100 mcg/ml level of H_2B_{1a} was effective in only two out of three assays. Avermectin B_{1a} was inactive at 1, 10 and 100 mcg/ml. Similarly, in an in vitro assay using Trypanosoma brucei, H_2B_{1a} again displayed growth inhibition in a 6-hour incubation at 100 mcg/ml, but no activity at 1 or 10 mcg/ml. Here again, avermectin B_{1a} was inactive at 1, 10 and 100 mcg/ml. In an in vivo assay of T. brucei in mice, doses of 50 mg/kg of H_2B_{1a} and avermectin B_{1a} were toxic. Doses from 0.4 to 10 mg/kg produced some toxic reactions. Over the dosing range of 0.1 to 10 mg/kg, H_2B_{1a} and avermectin B_{1a} provided no in vivo protection against T. brucei infection.

1. Describe the Proposed Action

(d) Toxicity

Ivermectin has been shown to be negative in the Ames Microbiological Mutation Assay and in a Mammalian Mutation Assay using a mouse lymphoma cell line. In addition, ivermectin did not induce unscheduled DNA synthesis in a human fibroblast cell culture. The results of these studies showed no genotoxic hazard associated with the use of ivermectin.

Ivermectin is teratogenic in rats, rabbits and mice at or near maternotoxic dose levels. Evidence of a teratogenic effect was limited to cleft palate that occurred at a low frequency in all three species and clubbing of the forepaws which occurred only in the rabbit fetuses. Mice are the species most sensitive to the effects of ivermectin with maternotoxicity at a dose of 0.2 mg/kg/day and teratogenicity at 0.4 mg/kg/day. A dose of 0.1 mg/kg/day was without maternotoxic or teratogenic effect in mice. In rabbits, 6 mg/kg/day was maternotoxic and teratogenic, and teratogenicity was also evident at a dose of 3 mg/kg/day. A dose of 1.5 mg/kg/day in the rabbit was without maternotoxic

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

or teratogenic effect. The threshold for materno-toxicity and teratogenicity in rats was 10 mg/kg/day; a dose of 5 mg/kg/day was neither maternotoxic nor teratogenic.

In a reproduction study in rats, as well as in acute studies, it was demonstrated that neonates are significantly more susceptible to the toxic effects of ivermectin than adult animals. The LD_{50} for infant rats is approximately 10-fold less than that of adults. In a rat reproduction study, there was increased neonatal mortality at a dose of 1.6 mg/kg/day. In a 14-week oral toxicity study in which weanling rats (about 4 weeks of age) derived from the reproduction study were given ivermectin at doses up to 1.6 mg/kg/day, there was no treatment-related mortality.

In the 14-week oral toxicity study in rats mentioned above, no treatment-related effects were observed at a dose of 0.4 mg/kg/day. At doses of 0.8 and 1.6 mg/kg/day, enlarged spleens resulting from congestion and extramedullary hematopoiesis occurred in a few

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

rats. This was accompanied by the accumulation of iron-positive pigment in the renal tubular epithelium and hyperplasia of the bone marrow.

In a 14-week oral toxicity study in dogs, no treatment-related effects were observed in animals given 0.5 mg/kg/day. Dogs given 1 and 2 mg/kg/day developed mydriasis and lost a small amount of weight. Four of 8 dogs given 2 mg/kg/day developed tremors, ataxia and anorexia and became dehydrated. These dogs were killed prior to termination of the study, and agonal gastro-intestinal hemorrhage and/or congestion was observed in 2 of the dogs. No other treatment-related histologic change was observed in any dogs.

Sheep given ivermectin orally in a micelle formulation did not evidence signs of serious reaction until doses (4 mg/kg) exceeded 20 times the use level. In cattle trials designed to examine the effects of ivermectin doses in up to 8,000 mcg/kg. Deaths occurred in 3 of 4 animals at this dose rate, but no clinical signs of

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

toxicosis were seen at 6,000 mcg/kg, which is 30 times use-level dose rate.

Trials indicate that signs of toxicity (partial mydriasis) may be seen in some horses at levels approximately 15 times the proposed use level, and toxicosis and some fatalities occurred in horses receiving doses (12 mg/kg) in the vicinity of 60 times use level.

The clinical signs of acute toxicity caused by ivermectin in the pig are lethargy, followed by ataxia, mydriasis, intermittent tremors, labored breathing and lateral recumbency. These signs appeared in pigs injected subcutaneously with ivermectin at 30 mg/kg body weight (100 times the recommended use level). Pigs treated with ivermectin at levels up to 15 mg/kg body weight (50 times the recommended use level) did not exhibit signs of toxicity.

The oral acute LD_{50} of ivermectin in the mallard duck is 85 mg/kg, with 95% confidence limits, 67 to 120

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

mg/kg. At the lowest dosage level tested of 10.0 mg/kg, slight lethargy and loss of coordination occurred immediately after dosing and lasted through day one. Toxicity symptoms were pronounced and lasted longer as the dosage levels of the drug increased. Symptoms of toxicity observed included lethargy, loss of coordination, prostrate posture, lower limb rigidity, loss of righting reflex and depression.

The subacute LC_{50} in this avian species in an eight-day dietary study is 383 ppm, with 95% confidence limits, 302 ppm to 487 ppm.

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

At 162 ppm, the lowest concentration tested, lethargy, reduced reaction to external stimuli (sound and movement) wing droop, loss of coordination and lower limb weakness occurred within three hours of exposure to the treated diet, and lasted for five days. However, birds appeared normal 24 hours after being on basal diet; there was 20% mortality at the 288 ppm concentration level, 80% at the 511 ppm concentration level, and 100% at higher concentration levels. Duration of toxicity symptoms increased as the levels of avermectin B_{1a} increased.

The acute oral LD_{50} of ivermectin in the bobwhite quail is estimated to be greater than 2000 mg/kg.

At an avermectin B_1 level of 62.5 mg/kg, the birds exhibited lethargy, reduced reaction to external stimuli (sound and movement) and loose droppings that lasted for two days. There also was no mortality at the lowest avermectin B_1 level (62.5 mg/kg) tested.

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

At higher dose levels of avermectin B_1 (125 mg/kg), toxicity symptoms appeared within 1-2 hours, well established within the first day, and lasted for five days. These symptoms included loss of coordination, shallow and rapid respiration, depression, reduced reaction to external stimuli (sound and movement) wing droop, lower limb weakness, prostrate posture, ruffled appearance, lethargy, comatose state and some loose droppings. However, no mortalities occurred at the 125 mg/kg dose level. Mortality incidents were inconsistent with increased avermectin B_1 levels.

Avermectin B_1 dosage levels of 250 mg/kg and 1000 mg/kg caused only 10% mortality, but levels of 500 mg/kg and 2000 mg/kg caused 40% mortality. However, severity of the toxicity symptoms increased as Avermectin B_1 dosage levels increased.

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

In an eight-day dietary study in this species, the subacute LC_{50} value of ivermectin was determined to be 3102 ppm, with confidence limits (95%) of 2338 to 4393 ppm.

The lowest concentration tested (288 ppm) induced toxicity symptoms within four hours of exposure to the treated diet. These toxicity symptoms included lethargy and reduced reaction to external stimuli (sound and movement) and lasted for three days after which birds appeared normal. Duration of the toxicity symptoms increased as a function of increased avermectin B_1 concentration in the diet. Mortalities occurred at concentration levels $\geq$ 1620 ppm.

(See Table 1 on following page -- 20.)

TABLE 4

Results of Acute, Subacute, Oral Teratology, and Genotoxic Studies of Ivermectin

TYPE OF STUDY	SPECIES	DURATION	SIGNIFICANT FINDINGS		NO EFFECT LEVEL
			ANTEMORTEM	POSTMORTEM	(mg/kg/day)
Acute Oral	Mouse (M,F)	-	-	LD_{50} 11.6-41.6 mg/kg	-
Acute Oral	Rat (M,F)	-	-	LD_{50} 42-53 mg/kg	-
Ocular Irritation	Rabbit	-	-	Slightly irritating	-
Dermal Irritation	Rabbit	-	-	Non-irritating	-
Dermal LD_{50}	Rabbit	-	-	LD_{50} 406 mg/kg	-
Dermal LD_{50}	Rat	-	-	LD_{50} 660 mg/kg	-
Acute Inhalation	Rat	1 hr. exposure	Transient irritation mucous membranes	No deaths	Actual exposure based on respirable particles 0.4 mg/kg
Acute Subcutaneous	Young Dogs	-	-	LD_{50} ±10 mg/kg	LD_{50} 4.8 mg/kg
Toxicity	Rat	In utero phases	Hypothermia, absence of milk in epigastric region	Increased pup mortality	0.8
		14-Week phase	None	Enlarged spleen, possible intravascular hemolysis	0.4
Toxicity	Dog	14-Week	Tumors, ataxia, dehydration, mydriasis	Dogs sacrificed due to poor condition, agonal changes in 2 dogs - no other changes	0.5
Teratology	Mouse	Day 6-15 of gestation	Mortality, tremors, convulsions, coma	Cleft palate	Maternal effects - 0.1 Teratogenic effect - 0.2
Teratology	Rat	Day 6-17 of gestation	Sedation; 3 rats sacrificed in poor physical condition	Cleft palate	Maternal effects - 5.0 Teratogenic effect - 5.0
Teratology	Rabbit	Day 6-18 of gestation	Sedation, decreased body weight	Decreased fetal weight, increased number fetal deaths, cleft palate, clubbed forepaws	Maternal effects - 3.0 Teratogenic effect - 1.5
Teratology	Dog	Day 5, 15, 25, 35 or Day 10, 20, 30, 40 of gestation	None	No treatment- related external, visceral or skeletal malformations	0.5
Ames Bacterial Mutagen Assay	-	-	Negative	-	2 mg/plate
Mouse Lymphoma Mutagen Assay	-	-	Negative	-	80 g/ml
Unscheduled DNA Synthesis In Human Lung Fibroblast	-	-	Negative	-	10 mg/flask

1. Describe the Proposed Action

(e) Purpose and Benefits

The cost of parasitism, in terms of morbidity and resultant depression of growth and feed efficiency, has long been recognized as a significant factor in the economical production of both meat and wool. Thus, the increased significance of parasitism has led to the widespread use of antiparasitic drugs. Losses to the sheep and wool industry have been primarily attributed to the reduced feed efficiency, general debility and mortality caused by internal and external parasites.

Ivermectin is an effective, new antiparasitic agent which is not chemically related to any other drug now being marketed for sheep. In the proposed form, ivermectin provides the most convenient, ready-to-use method of controlling important species of gastrointestinal roundworms found in the United States, plus lungworm and nasal bots, a spectrum of activity not shared by any other currently available product.

1. Describe the Proposed Action

(e) Purpose and Benefits (Cont'd)

The unique activity of this product also permits control of parasite strains shown to be resistant to benzimidazole compounds. Clearly beneficial effects with economic value would result from its use, such as decreased morbidity, and increased wool production and weight gain, due to the removal of the parasites.

(f) Potential Market Handling and Storage

Currently, two products dominate over 80 percent of the estimated 16.3 million doses of anthelmintics administered to 12.4 million sheep in the United States. IVOMEC● 0.08% Oral Solution for Sheep is expected to attain a significant market share within the anthelmintic market, due to its unique control of a broad range of parasites.

1. Describe the Proposed Action

(f) Potential Market Handling and Storage (Cont'd)

There are no special storage requirements for IVOMEC® 0.08% Oral Solution for Sheep. Stability studies show that IVOMEC® 0.08% Oral Solution for Sheep will be stable for two years when stored under normal conditions.

Spills of IVOMEC® 0.08% Oral Solution for Sheep should be contained and soaked up with absorbent towels or into loose soil. Gloves should be worn to prevent skin exposure. All the collected materials (contaminated towels and soil) should be bagged in plastic film bags and disposed of by incineration or in an approved landfill.

1. Describe the Proposed Action

(f) Potential Market Handling and Storage (Cont'd)

The proposed trade channels for distribution of IVOMEC® Oral Solution for Sheep will be similar to other currently marketed anthelmintic products. Animal health wholesalers, dealers, their distributors and licensed veterinarians will be utilized.

(g) Literature - Avermectins and Ivermectins as Insecticides, etc.

There are several reports in the literature describing the insecticidal as opposed to the parasiticidal activity of ivermectin and structurally related analogs, the avermectins.

The first report[3] tested the insecticidal activity of several avermectins against Tribolium confusum (confused flour beetle). Four avermectin analogues were 100% lethal by 28 days to T. confusum at 100 ppm, as compared to a 34% mortality in the controls. Malathion, the positive control, was more potent, showing similar activity at 10 ppm and less. This report and a second report[4] also reported on the ectoparasitic activity of avermectins on Cuterebra spp. (robust bot fly) larvae and Lucilia cuprina (sheep blow fly) larvae.

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc.

A report by Putter et al.[5] summarized the activity of avermectin B_{1a} against several mites, pesticidal worms, beetles, aphids, ants, larval flies and mosquitoes, and nematodes. Avermectin was active against all motile mite stages, but had no ovicidal activity. The lethal action of avermectin was slower than that of conventional organophosphates and pyrethroid insecticides. In the fire ant, avermectin permanently halted egg production in the queen at 0.12 g/ha, but was not 100% lethal to the worker ants. Larval flies and mosquitoes exposed to 2 to 50 ppb in their rearing medium failed to pupate. Another avermectin, B_{2a}, controlled soil nematodes at a rate of 0.16 to 0.24 kg/ha and was not observed to be phytotoxic to greenhouse tomatoes and cucumbers at doses as high as 10 kg/ha. It was postulated that the avermectins inhibit nervous signal transmissions at the neuromuscular junctions of arthropods and block signal transmission from ventral interneurons to excitatory motor neurons in the nematodes.

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc.

Reports[6-8] on the larvicidal activity of ivermectin, MK-933, towards horn, stable, face and house flies have been published. The first of these determined the LC_{50} and LC_{90} concentrations of insecticides for stable and horn flies in a larval medium of a dry mix, bovine feces and water. The larval susceptibilities were determined on the basis of emerging adults, corrected against the number of adults emerging from medium treated with acetone (insecticidal solvent) only. MK-933 displayed LC_{90} values of 0.186 ppm for stable flies and 0.006 ppm for horn flies by this method. The second report looked at larvicidal activity of MK-933 in the feces of steers given daily oral or subcutaneous doses, or a single subcutaneous or oral (bolus) dose. Daily oral doses as low as 20 mcg/kg were sufficient to prevent development of the immature stage of the stable fly, while as little as 0.5 mcg/kg/day provided horn fly control. A single injection of 200 mcg/kg, the anthelmintic dosage, controlled horn flies in the manure for up to 4 weeks post-treatment. Oral doses of 1 mcg/kg/day killed all horn fly larvae in the manure, while a 5 mcg/kg/day

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc.

oral dose killed all the face flies, about 60% of the stable flies and 90% of the house flies in the manure. The third report on larvicidal activity of MK-933 reported that a 200 mcg/kg injection resulted in 100% corrected mortality of the face fly larvae developing in the feces for 9 days. Larvae emerging from feces sampled 10 to 15 days posttreatment developed into malformed pupae, with approximately 90% failing to undergo eclosion. Effectiveness of ivermectin decreased after 15 days posttreatment.

Results of tests of avermectin B_{1a} against the red imported fire ants, Solenopsis invicta Buren, have been reported.[9,10] The avermectin B_{1a}, fed to laboratory colonies at concentrations as low as 0.0025% in soybean oil bait, inhibited reproduction of queens. Some worker mortalities occurred at concentrations of 0.025% or greater. Field tests indicated only 8 out of 928 colonies that fed on bait applied at rates of 0.0077 to 7.41 g/ha had worker brood. The primary effect of avermectin B_{1a} was on the reproductive capacity of the queen rather than acute toxicity

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc.

for the workers. The damage to the queen was characterized by irreversible cell and tissue damage to the ovaries, resulting in complete sterility or reduction in the numbers and size of eggs laid.

The efficacy of avermectins for rootknot control in tobacco was reported.(11) Control of Meloidogyne incognita was studied in tobacco fields for two seasons. Applications of 0.05 to 0.50 kg/ha suppressed root galling and egg production.

And finally, a recent article in Science summarized data on the microbiology, isolation and structure determination, chemistry, antiparasitic efficacy, mode of action, safety and metabolic disposition of the avermectin family of compounds.(12)

(h) Brief Description of Primary (and Secondary) Environment Affected

This subject is discussed more fully in Section D-2 following. The primary impact from the use of ivermectin in sheep on the natural environment will be the excretion of the drug by treated sheep via their feces and urine. Data have been collected relevant to

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected

the following properties and the drug sponsor has concluded that the use of ivermectin in sheep does not represent an action that would have a significant impact on the quality of the human environment.

In the analysis of the potential adverse impact on the environment from treating sheep with ivermectin, the following areas were examined and are reported in greater detail in Section D-2:

1) The Environmental Burden

The expected environmental burden based on the concentration of ivermectin and its metabolites in the accumulated waste in a sheep feedlot both under normal practices and in a "worst-case" situation. This environmental burden is calculated as the soil concentration (ppb) when feedlot waste is spread on a field at a level of nine tons per acre and mixed with the top 6 inches of soil.

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected

2) Stability in Soil

Half-life of ivermectin and ivermectin in feces was measured when treated soil was exposed to outdoor conditions in New Jersey, both in the winter and in the summer. Analysis of water percolated through these samples permitted characterization of the effluent.

3) Stability in Water

A sample of feces from steers dosed with radiolabeled ivermectin was extracted with water and the extractability and stability over eleven days were measured.

4) Soil Column Leaching

Experiments were carried out to determine the aqueous leaching of ivermectin and its metabolites from feces of sheep dosed with ivermectin. Feces alone and feces mixed 1:1 (w/w) with Iowa silt loam soil and placed on top of a 2 cm depth of this soil were leached for a few days with water. The leachates were assayed for total percolated drug and metabo-

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected

4) Soil Column Leaching (Cont'd)

lites, for extractability of radioactivity into dichloromethane, for percent of ivermectin in the leachates, and for toxicity of leachates towards Daphnia magna.

5) Soil Toxicity -- Microbial Effects

Feces from steers dosed with ivermectin were mixed with either pasture or forest soil and the effects on soil nitrification and soil respiration were measured.

6) Phytotoxicity of Ivermectin

A fresh-water, unicellular, non-motile chlorophyte, Chlorella pyrenoidosa was used in an algal toxicity test to measure the effect of ivermectin on overall cell growth, mean specific growth rate, maximum standing crop, algal biomass and lag period. Observations were also recorded relative to the phytotoxicity of avermectin on a variety of food crops during the conduct of insecticidal efficacy studies.

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected

7) Toxicity to Aquatic Organisms

The toxicity of ivermectin toward three aquatic species was measured: the bluegill sunfish, rainbow trout and the arachnid, Daphnia magna or water flea.

8) Toxicity to Nematodes, Arachnids and Insects

The effect of ivermectin and related compounds on a number of insects, phytophagous mites, and soil nematodes, was measured in a variety of tests.

9) Toxicity to Annelids

Studies were conducted to determine the LC_{50} for ivermectin to the earthworm, Eisenia foetida in artificial soil under controlled laboratory conditions.

From the results of these studies, it was concluded that the greatest potential for adverse environmental impact would be on aquatic organisms should ivermectin be permitted direct entry into ponds, streams, or rivers. The following statement was added to the labelling to avert such an action:

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected

9) Toxicity to Annelids (Cont'd)

"ENVIRONMENTAL SAFETY

Studies indicate that when ivermectin comes in contact with the soil, it readily and tightly binds to the soil and becomes inactive over time. Free ivermectin may adversely affect fish and certain water-borne organisms on which they feed. Do not permit water runoff from feedlots to enter lakes, streams, or ground water. Do not contaminate water by direct application or by the improper disposal of drug containers. Dispose of drug container by incineration or in an approved landfill."

A secondary and minor potential adverse impact on the evnironment could occur in the manufacture of ivermectin and in formulating IVOMEC 0.08% Oral Solution for Sheep. The environmental controls imposed during each of these operations at four locations have been examined and have been found to meet or exceed all of the requirements set forth by the respective governmental regulatory authorities.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden

The projected use of ivermectin in sheep involves the oral administration of the drug at a level of about 0.2 mg/kg body weight. The animals may be contained in a pasture or concentrated in a commercial feedlot. Generally, the sheep will receive only one dose of the drug; however, year-round parasite control programs could involve up to 3 or 4 treatments per year in young replacement stock.

Many sheep will be dosed with ivermectin in a large feedlot. The following calculations based on the U.S. Environmental Protection Agency publication[13] show the expected concentration of ivermectin and metabolites in the "Raw Waste" (manure) of a feedlot and the concentration in a field when the manure is spread as fertilizer. These calculations show that the concentration in the manure will be only 18 parts per

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

billion and in field, when spread as a fertilizer at a level of 9 tons per acre and mixed with the top 6 inches of soil, will be only 0.16 parts per billion.

Below (Figure 1) is a flow diagram from the above cited reference showing the daily raw waste produced in a typical feedlot operation in which a 36 kg lamb entered the operation and in 120 days reached a market animal weight of about 52 kg. During this period, the animal would be treated once with ivermectin at a dose level of 0.2 mg/kg.

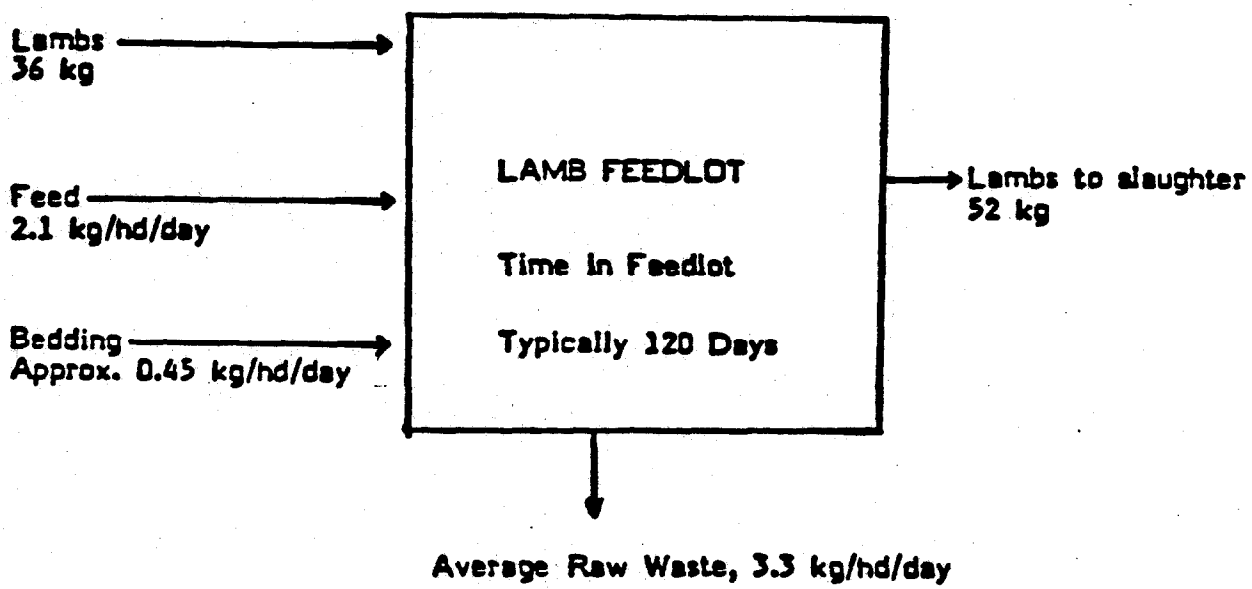


Figure 1

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

The following calculations show the average concentration of ivermectin and its metabolites in the waste produced by the single animal. The concentration, of course, will not change regardless of the actual number of sheep treated in the feedlot.

Weight of lamb	36 kg
Dose of ivermectin	x 0.2 mg/kg
Weight of ivermectin dosed	= 7.2 mg
Waste produced per sheep per day	3.3 kg
Total time in feedlot	x 120 days
Total waste produced per sheep	= 396 kg

Concentration of drug and metabolites in waste:

$$\frac{7.2 \text{ mg dose}}{396 \text{ kg waste}} = \frac{0.018 \text{ mg}}{\text{kg}} = 18 \text{ ppb}$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

If the manure from the feedlot were spread on a field as fertilizer at a rate of 9 tons per acre, equivalent to 190 to 250 lbs nitrogen per acre, [14-15] the total ivermectin plus metabolites would be 153 mg/acre or 3.5 micrograms per square foot:

Dose of ivermectin per lamb:	7.2 mg
Waste produced per lamb:	396 kg

Concentration of ivermectin and metabolites in waste:

$$\frac{7.2 \text{ mg}}{396 \text{ kg waste}} \times \frac{1 \text{ kg}}{2.2 \text{ lb}} \times \frac{2000 \text{ lb}}{1 \text{ ton (U.S.)}} = \frac{17 \text{ mg*}}{\text{ton (U.S.) waste}}$$

at a rate of 9 tons/acre:

(9 tons/acre)(17 mg/ton) = 153 mg/acre

$$\frac{153 \text{ mg}}{\text{acre}} \times \frac{1 \text{ acre}}{43560 \text{ sq.ft.}} \times \frac{1000 \text{ µg}}{\text{mg}} = 3.5 \text{ micrograms/sq.ft.}$$

*Equivalent to $\frac{18 \text{ mg}}{\text{ton (metric) waste}}$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

When mixed into soil at a depth of 6 inches, the total concentration of ivermectin plus metabolites would be only 0.16 ppb.

1 sq.ft. x 6 in. depth = (144 sq.in.)(6 in.) = 864 cu. in.

$$864 \text{ cu.in.} \times \frac{16.4 \text{ cc}}{\text{cu.in.}} \times \frac{1.5 \text{ g}}{\text{cc soil}} = 21{,}254 \text{ g soil}$$

$$\frac{3.5 \text{ µg}}{21{,}254 \text{ g}} \times \frac{1000 \text{ ng}}{1 \text{ µg}} = 0.16 \text{ ng/g soil} = 0.16 \text{ ppb}$$

Based on the analysis of the feces for the presence of unaltered drug (see Section b below) the concentration of ivermectin in the feces/soil mixture described above would be only about 0.10 ppb.

A "worst-case" situation in the utilization of manure from a sheep feedlot might include the use of the first three day's manure after dosing, which contains about 60% of the administered dose. The ivermectin plus metabolite level in the feces would then be:

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

$$\frac{\text{7.2 mg dose x 60\% excreted in first three days}}{\text{3.3 kg waste/day x 3 days}} = 0.436 \text{ mg/kg}$$

$$= 436 \text{ ppb}$$

Application of over 12 tons of livestock manure per acre per year is not recommended by the United States Department of Agriculture.(15) The Wisconsin Manure Management plan estimates the amount of nitrogen that can be applied annually without accumulating nitrates as 250 lbs. per acre.(16) This would be 9 to 12 tons per acre for sheep feces, based upon 21 to 28 lbs. nitrogen per ton fresh sheep manure.(14-15) Using 12 tons sheep manure/acre as a "worst-case," and the level of 436 ppb ivermectin and metabolites in the first three days post-dosing feces, would lead to an application of 109 micrograms of drug and metabolites per square foot:

$$0.436 \text{ mg/kg} \times \frac{2000 \text{ lb/ton}}{2.2 \text{ lb/kg}} = 396 \text{ mg/ton waste}$$

$$\frac{396 \text{ mg/ton} \times 12 \text{ tons/acre}}{43{,}560 \text{ sq.ft./acre}} = 109 \text{ micrograms/sq.ft.}$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

Plowing this application into a depth of 6 inches would lead to a "worst-case" level of 5.1 ppb of ivermectin and metabolites in soil.

$$\frac{109 \text{ micrograms/sq.ft.}}{21{,}254 \text{ g soil/1 sq.ft. x 6 in. depth}} = 5.1 \frac{\text{ng}}{\text{g soil}} = 5.1 \text{ ppb}$$

The "worst-case" of ivermectin application in pastured sheep would be an intensive pasturing situation with as many as 60 sheep/acre.[17-18] If the sheep were held at this level for 70 days, as is possible on Swede turnips, [18] then most or all of the 7.2 mg dose would be excreted, leading to an ivermectin and metabolite level or 432 mg/acre, or about 10 micrograms/sq.ft.:

$$\frac{7.2 \text{ mg}}{\text{lamb}} \times 60 \text{ lambs/acre} = 432 \text{ mg/acre}$$

$$\frac{432 \text{ mg/acre}}{43{,}560 \text{ sq.ft./acre}} = 10 \text{ microgram/sq.ft.}$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Metabolism of Ivermectin

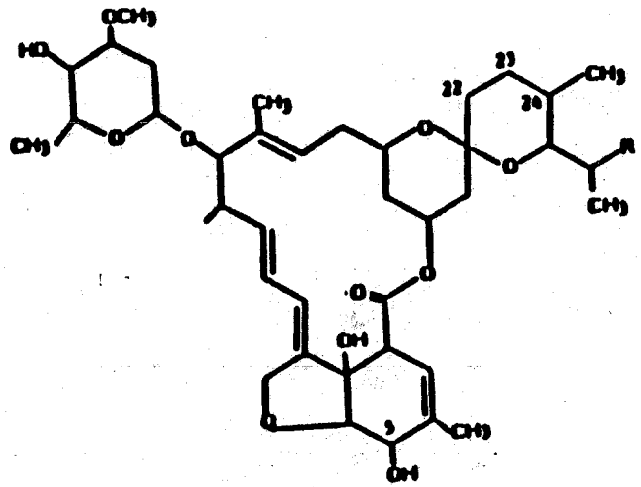
Essentially all of the dosed tritium-labeled ivermectin is excreted via the feces, either as the unaltered drug or as metabolites; only about 0.5% of the dose is excreted in the urine (see Table 2). Analyses of a 1-3 day feces composite by the reverse isotope dilution assay accounts for about 61% of the total radioactivity in the feces as unaltered drug. The remaining 39% of the radioactivity consists of ivermectin metabolites. These compounds are soluble in methylene chloride but are generally more polar than ivermectin. Based on extensive analyses of the residues found in the liver and fat of steers dosed with ivermectin, one might expect that the metabolites present in feces consist of the hydroxylated ivermectin and also the monosaccharides and aglycones of ivermectin and the hydroxylated analogs (see Figure 2). Figure 2 also shows the structural similarities between ivermectin and the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

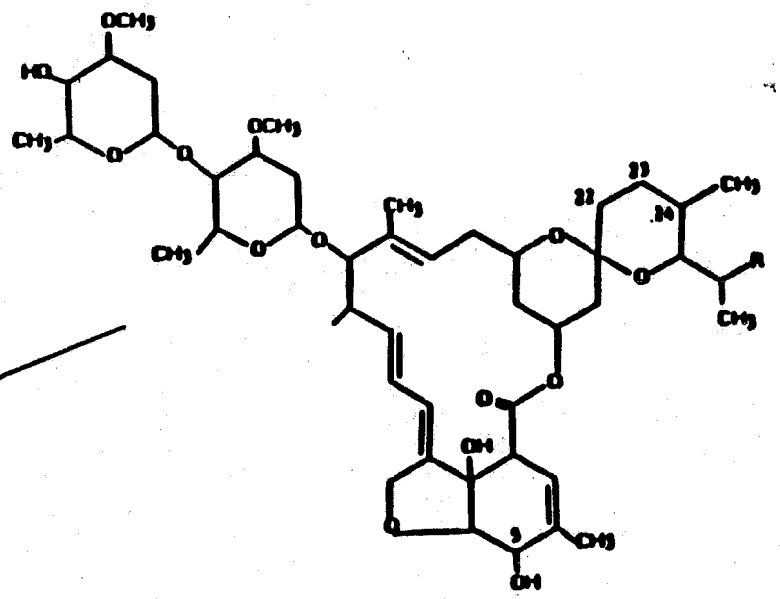
(b) Metabolism of Ivermectin (Cont'd)

products of its hydrolysis. Section D-2(k) summarizes the results of insecticide assays with ivermectin, ivermectin monosaccharide, ivermectin aglycone and avermectin B_{1a}.

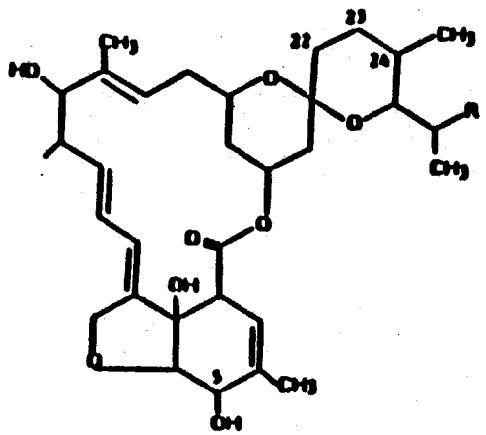
FIGURE 2: PRODUCTS OF HYDROLYSIS OF DIHYDROAVERMECTIN B_1



Dihydroavermectin-B_1-Monosaccharide



Dihydroavermectin-B_1



Dihydroavermectin-B_1-Aglycone

Dihydroavermectin-B_{1a}: $R = C_2H_5$

Dihydroavermectin-B_{1b}: $R = CH_3$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Metabolism of Ivermectin (Cont'd)

Biological assays of many compounds related to ivermectin indicate that these compounds appear to be less toxic than ivermectin towards Daphnia magna. As discussed in Section D-2(j), Toxicity to Aquatic Organisms, preliminary uncontrolled studies found that eluates (some diluted 1:1) from soil columns containing ivermectin and its bovine metabolites did not elicit a lethal effect in 48 hours on Daphnia.

A variety of biological assays of several compounds related to ivermectin showed that all these products are less active than the parent drug. Similarly, toxicity of monosaccharide and the aglycone of ivermectin toward Daphnia magna is less than that of parent compound. (See Table 3)

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Metabolism of Ivermectin (Cont'd)

Table 2

Comparison of Dosing and Excretion
of MK-0933 for Swine, Steer and Sheep

Animal	Dose Route in Study	Dosage Given In Study mg/kg	Dosage Sought In NADA mg/kg	% of Dose Excreted in First 7 Days		Percent of Parent Drug in Feces
				Feces	Urine	
Swine	Subcutaneous	0.4	0.3	36	0.4	39
Steer	Intraruminal	0.3	0.2	80	0.5	N.D.
Steer	Subcutaneous	0.3	0.2	62	1.5	40-50
Sheep	Intraruminal	0.3	0.2	69	0.5	61

N.D. = Not Determined

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Metabolism of Ivermectin (Cont'd)

Table 3

Comparison of the Toxicity of Ivermectin and the Monosaccharide and Aglycone of its Major Isomer

Compounds	48-Hour Daphnia Magna Results	
	LC_{50}, ppb	Approximate No Toxicity Level, ppb
MK-933 ($\geq$ 80% H_2B_{1a}, $\leq$ 20% H_2B_{1b})	.02	.01
H_2B_{1a}-MS (L638,724)	0.4	0.1
H_2B_{1a}-AG (L638,723)	> 17	10

Chemical structures may be found on page 43.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil

Experiments were conducted at the Merck Sharp & Dohme Research Laboratories to determine the stability of ivermectin in soil and of ivermectin in samples of composite sheep and composite steer feces in soil during the summer. Iowa silt loam soil was used for all samples. The samples were placed outdoors at the Merck site on June 24, 1981. Percolated rain water was collected as necessary and pooled until time of assay. Assay times were at 0, 1, 2, 4, and 8 weeks. Soil samples were assayed for total radioactivity and for the percent of total radioactivity represented by ivermectin. Percolated water samples were assayed for total radioactivity, for extractability into dichloromethane, and for the percent of total radioactivity contributed by ivermectin.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

The ivermectin in soil samples were prepared by mixing 1.13 µg ivermectin into 50 g of Iowa silt loam soil, for an ivermectin level of 22.6 ppb. Four 10-g portions were placed on top of glass fiber filters in coarse sintered glass funnels. One 10-g portion was reserved as a zero time sample. The funnels were placed on erlenmeyer flasks in a plastic tub at the outdoor site.

The composite feces in soil samples were all prepared by mixing 2.5 g of the appropriate feces with 47.5 g of Iowa silt loam soil. After thoroughly mixing, one 10-g portion was reserved as a zero time sample, while four 10-g samples were placed in coarse sintered glass funnels containing glass fiber filters. The funnels were placed on erlenmeyer flasks in the tub at the outdoor site. The feces/soil samples were 50 mg feces/g and total drug equivalent levels were 23 ppb for the com-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

posite steer feces in soil 52 ppb for composite sheep feces in soil.

The soil/feces samples were assayed by weighing, homogenizing with an equal weight of water, and combusting triplicate portions to determine total radioactivity per gram. The rest of the homogenate was spiked with unlabeled ivermectin and mixed with an equal volume of acetone. After centrifuging, the acetone was removed and a second acetone extraction was performed. The pooled acetone extracts were evaporated after an aliquot was removed for scintillation counting. The residue was dissolved in methanol, filtered and redissolved in methanol. Ivermectin components were isolated by high performance liquid chromatography (HPLC). The specific activity of the H_2B_{1a} and H_2B_{1b}

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

components of the ivermectin were determined to allow calculation of the percent of ivermectin in the sample.

The rain water which percolated through the samples was collected as needed and frozen until assay time. After thawing, the volumes were determined, aliquots were removed for scintillation counting, and unlabeled ivermectin was added. The aqueous samples were extracted twice with 10% by volume of dichloromethane. The pooled dichloromethane extracts were evaporated after aliquots were removed for scintillation counting and chromatographed to determine the percent of ivermectin in the percolates. In all cases, no ivermectin was observed in the aqueous percolates; only very polar compounds were present. The percent of ivermectin in the entire sample (feces/soil mixture plus percolated water) was determined by correcting the percent of ivermectin in the feces/soil mixtures for the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

amount of non-ivermectin radioactivity which percolated. Table 4 presents the results which are plotted in Figure 3. As can be seen, the level of ivermectin in each sample decreased "pseudo-exponentially" with apparent half-lives of 1 to 1.5 weeks in every case. The decomposition products were more polar compounds.

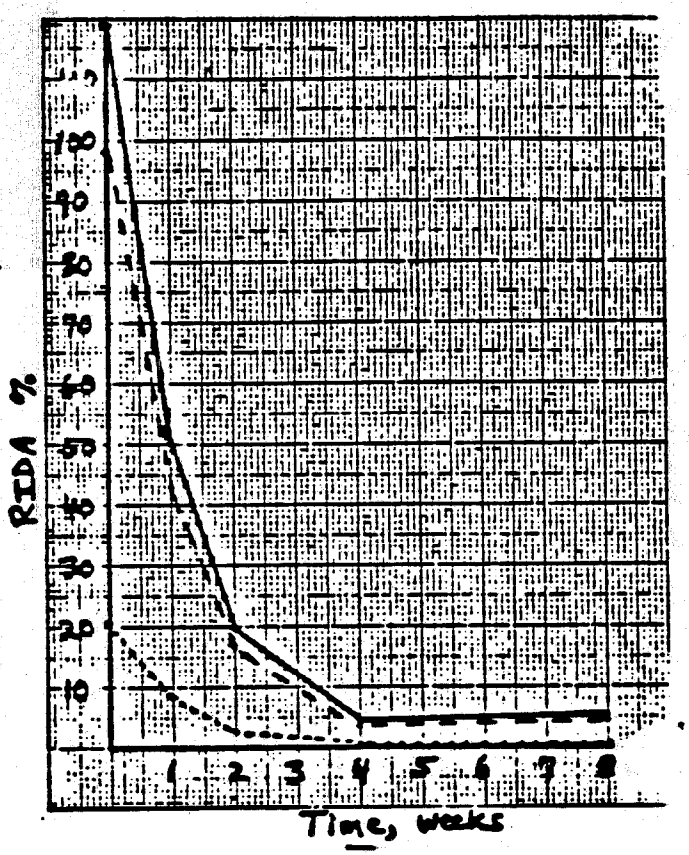
2. Discuss the Probable Impact of the Action on the Environment including primary and secondary consequences)

Table 4: RIDA Data for Outdoor Stability Study of Ivermectin

Sample	Time (weeks)	RIDA % of Soil/Feces H_2B_{1b}	RIDA % of Soil/Feces H_2B_{1a}	(1.00)- (Fraction of DPMs in percolated H_2O)	Corrected RIDA % H_2B_{1b}	Corrected RIDA % H_2B_{1a}	Total % MK-0933
Ivermectin in Soil (MK-933)	0	20.7	98.0	1.0	20.7	98.0	118.7
	1	9.7	47.5	.889	8.6	42.2	50.8
	2	3.5	21.0	.804	2.8	16.9	19.7
	4	1.2	5.5	.665	0.8	3.7	4.5
	8	1.5	7.9	.552	0.8	4.4	5.2
Composite steer feces in soil	0	14.0	30.1	1.0	14.0	30.1	44.1
	1	14.5	33.1	.634	9.2	21.0	30.2
	2	6.0	16.0	.725	4.4	11.6	16.0
	4	5.1	9.7	.468	2.4	4.5	6.9
	8	1.0	2.4	.423	0.4	1.0	1.4
Composite sheep feces in soil	0	13.3	61.2	1.0	13.3	61.2	74.5
	1	8.9	42.7	.841	7.5	35.9	43.4
	2	4.3	24.2	.766	3.3	18.5	21.8
	4	2.4	15.1	.649	1.6	9.8	11.4
	8	0.4	3.3	.650	0.3	2.1	2.4

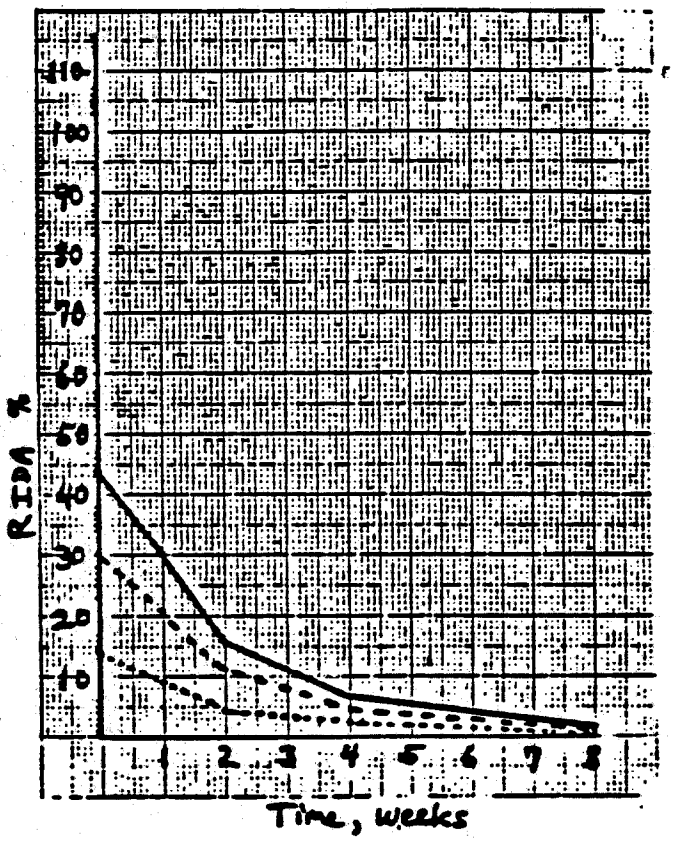
Figure 3. Corrected RIDA Data for Stability Study of Ivermectin

A. 11671-103A Ivermectin in Soil



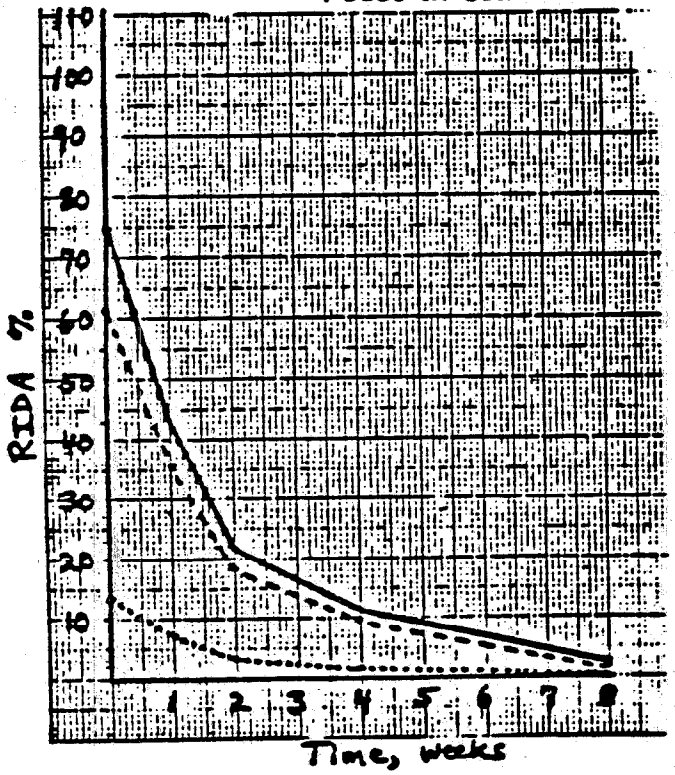
H_2B_{1b} —— H_2B_{1b} ·····

B. 11671-103B Composite Steer Feces in Soil



Total Ivermectin ——

C. 1167-103C Composite Sheep Feces in Soil



2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Experiments were also carried out at Merck Sharp & Dohme Research Laboratories (MSDRL) to determine the stability of ivermectin in soil, of ivermectin mixed with control steer feces in soil, of ivermectin and its metabolites in feces from steers dosed with tritiated ivermectin (composite feces) in soil, and of ivermectin in control steer feces when these samples were exposed to an outdoor winter environment. The amount of ivermectin remaining in these outdoor stability samples was determined by reverse isotope dilution assay (RIDA) at various times. Rain water, percolated through these samples, was collected and assayed for dichloromethane-extractable materials, including ivermectin, and for toxicity of the eluated materials towards Daphnia magna.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Samples of ivermectin in soil, of ivermectin in control feces in soil, and of composite steer feces in soil, were prepared. Triplicate, weighed portions of each sample were placed into 8 cm coarse-fritted sintered glass funnels. Triplicate samples of ivermectin mixed into control feces were weighed into 4 cm medium-fritted, sintered glass funnels.

The glass funnels were placed into filter flasks and the flasks were placed into a plastic tub and set outdoors in a fenced-in area at MSDRL where the samples were exposed to the outside environment. A graduated cylinder was also placed in the tub to measure rainfall.

The samples were placed outdoors on October 30, 1980. Outdoor stability samples were taken after 1 week, 3 weeks, 7 weeks and 13 weeks. Rain water and melted snow were collected as necessary.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Outdoor samples were assayed by adding a known amount of unlabeled ivermectin to a weighed sample. Sufficient acetone was added with mixing to equilibrate the added and endogenous ivermectin. Acetone was used to extract the cold carrier and endogenous tritium-labeled ivermectin. The extract was chromatographed on a high performance liquid chromatography (HPLC) system. The HPLC eluates which contained H_2B_{1b} and H_2B_{1a} components of MK-933 were collected.

The total radioactivity in the sample was determined by combustion of weighed samples. From the total radioactivity in the extracted sample, the amount of unlabeled H_2B_{1a} and H_2B_{1b} added, and the specific activities of the isolated compounds, the percent of

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

each ivermectin component in the sample could be calculated.

Percolated water samples were collected and the total concentration of ivermectin, its metabolites and degradation materials in solution was determined by counting aliquots. The amount of radioactivity extractable into dichloromethane was determined either by extracting the original solution, then adding unlabeled ivermectin for RIDA analysis, or by adding an equal volume of acetone and an aliquot of unlabeled ivermectin to the original solution, then extracting with dichloromethane. The material extracted into the dichloromethane was then chromatographed by HPLC, and the specific activities of the H_2B_{1a} and H_2B_{1b} components of ivermectin were determined. The percolated water samples were also assayed for toxicity towards Daphnia magna.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

The dichloromethane extraction and HPLC analysis of the leachate from outdoor stability samples of composite feces in soil indicated less than 10 ng ivermectin per liter in solution. This is below the 48-hour no-mortality level for toxicity toward Daphnia magna. This low level were confirmed in a preliminary, uncontrolled Daphnia toxicity experiment, where no mortality occurred in 48 hours. The dichloromethane extractions and HPLC analyses of the leachates from the outdoor stability experiments of ivermectin in soil, of ivermectin in control feces in soil, and of ivermectin in control feces also showed low levels of ivermectin in solution, and less than 50% mortality towards Daphnia magna after 48 hours.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

The polar compounds which eluted from the outdoor stability samples of ivermectin in soil represent extensive degradation of ivermectin. These compounds were more polar than the corresponding monosaccharides or aglycones of ivermectin. The amount of these polar decomposition materials in the soil samples when determining the rate of degradation of ivermectin in soil. Initially, ivermectin comprised 59% of the radioactivity in the ivermectin in soil sample and polar compounds accounted for only 2%. After 13 weeks, about 17% of the initial radioactivity had eluted as polar material. Inclusion of this eluted polar material with the polar compounds in the soil means that 46% of the radioactivity after 13 weeks was polar material, while 31% of the total radioactivity was ivermectin. Thus, in 13 weeks, the level of ivermectin decreased from 59% to 31% indicating a half-life of about 14 weeks.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Similarly, for the outdoor sample of composite feces in soil, 35% of the original radioactivity percolated in 13 weeks, and the decrease in remaining ivermectin in the sample suggested a half-life of about 31 weeks. Ivermectin in control feces in soil, the half-life estimate was 52 weeks, and for ivermectin in control feces without soil, it was 16 weeks.

Thus, there were low levels of ivermectin in the leachates from the outdoor winter stability samples of ivermectin in soil, ivermectin in control feces in soil, ivermectin in control feces without soil, and of composite feces in soil. The levels of ivermectin in these were below the LC_{50} level of 20 ng/l as determined by Daphnia magna assay. Most of the percolated radioactivity represented highly polar material, indicating extensive degradation of the original ivermectin. Half-life estimates for the degradation of

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

ivermectin in various soil/feces mixtures in an outdoor environment from November through January ranged from 14 weeks to 52 weeks.

Experiments on the stability of ivermectin in feces mixed with soil were carried out in aerated brown bottles at 22°C over a period of 3 to 18 days. The experimental set consisted of two types of samples: (1) feces from steers dosed with tritium-labeled ivermectin and (2) control feces spiked with tritium-labeled ivermectin. Each of these materials was mixed with either sandy loam pasture or gravelly clay forest soils. The experiments were set up with triplicate samples. On a prescribed schedule, the samples were extracted with water and then water/acetone. The extracts and spent solutions were assayed for radioactivity content and the water/acetone extract assayed by an HPLC/fluorometric procedure for the ivermectin content.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Considerable intragroup and intergroup variation in the radioactivity of ivermectin assays was encountered. Calculations of the half-life for the degradation process were by the linear least squares fitting of the logarithm of concentration at sample time. The half-lives indicate a relatively slow, but significant, degradation of ivermectin. Thus, there would not be a gradual accumulation of ivermectin even if it were introduced into the soil on repeated but infrequent intervals.

Half-lives of Ivermectin Degradation in Feces/Soil Mixtures

Sample	Soil	Half-life-days
Feces from dosed animal	Pasture	196
Feces from dosed animal	Forest	111
Control feces plus ivermectin	Pasture	169
Control feces plus ivermectin	Forest	260

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

At the end of the experiment, a small fraction (2.4-7.0%) of the initial radioactivity had been collected in the ethanol used to trap volatile products. The formation of such products indicates that extensive degradation of the ivermectin and metabolites had occurred.

Further analysis of the radioactivity in these ethanol traps at MSDRL showed that the radioactive component was completely volatile. Mild heating of the trap samples gave distillate and residue fractions each with the same specific activity. Thus, this volatile radioactivity appears to be tritiated water. Since the tritium label is in a chemically inert portion of the ivermectin molecule, any loss of tritium would have to occur after extensive degradation of this portion of the molecule.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Stability of Ivermectin in Aqueous Extracts of Steer Feces

To simulate the situation expected to arise in runoff from feedlots containing ivermectin-treated cattle, the extractability of ivermectin and its metabolites from fecal material by water, and the stability of ivermectin in the aqueous phase, were studied.

Samples of feces from steers dosed with tritium-labeled ivermectin were extracted with either lake water or reverse osmosis water at a level of 50 mg of feces/ml of water. The feces used in the study were a composite collected 2 to 5 days after a 0.3 mg/kg dose, and the level of ivermectin and its metabolites was 600 ppb. This level is over 30 times the level of 18 ppb expected under typical feedlot conditions [see Section D-2(a), Environmental Burden].

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Stability of Ivermectin in Aqueous Extracts of Steer Feces (Cont'd)

After blending, centrifuging and filtering through glass fiber filters, the fecal extracts were transferred to 1000-ml amber bottles, sealed with rubber septa and stored at 22°C. Samples were flushed three times a week with dry air to prevent anaerobiosis. Weight losses caused by evaporation during flushing were corrected by addition of reverse osmosis water. Samples were assayed at 0, 2, 7, and 11 days.

At a loading of 50 mg feces/ml water, 30 ppb would be the maximum nominal concentration of ivermectin and metabolites in the extracts. Based upon the tritium-activity, lake and reverse osmosis water extracted 36.5 and 35.0%, respectively of the total radioactivity, for solution concentrations of about 10-12 ppb in total drug-equivalent. High performance liquid chromatographic analysis determined the concentration of ivermectin in lake water at 2.8 ng/ml (ppb) and at 2.6 ng/ml in reverse osmosis water, which are 9.3% and 8.7%

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Stability of Ivermectin in Aqueous Extracts of Steer Feces (Cont'd)

of the maximum nominal concentrations, respectively. Comparison of mean tritium-activity in the extracts and mean ivermectin concentration between lake and reverse osmosis water showed no significant differences. Also, there was no significant decline in the ivermectin or total residue level during 11-day study in either lake or reverse osmosis water.

The small amount of unaltered drug extracted from the feces into the water probably reflects sorption to the organic material in the cattle wastes.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching

Experiments were carried out to determine the aqueous leaching of ivermectin and its metabolites from feces of sheep dosed with ivermectin. Feces alone and feces mixed with 1:1 (w/w) with Iowa silt loam soil and placed on top of a 2 cm depth of this soil were leached for a few days with water. The leachates were assayed for total percolated drug and metabolites, for extractability of radioactivity into dichloromethane, for percent of ivermectin in the leachates, and for toxicity of leachates towards Daphnia magna.

For the leaching of composite feces mixed into soil, 50 g of composite sheep feces was mixed into 50 g of Iowa silt loam soil. The drug equivalent level was 516 ppb, over three thousand times higher than expected in fertilized fields. Ninety grams of this mixture was placed on top of a nine centimeter diameter by 2 cm

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

deep column of Iowa silt loam soil. A similar column was prepared with control sheep feces. A volume of 944 ml (over 10 column void volumes) of distilled water was passed through the control feces plus soil column. The total drug equivalent in the leachate from the composite sheep feces plus soil column was 2.9 ppb. A portion, taken for chemical analysis, was extracted with dichloromethane after 0.9 mg of ivermectin was added. Only 36% of the radioactivity was extractable into the dichlormethane, and chromatography of the extracted material showed only about 1% of the percolated drug-equivalent was ivermectin, or 0.029 ppb. Thus a total of 0.027 µg of ivermectin had percolated.

The remainder of the percolated water was used for a bioassay with Daphnia magna. The solution was assayed at full strength (2.9 ppb) and at several dilutions

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

with spring and river water. The leachate from the control sheep feces plus soil column was similarly diluted for control solutions in the bioassay. High *Daphnia* mortalities were observed in the leachates from the control feces/soil columns, especially undiluted and diluted with less than three equivalent volumes of water. This mortality in the concentrated leachates was presumably due to bacterial or osmotic effects. However, leachates from control feces/soil and composite feces/soil, diluted with 3 parts or with 7 parts of water caused no *Daphnia* mortalities in 48 hours. In these solutions, the total drug-equivalent concentrations were 0.73 and 0.37 ppb, respectively. Since ivermectin was chemically determined to be 1% of the drug-equivalent in the percolate, the respective solution concentrations of ivermectin were 0.007 and 0.004 ppb, both below the 48 hour no-mortality level of 0.010 ppb. Thus, the percolated drug metabolites were not

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

toxic in these two solutions at 0.73 and 0.37 ppb, indicating the water leachable metabolites are at least 37 to 73 times less toxic than the parent compound. These results are entirely consistent with those obtained in previous MSDRL studies with composite steer feces in soil.

For the leaching of sheep feces alone, forty five grams of composite sheep feces, containing 1032 ppb total drug equivalent (roughly 60% ivermectin and 40% polar metabolites) was place into a sintered glass funnel. This drug equivalent level is 57 times the level expected in feedlot feces. A total of 234 ml of distilled water was passed through the feces during the four days. The total drug equivalent concentration in the percolated water was 53.4 ppb and 26% of the total radioactivity percolated. Ten percent of the sample

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

was taken for chemical analysis, the remainder was used for bioassay. A sample of 222 ml of distilled water was passed through a sample of control sheep feces. This sample was used for the control for the bioassay tested.

To the percolated water sample used for chemical analysis was added 0.9 mg ivermectin in methanol and 20% (v/v) of dichloromethane. After shaking, the dichloromethane was removed. A second 20% (v/v) of dichloromethane was used for a second extraction. After removing an aliquot for scintillation counting, the combined extracts were evaporated, the residue was dissolved in methanol and chromatographed. Only 24% of the aqueous radioactivity was extractable into dichlormethane and chromatography showed about 13% of the percolated drug equivalent was ivermectin. Thus, 1.6 µg of ivermectin had percolated.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

The percolates through the control and composite sheep feces were diluted with a 1:1 mixture of spring and river water to provide solutions for toxicity assays using Daphnia magna. As with the leachates through the control feces/soil columns, there were unacceptably high Daphnia mortalities in many of the dilutions tested. There were no dilutions tested with sufficiently few Daphnia mortalities in the controls ($\leq$ 10%) and sufficient survivals in the correspondingly diluted percolate from composite sheep feces to meaningfully assess the toxicities of the leachable drug metabolites relative to parent drug. The composite feces used to generate the bioassay samples (1032 ppb in total drug-equivalent) had an ivermectin level 57 times that expected in feedlot feces (18 ppb) and over twice that calculated in a "worst case" feedlot situation (436 ppb). At the proposed target dose level of 0.2 mg/kg, feedlot run-off might be expected to have total drug

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

and metabolite levels on the order of 35.6 ppb, since leachates from the feces of sheep dosed at 0.3 mg/kg contained 53.4 ppb drug equivalent. Thus, run-off from a sheep feedlot may have levels of ivermectin toxic to aquatic organisms, but this run-off would be quickly detoxified by passing over or through relatively small amounts of soil.

Since both the feces column and the feces plus soil column contained 45 g of composite sheep feces, the removal of most of the aqueous ivermectin by soil can be seen. Whereas 1.6 µg of ivermectin percolated from the sheep feces column, only 0.027 µg percolated from the sheep feces plus soil column.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

Additional column leaching experiments were carried out at MSDRL to determine the mobility of ivermectin in soil, of ivermectin mixed with control feces in soil and of ivermectin and its metabolites from feces of ivermectin-dosed steer mixed with soil. Leaching experiments were also run with ivermectin mixed into control steer feces and with feces from steers dosed with ivermectin, both in the absence of soil. Leachates from the columns were assayed for extractability of radioactivity, as tritium-labeled ivermectin and its metabolites, into dichlormethane and for toxicity of eluates towards Daphnia magna.

Four types of columns were used. Two cm diameter glass tubes, with coarse-fritted disks and glass fiber filters at the bottom were closed off with rubber tubing and pinch clamps. Iowa silt loam soil was added to the column, the pinch clamp was opened, and the soil

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

was allowed to pack while the water drained. Initially, 22 cm long columns were prepared. However, the flow rates from these columns were so slow that shorter columns, five cm long, were prepared to obtain enough volume of leachate to assay.

Three 5 cm by 2 cm diameter columns were prepared. A control feces column was prepared by mixing 1.5 g of control steer feces into 1.5 g of soil. This mixture was added to the top of a soil column. A second column was prepared by mixing 1 μg of tritium-labeled ivermectin into 1.5 g of feces from dosed steers (composite feces) mixed into 1.5 g soil, and placed on top of a 5 cm soil column. The ivermectin and metabolite level in this sample was about 300 ppb.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

The effluent from the control column served as a control in the Daphnia assays. Only 0.3% of the radioactivity applied to the second column (as labeled ivermectin) was eluted. Over 20 void volumes of water percolated through the composite feces column, eluting 30% of the initial charge of applied radioactivity. Only 17% of the eluate's radioactivity was extractable from a 1:1 mixture of eluate and acetone by dichloromethane. High performance liquid chromatography (HPLC) analysis of this dichlormethane extract showed no detectable ivermectin. The original eluate, diluted 1:1 with spring water, was not toxic to Daphnia magna at 0.48 ppb (or 24 times the LC_{50} concentration), in a preliminary uncontrolled assay.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

The 5 cm long columns were segmented. In the ivermectin column 85% of the recovered radioactivity was still in the soil layer on top of the column. On the other hand, the original feces/soil mixture on top of the composite feces column contained only 41% of the recovered radioactivity. This column showed greater amounts of radioactivity along the column than did the ivermectin column.

Even shorter columns, 2 cm high by 9 cm diameter, were prepared to increase the amounts of effluents. Again, feces from steers dosed with tritium-labeled ivermectin, when mixed 1:1 with soil for a drug and metabolite level of about 300 ppb, was applied to the top of a 2 cm deep column. A control column of control steer feces plus soil was also prepared. A third column, consisting of 39 µg of tritium-labeled ivermectin was prepared at an ivermectin level of 455 ppb. A soil

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

blank, without drug or feces, was also prepared. About 15 to 20 void volumes of water percolated through these columns. About 9% of the radioactivity eluted from the composite feces column, while only 0.5% of the radioactivity eluted from the ivermectin column. The effluent from the column charged with the feces from steers dosed with ivermectin was again non-toxic to Daphnia magna at 3.2 ppb. This is about 160 times the 48-hour LC_{50} value for ivermectin of about 0.020 ppb. The effluent from the column charged with 455 ppb of ivermectin did show toxicity towards Daphnia magna, with an apparent 48-hour LC_{50} of about 0.12 ppb. This would indicate about 32 ng of ivermectin per liter of effluent from this high level of ivermectin in soil.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

Another experiment involved the leaching of ivermectin and its metabolites from steer feces. No soil was used to reduce the amount of ivermectin or its metabolites in the leachate. Samples of 45 g of control feces, 45 g of control feces into which 30 µg of ivermectin had been mixed, and 45 g of feces from steer feces dosed with ivermectin (composite feces) were placed into 6 cm diameter sintered glass funnels of medium porosity. The level of ivermectin and metabolites in the composite feces sample was about 630 ppb. About 100 ml of water was slowly percolated through each sample, eluting only 0.2% of the radioactivity from the tritium-labeled ivermectin/feces sample, and about 11% of the radioactivity from the composite feces sample.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

The eluates from the three samples were assayed in a preliminary, uncontrolled assay for toxicity towards Daphnia magna. All eluates were diluted with an equal volume of distilled water to provide sufficient volume for assay. The diluted eluate from the control feces was not toxic to Daphnia magna, but the diluted eluate from ivermectin plus feces sample was toxic to about half the Daphnia at 0.35 ppb. The eluate from the composite feces sample was toxic at 13 ppb. Further dilution of this sample suggested an LC_{50} of about 7 ppb, which is about 350 times the 48-hour LC_{50} of ivermectin. From this LC_{50} of value, the amount of ivermectin in the original eluate would have been about 0.07 ppb, or less than 0.3% of the total drug-equivalent present.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

Thus, some ivermectin can be leached out of feces when the original ivermectin level is at 600-1000 ppb. This is much higher than the 18 or 19 ppb for ivermectin and metabolites expected in sheep and steer feedlot feces. Passing these eluates through a very short layer of soil appears to lower the amounts of ivermectin in solution, leaving soluble metabolites less toxic than ivermectin to Daphnia magna.

In another study, soil of four different types was charged in triplicate to columns of 19 mm ID nylon film tubing to a depth of 30 cm. A mixture consisting of 0.5 g of a feces composite from three steers dosed with ivermectin and 1.0 g of soil was placed on top of each

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

of the 12 columns of soil. The charge of ivermectin plus metabolites in the feces sample was 330 ng based on the total radioactivity in the sample. Water was added to the top and allowed to percolate through the column.

The experiment continued for about 7 weeks. The total amount of water passing through the column depended on the type of soil in the column and varied from 2-7 column volumes (180-600 ml).

The leachate from each column was collected in fractions and each was assayed for radioactivity content. The radioactivity profiles of the leachate are shown in the appended report. The data in Table 5 below summarized the recovery of the radioactivity charged to the column and the analysis of the leachate for unaltered ivermectin. It will be noted that the per-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

cent of radioactivity recovered in the leachate plus column (total recovered) was satisfactory (90-105%). There was no detectable unaltered ivermectin in the leachate suggesting that the drug had undergone decomposition in the soil/feces mixture and/or had absorbed firmly to the soil in the column.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

Table 5

Soil Column Leaching - Summary of Results

Soil	Leachate ml	Percent of Charged Radioactivity* Leachate Total	Leachate Per ml	Column	Total Recovered	Analysis of ivermectin in Leachate
Silt Loam	295	27.0	.091	69.6	96.6	ND**
Clay Loam	595	48.1	.081	57.3	105.4	ND
Sandy Loam	591	42.7	.072	52.9	95.6	ND
Loam	193	9.8	.051	81.5	91.3	ND
Sandy Loam***	632	46.8	.074	43.8	90.6	ND

* Total Radioactivity charged to columns: $6.60\text{-}6.62 \times 10^4$ dpm.

** ND - ivermectin not detected at the limit of 13 ng per assay.

*** Applied soil/feces mixture which had been aerobically aged for 30 days.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Soil Column Leaching (Cont'd)

In these soil percolation experiments the concentration of ivermectin plus metabolites in the steer feces was 660 ppb. This value is substantially higher than the value of 19 ppb calculated for the residue concentration in the cattle feedlot waste. In the laboratory the fecal sample was a composite of 2-5 day post-dose collection, a period when most of the drug and metabolites are excreted. The 19 ppb arises from the calculation based on the more realistic dilution that occurs during the 130 days in the feedlot.

Because of the extremely low concentrations of residues in the feedlot wastes and the detection limits of the assays, it was not feasible to dilute the feces containing the 660 ppb of residue. The detection limits were about 1 ppb for the radioactivity measurements and about 10 ppb for the fluorescence assay of ivermectin. However, even at this excessively high column loading, about 35-fold higher (660 ppb/19 ppb) than that expected in the feedlot, there was no detectable ivermectin in the leachate.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Distribution of Ivermectin Between Water and Soils

The absorption of ivermectin from water by soil was investigated using tritium-labeled ivermectin at an initial concentration of about 2 µg/ml H_2O (ppm) with varying amounts of soil. The final aqueous phase ivermectin concentration ranged from about 0.9 to 0.06 µg/ml H_2O, depending on the amount and nature of the soil. Soils from Iowa and New Jersey were investigated. The Freundlich constants K, an empirical constant analogous to a distribution coefficient (soil/water), were found to be approximately 320 and 540 for Iowa and New Jersey soils, respectively.

The absorption and desorption of tritium-labeled ivermectin was investigated in greater detail with the Iowa soil. Triplicate samples containing 1.5 g of soil of clay loam texture, 7.5 ml 0.01 M $CaCl_2$ solution and 10, 25, 50 or 100 µl of ivermectin in methanol at

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Distribution of Ivermectin Between Water and Soils (Cont'd)

0.463 mg/ml were mixed for 16 hours in the dark, then centrifuged. The ivermectin in solution was determined by scintillation counting of the aqueous layer, while the bound ivermectin was determined by combustion of a portion of the soil. The soil samples were desorbed twice by replacing the removed aqueous layer with fresh $CaCl_2$ solution. The solution concentrations of ivermectin ranged from 0.001 µg/ml to 0.17 µg/ml while the bound concentrations ranged from 3.1 µg/g to 40.4 µg/g. The ratio of bound ivermectin to free (K_d) was slightly less during adsorption than desorption steps, but the average value for both adsorption and desorption was 332.7. From this value (the distribution constant) the binding constant normalized to the organic carbon content, K_{oc}, was calculated to be 12600, since $K_{oc} = 100 \times K_d/\%oc$ and %oc = % organic matter/1.724 (19). For this Iowa soil, the % organic matter was 4.56%.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Distribution of Ivermectin Between Water and Soils (Cont'd)

The adsorption and desorption data was also fit to the Freundlich equation:

$$\frac{x}{m} = K_c^{1/n}$$

where x/m is the μg bound/g soil, c is the solution concentration and K and n are constants. If n=1, then the Freundlich K equals the distribution coefficient, K_d. Using all the adsorption and desorption data, overall values of K = 207 and 1/n = 0.8652 were obtained.

The value of K_{oc} can also be used to estimate the R_f value for ivermectin on soil, thin layer chromatography plates as follows:

$$R_f = \frac{1}{1 + (K_{oc})(\%\ oc/100)(d_s)(1/\theta^{2/3} - 1)}$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Distribution of Ivermectin Between Water and Soils (Cont'd)

where Θ is the soil pore fraction, d_s is the density of the soil solids, and % oc is the % organic matter/1.724.(19). Using Θ = 0.5, d_s = 2.5 g/cc, % oc = 1.40, values appropriate for a Hagerstown silty clay loom (19), and K_{oc} = 12600 for ivermectin, R_f is calculated to be 0.0038. This value classified ivermectin as "immobile" (19) and is consistent with unpublished soil TLC studies with the related compound, avermectin B_{1a}. In those studies avermectin displayed no mobility on TLC plates of six soil types.

At the level of interest, 0.10 ppb in the plowed field, calculations based on the distribution coefficient, K_d show that the total concentration of drug and metabolites in the water equilibrium with the top 6 inches of soil would be about 0.3 parts per trillion. That is, K_d = soil ivermectin concentration ÷ solution ivermectin concentration = 332.7 for Iowa clay loam soil, therefore:

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Distribution of Ivermectin Between Water and Soils (Cont'd)

$$\text{Solution concentration} = \frac{\text{soil concentration}}{K_d}$$

$$= \frac{0.10 \text{ ppb}}{332.7} = 0.00030 \text{ ppb}$$

$$= 0.30 \text{ parts per trillion.}$$

Thus, in plowed fields under expected fertilizer applications, the ground water in contact with the fertilized soil would have ivermectin concentrations 33-fold lower than the 48-hour no-mortality level for Daphnia magna which is about 10 parts per trillion.

The results from these soil-binding experiments are consistent with those obtained in the column leaching studies. In the latter experiment, feces containing ivermectin was placed on top of 30 cm columns of soil. The columns were washed with water but the ivermectin was bound to the soil and no detectable amount was found in the leachate.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Distribution of Ivermectin Between Water and Soils (Cont'd)

The binding of ivermectin to soil and the practical implications of this binding was further demonstrated in toxicity studies using Daphnia magna as the test organism. The toxicity of ivermectin to the Daphnia was reduced by more than 99% by mixing the test solution with 2.5 g of soil/100 ml.

(g) Biological Activity and Toxicity of Ivermectin Metabolites

A variety of biological assays of several compounds related to ivermectin showed that all these products are less than active than the parent drug. Similarly, toxicity of the monosaccharide and the aglycone of ivermectin toward Daphnia magna is less than that of the parent compound by a factor of 20-40 fold (monosaccharide) and at least 200-400 fold (aglycone). Analysis of the feces from a sheep dosed with tritium-labled ivermectin shows that only 60% of the total

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Biological Activity and Toxicity of Ivermectin Metabolites (Cont'd)

fecal residue is intact drug. Thus, 40% of the fecal residue will probably be many fold less toxic to aquatic organisms than is ivermectin. This was the conclusion from the bioassay of percolated steer and sheep metabolites.

A substantial reduction in toxicity was observed in experiments carried out at the Merck Sharp & Dohme Research Laboratories with feces from ivermectin-dosed steers mixed into soil at ivermectin residue levels of 33 ppb, over 360 times the level expected in the field. Aqueous leachate from this experiment showed no toxicity towards Daphnia magna at a solution concentration of 260 parts per trillion drug-equivalent, about 13 times the LC_{50} of the parent drug.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Biological Activity and Toxicity of Ivermectin Metabolites (Cont'd)

Feces from dosed steers were also mixed with soil in 1:1 ratio. This provided a drug residue level of over 300 ppb, three-thousand times the level expected in a field plowed with dosed-steer feces. Leachate from this mixture, after passing through a 2 cm deep soil column, was also not toxic to Daphnia magna at a concentration of drug-equivalent of 3160 parts per trillion, almost 160 times the LC_{50} level of the parent drug.

(h) Soil Toxicity-Microbial Effects

A laboratory screening test was conducted to determine the potential for ivermectin residues present in wastes from treated cattle to affect two soil processes: the microbial conversion of soil ammonia to nitrate (nitrification) and the overall conversion of carbonaceous soil organics to carbon dioxide (soil microbial respiration). Other soil microbial community processes and activities were not examined, nor were any tests conducted with sheep feces.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Soil Toxicity-Microbial Effects (Cont'd)

In pasture and forest soils to which approximately 5% feces from a steer treated with ivermectin was added, no biologically significant effects on nitrification or overall soil community respiration were observed during the one-month test period when those results were compared to control soils amended with steer feces that did not contain ivermectin residues. The 5% amendment rate was approximately ten times the rate normally used when cattle manure is applied to agricultural soils as a fertilizer. No other amendment levels or ivermectin doses were screened in this test.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Soil Toxicity-Microbial Effects (Cont'd)

(1) Nitrification

The effect of steer feces containing ivermectin and its metabolites on the nitrification process in two types of soil was determined by measuring their effect on the reaction.

$$NH_4^+ \text{ ——— } NO_2^- \text{ ——— } NO_3^-$$

An aqueous homogenate of steer feces was added to either pasture or forest soil at a level of 50 mg feces/g soil (or 30 ppb of ivermectin plus metabolites). After the mixture aged for periods from 0 to 4 weeks, $(NH_4)_2SO_4$ (at a level of 100 ppm N) was added and the system further aged for 1-2 weeks. The samples were extracted and the concentration of ions in solution measured by the use of ion-specific electrodes. Sodium azide (NaN_3) at a concentration of 1000 ppm was used as a positive control.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Soil Toxicity-Microbial Effects (Cont'd)

The data summarized in Table 6 show a very small, variable effect of ivermectin on the nitrification process in soil as indicated by the measured ion concentrations. The positive control NaN_3, at 2 and 4 weeks consistently showed reduced concentrations of NO_3^- and increased concentrations of NH_4^+ and NO_2^- compared with the controls.

Even though there is essentially no effect of ivermectin and its metabolites on the nitrification process in soil at the level of 30 ppb, the actual concentrations in soil (assuming a mixture down to 6 inches), is about 0.1 ppb or a factor of 300-fold lower.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Soil Toxicity-Microbial Effects (Cont'd)

Table 6: Effect of Ivermectin and Metabolites in Feces on Soil Nitrification Process

Assay Weeks	Soil Type	Treatment	Mean - ppm N in Soil NO_3^-	NH_4^+	NO_2^-
0	Pasture	Control Feces	42	35	10
		Feces + drug	51	46	9
		NaN_3 1000 ppm	41	94	9
0	Forest	Control	14	85	0.5
		Feces + drug	14	101	0.5
		NaN_3	14	119	13
2	Pasture	Control	85	9	2
		Feces + drug	120	5	1
		NaN_3	30	100	12
2	Forest	Control	38	73	2
		Feces + drug	43	92	2
		NaN_3	25	141	26
4	Pasture	Control	127	10	6
		Feces + drug	155	7	6
		NaN_3	51	115	11
4	Forest	Control	43	69	1
		Feces + drug	33	71	1
		NaN_3	18	145	19

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Soil Toxicity-Microbial Effects (Cont'd)

(2) Respiration

The effect of steer feces containing ivermectin and its metabolites on the respiration process in two types of soils was determined by periodically measuring the CO_2 content in the head gas of the bottles containing the feces-soil mixture.

The steer feces had no effect on respiration compared to the controls in pasture soil and caused only a very small increase in respiration in forest soil. Sodium azide clearly depressed respiration in both soils. The data are summarized in Table 7.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Soil Toxicity-Microbial Effects (Cont'd)

Table 7: Effect of Ivermectin and Metabolites in Feces on the Soil Respiration Process

Assay Days	Soil Type	Mean Accumulation of % CO_2		
		Control	Feces	Sodium Azide
1	Pasture	1.9	1.8	1.1
	Forest	3.5	3.1	1.6
2	Pasture	3.0	2.7	1.4
	Forest	5.3	4.7	2.1
6	Pasture	6.9	6.1	1.9
	Forest	10.4	9.9	3.0
10	Pasture	10.8	10.1	2.2
	Forest	15.7	15.7	3.5
20	Pasture	19.4	17.6	2.9
	Forest	27.8	30.2	4.6
30	Pasture	24.3	21.5	3.2
	Forest	34.2	37.8	5.1

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Soil Toxicity-Microbial Effects (Cont'd)

As with the nitrification experiment, the test level of 30 ppb is a factor of 300-fold higher than that which would be expected in a field fertilized with manure from a feedlot (at 9 tons/acre) and plowed to a depth of 6 inches (a calculated concentration of about 0.16 ppb).

(i) Phytotoxicity of Ivermectin

Chlorella pyrenoidosa, a fresh water unicellular, non-motile chlorophyte, was used in an algal assay bottle test to determine the toxicity of ivermectin toward algae. The experiment was carried out by preparing a stock solution of ivermectin in N,N-dimethylformamide (DMF) at a concentration of 20 mg/ml. The test concentrations were prepared by mixing the required volume of stock solution with synthetic algal nutrient medium to yield the appropriate final concentration.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

A medium control, a solvent control (0.5 ml DMF/l) and ivermectin test concentrations of 1.0, 1.8, 3.2, 5.6, and 10.0 mg/l (ppm) were prepared.

The tests were carried out in Erlenmeyer flasks, continuously agitated under fluorescent lighting and maintained at 24°C. Cell counts were made on 0, 2, 3, 4, 7, 9, 11, and 14 test days. The following results were obtained:

(1) Effect on overall cell growth: None

(2) Effect on μmax (mean specific growth rate): None

(3) Effect on Maximum Standing Crops (MSC), cell/ml: Significantly reduced when compared to controls.

(4) Effect on algal biomass: Significantly reduced in the 10.0 mg/l concentration.

(5) Effect on lag period: None

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

It is obvious from these results that ivermectin, at these relatively high concentrations, has moderate effect on the growth characteristics of this alga.

Only a very limited amount of studies have been conducted on the application of ivermectin to plant foliage or roots. However, a considerable volume of data has been generated on three ivermectin analogs, avermectin B_1, avermectin B_{2a}, and avermectin B_{2a} 23-ketone, of interest in plant agriculture which demonstrates the complete lack of phytotoxic effects at application rates as high as 9.0 lb active ingredient per acre. A summary of the studies follows:

To date the three avermectin compounds of interest to row crop agriculture, avermectin B_1, B_2, and B_2 23-ketone, have been evaluated in efficacy screens on

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

plants in more than 50 greenhouse trials and more than 30 field trials on a total of 17 crops. There has been one as yet unexplained incident of phytotoxicity with avermectin in a cooperator trial on tomatoes at Bradenton, Florida. In the remaining approximately 80 greenhouse and field studies, no phytotoxicity nor other adverse effects on plant growth have been observed due to avermectin treatment.

Alfalfa: Foliar applications of avermectin B_1 to alfalfa at rates as high as 0.1 lb ai/acre in four field trials in 1980 resulted in no observable phytotoxicity.

Apples: Eighteen field studies have been conducted with avermectin B_1 on apples during the years 1979 and 1980.. The highest rate was 0.02 lb ai/100 gallons

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

(approximately 0.16 lb B_1/acre) applied to foliage in dilute spray. No phytotoxicity was recorded. A single case of phytotoxicity was observed when avermectin B_1 was combined with oil and followed three days later with an application of fungicide (Captan). The injury was similar to that caused by the interaction of oil and Captan on apple foliage, and the investigator attributed the phytotoxicity to the oil/Captan combination and not to avermectin B_1.

Cabbage: Two trials were conducted with avermectin B_1 as foliar application on cabbage in 1980. The highest use rate was 0.05 lb ai/acre. No phytotoxicity was observed. In soil incorporation tests conducted in 1979, avermectin B_1 and B_{2a} at rates of 2.5 lb active/acre did not adversely affect cabbage growth.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

Collards: A single trial was conducted at 0.05 lb B_1/ acre. No phytotoxicity was observed.

Corn: A single greenhouse and one field trial have been conducted on corn. In the greenhouse study no phytotoxicity was observed with avermectin B_1 or avermectin B_2 when soil incorporated at 1.5 lb ai/ acre. In the field trial, no phytotoxicity was observed with multiple applications of 0.05 lb ai/acre of avermectin B_1 to corn foliage.

Cotton: Seven field trials have been conducted on cotton. Multiple applications of 0.05 lb ai/acre and a single application of 0.1 lb ai/acre resulted in no observable phytotoxicity with avermectin B_1.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

Cucumbers: Several greenhouse trials have been conducted with avermectin B_1 and avermectin B_2. Single applications of these materials to soil at rates as high as 9.0 lb ai/acre resulted in no observable phytotoxicity.

Grapefruit: Field trials have been conducted with avermectin B_1 in 1979 and 1980 at rates as high as 6 ppm (0.04 lb B_1/acre) in dilute sprays. No phytotoxicity has been observed.

Lima Beans: Approximately 25 greenhouse trials have been conducted with avermectins on lima beans. This has been the major greenhouse screening plant for foliar miticidal activity. No phytotoxicity has been observed at any rate on foliage or in the soil with any avermectin or avermectin formulation on this plant species.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(1) Phytotoxicity of Ivermectin (Cont'd)

Oranges: Field trials have been conducted with avermectin B_1 at rates as high as 25 ppm (approximately 0.16 lb B_1/acre) applied to foliage and fruit. No phytotoxicity has been observed.

Peaches: One field trial was established on peaches in 1980. A single application of 16 ppm (approximately 0.10 lb B_1/acre) of avermectin B_1 in dilute spray results in no phytotoxicity symptoms.

Pears: Three field trials were conducted on pears during 1980. Rates of avermectin B_1 as high as 16 ppm (ca. 0.10 lb B_1/acre) in dilute spray were non-phytotoxic to fruit or foliage.

Peanuts: A single field trial was conducted on peanuts during 1979 with avermectin B_{2a}. No phytotoxicity or adverse plant growth was observed at rates as high as 1.35 lb B_{2a}/acre incorporated into soil.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

Potatoes: Five field trials were conducted on potatoes during 1980. Multiple foliar applications of avermectin B_1 at rates as high as 0.05 lb ai/acre did not cause phytotoxicity.

Sweet Corn: Three field trials were conducted on field corn during 1980. Multiple spray applications of avermectin B_1 at rates as high as 0.05 lb ai/acre did not cause phytotoxicity.

Tobacco: During 1979 and 1980 five field trials were conducted with avermectin B_1, B_2 or B_2 23-ketone incorporated into soil at 0.45 lb active/acre. No sign of phytotoxicity was observed under a variety of soil conditions.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

Tomatoes: Several greenhouse trials have been conducted with avermectin B_1 and/or avermectin B_2. Rates as high as 9.0 lb ai/acre of these materials incorporated into the soil did not result in phytotoxicity.

Three field trials were conducted with the avermectins on tomatoes at the same location in Bradenton, Florida. In the first two trials no phytotoxicity was observed when soil incorporated at rates as high as 3.0 lb ai/acre for avermectin B_1 and avermectin B_2 and at 1.0 lb ai/acre of avermectin B_2 23-ketone. However, in the third trial conducted under similar conditions by the same researcher slight stunting was reported with B_1 when incorporated in soil at 0.3 lb ai/acre.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Phytotoxicity of Ivermectin (Cont'd)

It is believed that 153 milligrams/acre, the rate projected from applications of sheep manure, would have no phytotoxic effect on naturally occurring field species or cultivated plant species.

(j) Toxicity to Aquatic Organism

(1) Bluegill Sunfish

Bluegill sunfish, Lepomis macrochirus Rafinesque, a warm-water fish of wide geographic distribution and important as a food-web organism, is recommended as a bioassay organism. Range finding tests were carried out with 4 liters of water to which had been added suitable aliquots of ivermectin dissolved in N,N-dimethylformamide (DMF). A sample of pure dilution water and also one containing DMF (as in the highest test concentration) were used as controls. Four test organisms were added to each solution and mortalities were recorded at 24, 48, 72 and 96 hours.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Aquatic Organism (Cont'd)

The definitive test was carried out in 15 liters of water contained in a 19.6 liter glass jar.

Ivermectin dissolved in DMF was added to the water to give concentrations of 5.6, 10.0, 18.0, 32.0 and 56.0 µg/l. Pure dilution water and water containing DMF at the highest concentration used in the test served as controls.

Ten bluegill sunfish, approximately 4 months old and 35 mm in length, were introduced into each test and control jar maintained at 20-21°C and the mortality and abnormal behavior of the fish observed at 24, 48, 72 and 96 hours.

The 96-hour LC_{50} (with 95% confidence limits) for ivermectin was 5.3 (4.4 - 6.4) µg/l.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Aquatic Organism (Cont'd)

(2) Rainbow Trout

Rainbow trout, Salmo gairdneri, prefers water temperatures below 20°C, has a wide geographic distribution and occupies an important place in the aquatic food web. For these reasons, the rainbow trout is recommended as a bioassay test organism.

The assay was carried out in the same manner as that described for the bluegill sunfish except that the age of the trout was approximately 3 months, the mean length about 45 mm, and the temperature of the water 11.5-12°C. The 96-hour LC_{50} (with 95% confidence limits) for ivermectin was 3.3 (2.8-4.0) µg/l.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Aquatic Organism (Cont'd)

(3) Daphnia magna Strauss

Daphnia magna Strauss, because of its wide geographic distribution and importance in the food-web, is recommended as a bioassay test organism.

Two laboratory studies were conducted to determine the toxicity of ivermectin towards Daphnia. In the range-finding test for the first study, suitable aliquots of a solution of ivermectin in DMF (1.0 mg/ml) were added to 500 ml of dilution water. The diluted solution was thoroughly mixed and divided equally in two replicate polypropylene beakers. Fourteen concentrations were tested, plus a water control and a water/solvent control, containing the same DMF concentrations as in highest range-finding concentrations. Five newly released instar daphnids, less than 20 hours old,

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Aquatic Organism (Cont'd)

were carefully added to each beaker of test solution and controls. Mortalities were recorded at 24 and 48 hours.

The definitive test was conducted, based on the range-finding tests, in 250 ml glass beakers with five concentrations of ivermectin, a water control, and a solvent/water control with four replicates of each. Five organisms were placed in each of the 20 test solutions, four water controls, and four solvent/water controls. The temperature was maintained at 21°C. Mortalities at 24- and 48-hour exposure were recorded.

The 48-hour LC_{50} (with 95% confidence limits) was 0.0158 (0.0127-0.0196) µ/l (ppb).

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Aquatic Organism (Cont'd)

For the second laboratory study, a definitive static test of the acute toxicity of ivermectin to the neonate Daphnia magna was performed. Fifteen Daphnia were placed into each 2-liter battery jars containing 0.013, 0.022, 0.036, 0.060, and 0.10 µ/l (ppb) of ivermectin at 22°C. Water quality criteria were regularly monitored over the 48-hour test.

The 48-hour LC_{50} (with 95% confidence interval) was 0.036 (0.030-0.043) µ/l (ppb), while the no-discernible-effect concentration through 48 hours was 0.013 µ/l.

To more nearly reproduce circumstances which will exist under actual field conditions, feces from steers administered ivermectin containing a to-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Aquatic Organism (Cont'd)

tal drug-equivalent level of 620 ppb (33-fold higher than the calculated feedlot concentration of 19 ppb) were mixed 1:1 with Iowa silt loam soil. The mixture was placed on top of either 5 cm or 2 cm columns of the same soil, and water was percolated through the columns. The eluate from the 5 cm column was diluted 1:1 with spring water.

These percolated water solutions were assayed by total radioactivity at 0.48 and 3.16 ppb in total drug-equivalent, respectively. In a preliminary, uncontrolled study, these solutions produced no 48-hour mortalities towards Daphnia magna.

Elution of composite feces alone, with no soil present, provided a solution with a total drug- equivalent of 26.0 ppb. This solution, diluted 1:1 with distilled water, was toxic to Daphnia magna. Further dilution with distilled

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Aquatic Organism (Cont'd)

water indicated a 48-hour LC_{50} near 7 ppb of total drug-equivalent, which is about 350 times the 48-hour LC_{50} of ivermectin.

Pure ivermectin, when mixed into Iowa silt loam soil at the very high level of 455 ppb, placed on top of a column of the same soil only 2 cm deep, produced eluate of only 0.18 ppb in total drug- equivalent. Dilution of this solution with an equal volume of distilled water produced a solution which was non toxic to Daphnia magna in a 48-hour bioassay.

Augmented with the soil sorption and column leaching studies discussed above, it can be concluded that ivermectin binds strongly to soils. Preliminary uncontrolled studies found that eluates (some diluted 1:1) from soil columns containing ivermectin and its bovine metabolites did not elicit a lethal effect in 48 hours on Daphnia.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects

Data in our files, as well as published data[20], indicate that ivermectin and some analogs are effective against a number of insect pests, phytophagous mites, and soil nematodes.

Several compounds were tested for activity against the Mexican bean beetle (Epilachna varivestis), the Southern armyworm (Spodoptera eridania), the black bean aphid (Apohis fabae), the two-spotted spider mite (Tetranychus urticae), the corn rootworm (Diabrotica undecipunctata) and the rootknot nematode (Meloidogyne incognita). Average percent of kill or feeding inhibition, or an effectiveness rating from 0 (no kill or feeding inhibition) to 10 (complete kill or total inhibition of feeding), were noted for each compound.

Aphid contact and systemic poison tests were made on the black bean aphid while feeding on nasturtium plants

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

grown in 2 1/2 inch pots. Tests were made on aphids that migrated to the test plant within the prior 24 hours. The foliage and aphids were exposed to a spray of the test chemical at 250 ppm while the plant was rotating on a turntable. Immediately thereafter, 21 ml of a 250 ppm stock suspension was poured onto the surface of the soil (25 lb/acre rate).

The plants were held under fluorescent light over a paper collar so dead aphids could be collected. The systemic effects were also tested separately without an accompanying foliar application.

Mite contact and systemic tests were performed on bean plants growing in 2 1/2 inch pots and infected with the two-spotted spider mite 24 hours previously. Plants were dipped in a suspension of the test material at 250 ppm. Immediately afterward, 21 ml of a 250 ppm suspen-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

sion was poured on the surface of the soil (rate equivalent to 25 lb/acre) to provide both a contact systemic effect. From this test, observations are made on adult kill (initial), immature mite kill (residual) and egg development (failure to hatch). The systemic effects were also tested separately without an accompanying foliar application.

For the Mexican bean beetle, a combination of stomach poison and feeding-deterrent effects was measured on larvae about 5 to 7 days after emerging from eggs. Leaves of young bean plants were removed from the plants by cutting the petioles and were dipped in a suspension of the chemical at 250 ppm in the tests. Petioles of the excised leaves were placed in a water reservoir to maintain leaf turgidity, and 5 larvae were placed upon them as soon as the chemical deposit was dry. Observations were made on the mortality of the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

beetles and the extent of inhibition of feeding two or three days later. For the Southern armyworm, materials were tested as stomach poisons for 5- to 7-day-old larvae of the armyworm. The larvae were transferred from stock cultures to bean leaves that had been dipped in suspension of the test material. The procedures were essentially as outlined for Mexican bean beetle larvae.

In the corn rootworm test, the formulation was mixed with the soil, and corn seedlings and larvae were introduced 3 days after the soil was treated.

To test nematicide activity, the rootknot nematode has been chosen as a preferred test subject among some 200 plant parasitic nematodes. This nematode is distributed worldwide on a wide assortment of crops. Al-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

though it resides in root tissues as a parasite where it incites formation of galls, it may also survive in the soil for many months as a scavenger.

The test described below was designed to destroy free-living forms and to a lesser extent disinfect gall tissue.

Air-dried soil and sand were blended in a ratio of 2:1, and 7 grams of chopped galls and root tissues from an infected stock of plants was added to each gallon of mixture. The inoculum was blended with the mixture and 130 ml was added to each styrofoam cup (10 oz. size). In the test, 10 ml of a 520 ppm suspension (equivalent to 50 lb/acre) was added to each cup which was then covered with a lid, shaken vigorously 2 hours later to assure uniform distribution, incubated 1 to 2 days and again shaken. The covers were removed and the soil leveled. In the cucumber standard test, four cucumber

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd) seeds were sown in each cup and covered with 30 ml of sand to a depth of about 1/4 inch. The sand was then sprinkled with a nutrient solution (Miracle Gro at 1 tsp./gal.) containing a damping-off preventive (Dexon at the rate of 1 tsp. of 35% material/gal.) to permit growth of vigorous, healthy roots. After a holding period of 18-25 days, the roots were washed free of soil-sand and rated according to the severity of infection on a scale of 0 (severe galls) to 10 (no infection). In the tomato translocation test, young plants were transplanted into infested soil and sprayed. After a period of 18-25 days, the roots were scored for galling on a scale of 1 to 10.

The major isomer of ivermectin, 22,23-dihydroavermectin B_{1a} (H_2B_{1a}, L-638,709) was tested against the Mexican bean beetle, Southern armyworm, aphids and mites at application levels of 33, 8, 2 and 0.5 ppm.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

Even at the 0.5 ppm level, H_2B_{1a} produced 100% mortalities against the bean beetle, and adult and immature mites, and 90% mortality after 5 days to the aphids. Against the armyworm, H_2B_{1a} afforded 90% mortality at the 8 ppm level. In the systemic test against aphids and mites, however, H_2B_{1a} produced no mortalities at either 0.38 or 1.5 lb/acre, and only 15% mortalities against the corn rootworm at 3.1 and 12.5 lb/acre. These results indicated little or no uptake of H_2B_{1a} by the plants.

In the standard rootknot nematode test H_2B_{1a} exhibited galling ratings of 9 at or above 0.75 lb/acre, and ratings of 7 to 8 at 0.19 lb/acre (86 g/acre).

As a comparison, avermectin B_{1a} (B_{1a}, L-676,895) produced 100% mortalities at 0.5 to 33 ppm application level against the bean beetle, adult and immature mites, and aphids by Day 5. Against the armyworm,

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

B_{1a} afforded 100% mortality at the 8 ppm level, but only 40% mortality at 2 ppm. Subsequent tests indicated B_{1a} was active against the bean beetle at an application level of 0.2, but began to lose its activity below that level. Also, subsequent trials showed B_{1a} to cause only about 50% mortalities at the 0.5 ppm level against aphids on Day 5. Also, the activity against the spider mite fell off below 0.2 to 0.5 ppm. B_{1a}, like H_2B_{1a}, was also ineffective in the systemic test against aphids and mites at 0.38 and 1.5 lb/acre and against the corn rootworm at 3.1 and 12.5 lb/acre, again indicating little or no uptake of the compound by the plants.

In the standard rootknot nematode test with cucumber seedlings, B_{1a} had similar activity to that of H_2B_{1a}, demonstrating galling ratings of mostly 9 to 10 at levels down to about 0.75 lb/acre and galling ratings of 8 to 9 at 0.19 lb/acre. In the tomato tran-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

slocation test, the galling ratings were 10 (no infection) at 0.75, 1.5 and 3.1 lb/acre.

Another compound, related to H_2b_{1a} via the loss of one oleandrose unit, H_2B_{1a}-monosaccharide (H_2B_{1a}-MS, L-638,724) was also active against the bean beetle, the aphid and mites in the application range of 0.2 to 33 ppm. This compound displayed better activity than H_2B_{1a} towards the Southern armyworm, displaying 100% mortality at 0.2 ppm. The H_2B_{1a}-MS also displayed greater than 90% mortalities against adult two-spotted spider mites down to 0.05 ppm, although it was not effective against immature mites at this level. The H_2B_{1a}-MS, like H_2B_{1a} and B_{1a}, was not effective against aphids or mites when applied systemically at 0.38 or 1.5 lb/acre, or against corn rootworm in the soil even at 3.1 or 12.5 lb/acre.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

In the standard rootknot nematode test with cucumber seedlings, H_2B_{1a}-MS displayed galling ratings of 9 at 3.1 and 0.75 lb/acre.

A fourth compound, related to H_2B_{1a} by the loss of two oleandrose units H_2B_{1a}-aglycone (H_2B_{1a}-AG, L638,723) was tested at an application rate of 0.5 ppm against the Mexican bean beetle, where it displayed a mortality rating of zero. Against the Southern armyworm, applications of 0.5 and 0.25 ppm showed conflicting results in two tests, having no mortality at 0.5 ppm, but a mortality rating of 9 at 0.25 ppm. At 0.1 ppm, there was no activity against aphids or mites, a level where H_2B_{1a}-MS and B_{1a} were active.
Against the corn rootworm, a level of 0.19 lb/acre in the soil was inactive. In two trials of the standard rootknot nematode test with cucumber seedlings at 0.19 lb/acre, H_2B_{1a}-AG displayed galling ratings of only 3 to 5.5.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

Thus, toward a variety of insect pests and nematodes, the activities of H_2B_{1a}, B_{1a} and H_2B_{1a}-MS were quite similar. Less data was accumulated on H_2B_{1a}-AG, but it appeared less active than the other compounds against the Mexican bean beetle, the Southern armyworm, aphids and mites. Both B_{1a} and H_2B_{1a}-MS were more active than H_2B_{1a} against the Southern armyworm. None of the compounds was active against the corn rootworm, the black bean aphid or the two-spotted spider mite when applied to or mixed into the soil, indicating that the compounds were not readily taken up by the plants. All the compounds displayed activity in the soil against the rootknow nematode at levels of 0.75 lb/acre and lesser activity at 0.19 lb/acre. These levels are over 2000- and 550-fold, respectively, the application levels expected in fields fertilized with feces from dosed sheep (153 mg/acre - see Section D-2(a), Environmental Burden).

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(1) Toxicity to Annelids

Studies were conducted to determine the LC_{50} for ivermectin to the earthworm, Eisenia foetida in artificial soil under controlled laboratory conditions.

In an initial study, ivermectin was used at 0.1, 1.0, 10.0, 100.0, and 1000.0 mg/kg soil in range-finding tests for toxicity to the earthworm. The definitive test was conducted using four replicates at 12, 25, 50, 100, and 200 mg ivermectin/kg soil with four replicate solvent controls. Copper sulfate was used as a reference toxicant. The test soil consisted of 100 g peat, about 50 g bentonite clay, 5 g cow manure, about 10 g $CaCO_3$ (to maintain a pH of 7.0) and quartz sand added to reach a final weight of 1 kg per test replicate.

Ten worms were added to the surface of each test vessel containing 1 kg of the dosed test soil. In one replicate, the worms were weighed individually and in the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(1) Toxicity to Annelids (Cont'd)

other three replicates, all 10 worms were weighed as a group. The same replicate was checked for the before and after test weight range. Test vessels were covered with watch glasses and maintained at 20°C in continuous light. Mortality was assessed on days 7, 14, and 28. Weights of live worms and moisture content were determined only when the test was terminated (day 28).

The concentration of ivermectin lethal to 50% of the earthworms (LC_{50}) was estimated for the 28-day exposure period by the method of Litchfield and Wilcoxson[21] and found to be 315 mg/kg soil. However, the confidence limits could not be determined. No pathological symptoms or behavioral changes in the worms were noted during the definitive test. However, worms in all of the ivermectin-treated soils did not gain as much weight as the control worms, and the worms in the highest dose (200 mg/kg) actually lost weight over the 28-day test period. It is therefore concluded that all

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(l) Toxicity to Annelids (Cont'd)

of the ivermectin doses tested appeared to suppress rate of weight gain in the test organisms and that this suppression was dose-related.

An almost two-million-fold difference exists between LC_{50} level for earthworms and the environmental burden expected to exist in the soil as a result of fertilization with manure from sheep treated with ivermectin at the intended use level.

(m) Bioconcentration by Bluegill Sunfish

A dynamic 42-day study was conducted to evaluate the bioconcentration of ^{3}H-Avermectin B_{1a} by bluegill sunfish (Lepomis macrochirus). A flow-through proportional diluter system was used to maintain a mean water concentration of 0.099 µg/l ^{3}H-Avermectin B_{1a} for a 28-day exposure period. Radioanalysis of whole fish, fillet and visceral portions throughout the exposure

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Bioconcentration by Bluegill Sunfish (Cont'd)

period indicated a gradual uptake of ^{3}H-Avermectin B_{1a}. Daily bioconcentration factors ranged from 19-69, 6.6-33, and 24-110 for whole fish, fillet, and viscera, respectively. Uptake tissue concen-trations of ^{3}H-Avermectin B_{1a} ranged from 1.9-6.8 ppb for whole fish, 0.66-3.3 ppb for fillet, and 2.4-11 ppb for viscera. The fish ceased accumulating ^{3}H-Avermectin B_{1a} at about day 10. The compound appeared to have reached a steady-state plateau as indicated by a linear regression analysis of days 10, 14, 21, and 28 whole fish residue data.

To measure the elimination of ^{3}H-Avermectin B_{1a}, the test fish were placed in clean water for 14 days. Radioanalysis throughout the depuration period indi-cated 95, 91, and 95 percent clearance rates from whole fish, fillet, and viscera, respectively. The whole fish concentration of ^{3}H-Avermectin B_{1a} dropped

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Bioconcentration by Bluegill Sunfish (Cont'd)

from a day 28 uptake value of 6.8 ppb to 0.32 ppb by day 14 of depuration period. Fillet levels decreased from 3.0 ppb on day 28 to 0.27 by the end of the study; whereas, viscera concentrations dropped from 11 ppb on day 28 to 0.53 ppb by day 14 depuration.

A two-compartment kinetic model was used for analysis of the uptake-depuration whole fish data. The graphical method employed linear regression analysis and yielded an uptake rate constant (K_1) of 11 ppb in fish/ppb in water/day, a depuration rate constant (K_2) of 0.21 day $^{-1}$, and a calculated steady-state bioconcentration factor (BCF) of 52. This latter value was 75% of the actual day 28 whole fish bioconcentration factor of 69.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing

A secondary environmental effect would result from the discharge of by-products from the chemical manufacturing process for ivermectin.

The following summarizes the environmental effects of manufacture of ivermectin at the Danville, Pennsylvania Plant:

The manufacturing process generates two liquid-waste streams; one a combination of solvent-based waste streams, the other a combination of aqueous waste streams.

The solvent-based waste streams are generated in the isolation step and in the recovery of solvents used for the isolation. They will contain discarded organic

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

by-products and some residual avermectins in a solution of organic solvents such as hexane, ethanol and toluene.

The solvent-based stream will be destroyed by incineration. The incineration process will be subject to and in compliance with the following environmental regulations administered by the Pennsylvania Department of Natural Resources:

Pennsylvania Rules and Regulations for the Protection of Natural Resources, Title 25, Part I, Subpart C, Article I, Land Resources, Chapter 75, Solid Waste Management and Article III, Air Resources.

40 CFR Parts 264 and 265. Standards Applicable to Owners and Operators of Hazardous Waste Treatment, Storage and Disposal Facilities.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

The aqueous-based waste stream will consist of the spent fermentation broth and wash waters and will contain unconsumed fermentation nutrients, unrecovered by-products and traces of avermectins and dissolved solvents such as hexane, ethanol and toluene. The aqueous-based stream will be treated in a chemical pretreatment unit designed to destroy residual avermectins; the treated stream will receive final biological treatment in the existing two-stage secondary waste treatment plant and will be discharged under the requirements of and in compliance with NPDES Permit No. PA 0008419 which is administered by the Pennsylvania Department of Natural Resources.

Air emissions generated during the production process will consist of volatile organic compounds such as hexane, ethanol and toluene which will be controlled as appropriate by condensers. The air emissions will be subject to and in compliance with the regulations for

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

air emissions of the Pennsylvania Department of Natural Resources (Title 25, Part I, Subpart C, Article III, Air Resources).

Dry solid wastes generated during the production operations (paper, trash, etc.) will be disposed of in an incinerator which will be subject to and in compliance with the regulations for air emissions of the Pennsylvania Department of Natural Resources (Title 25, Part I, Subpart C, Article III, Air Resources).

Air, liquid and solid waste emissions will comply with the above-mentioned environmental control requirements.

The following summarizes the environmental effects of manufacturing ivermectin at the Barceloneta, Puerto Rico plant.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

The manufacturing process generates two liquid-waste streams: one a combination of solvent-based waste streams, the other a combination of aqueous waste streams.

The solvent-based streams are generated in the chemical processing steps. They will contain discarded organic compounds in a solution of solvents such as ethanol, formamide, toluene and water. The solvent based stream will be destroyed by incineration. The incineration process will be subject to and in compliance with the following environmental regulations:

Puerto Rico Environmental Quality Board Regulations for the Disposal of Solid Waste and Regulation for the Control of Atmospheric Pollution

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

U.S. Environmental Protection Agency

Regulation, 40 CFR Parts 264 and 265.

The aqueous-based waste stream will consist of wash waters generated by equipment washings. Two holding tanks are provided to contain these washings prior to disposal. Both tanks are installed in a concrete sump. The holding tanks are equipped with sodium hydroxide addition facilities and filters to remove solid ivermectin.

The tanks will be tested daily for ivermectin. The tested contents will normally be pumped out through a filter to the chemical sewer which discharges to the Barceloneta Regional Sewage Treatment Plant (BRSTP). If ivermectin is present in the tanks, the contents will either be chemically pretreated with sodium hydroxide to destroy the ivermectin or be incinerated.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

All water discharges from the operating area are directed to the holding tanks to contain any potential spills for treatment.

The holding tanks are installed in a concrete sump. Both tanks are equipped with overflow lines into the sump. In the event of the sprinkler system activation, the tanks will overflow into the sump which has an additional holding capacity of 20,000 gal.

Discharges to the BRSTP will be under the requirements and in compliance with NPDES Permit No. PR 0021237 which is administered by the U.S. Environmental Protection Agency.

Air emissions generated during the production process will consist of volatile organic compounds such as ethanol, formamide and toluene which will be controlled as appropriate by condensers. Exhaust air in the pro-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

cess building and the formulation and sterile areas will be filtered. Air emissions will be subject to and in compliance with the regulation for air emissions of the Puerto Rico Environmental Quality Board Regulations for the Control of Air Emissions.

Dry solid waste, generated during the production operation (paper, trash, etc.) will be disposed of in an incinerator which will be subject to and in compliance with the regulations for air emissions and solid waste disposal of the Puerto Rico Environmental Quality Board.

The following procedures and precautions are employed to protect workers in the Danville, Pennsylvania Plant and the Barceloneta, Puerto Rico plant from the potential occupational health hazards when working with ivermectin.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

1. Controlled Environment: Areas where exposure potential exists are physically separated from controlled areas so that spills and fugitive dust are contained. Liquid drains are intercepted with holding tanks, all exhaust is HPEA filtered and the rooms are at negative pressure relative to surrounding areas. Strict procedures are used to keep employees from carrying dust beyond the work area.

2. Equipment: Inside the work area, equipment is selected and designed to limit the amount of dust released into the work room. Where containment may not be complete, local exhaust ventilation is employed.

3. Personal Protective Equipment: As a redundant control, in case of accidental release, employees are required to wear full body coveralls including

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

gloves, shoe coverings and a hood. Where some release in anticipated such as at a packing station an airline mask is required. Where atmospheric levels have been shown to be consistently low, an approved negative pressure respirator is permitted. Change rooms are provided near or contiguous to the work area. Outer garments must be removed and hands/face washed before exiting the area and showers are required at the end of the shift.

4. Maintenance and Cleaning: The same procedures are followed by maintenance and cleaning personnel. In addition most tools are dedicated to the work area and clothing is laundered in a special area located there also.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

5. Surface Contamination: Objects which leave the work area and all equipment to be serviced must be cleaned so that the contamination level does not exceed the established level.*

6. Air Sampling: Air sampling is done to be sure that atmospheric concentrations do not exceed the established level.*

*Note: Until it can be consistently demonstrated over a period of about one year that control measures actually maintain levels below those which have been set, females of child bearing capacity are not permitted to work with ivermectin.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

7. Medical Surveillance: As with many chemicals handled at our plants, a medical surveillance programs has been established and participation is required.

8. Training: All employees assigned to work with ivermectin have been trained in the hazards, precautions and emergency procedures associated with the agent.

9. Laboratories: In laboratories where analysis is done, areas have been designated for ivermectin. Upgraded handling procedures are required as well as disposable lab coats and gloves. To the maximum extent possible work is to be done in a hood.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

10. Formulations: Once the product is formulated (0.08%) and the surface contamination level is acceptable, the work area is essentially decontrolled from an employee health standpoint. Gloves and safety glasses are required and spill and splash procedures are in effect, but other precautions are the same as would be taken for any drug.

11. Signs: The perimeter of the work area is posted to control unauthorized traffic and to remind employees of the procedures and equipment needed.

12. Emergencies: Emergency procedures have been prepared for spills, etc. and both local first aid and hospital personnel have been trained on how to handle emergencies.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

Ponders End, England Plant

The manufacturing process is guarded against contamination of the environment with respect to gaseous, liquid and solid materials, in the following way:

The solvents (toluene, ethanol and formamide) are handled in atmospheric tanks vented via flame arresters to a safe location. Hazards inherent in the use of hydrogen are minimized by maintenance of a low inventory and use in an open construction ensuring ready disposal should a leak occur.

Where the avermectin intermediate (C-076) is handled, a ventilated glove box is used which has its exhaust connected to a water scrubber. Water from the scrubber is drained to the captive drain system. The product handling area is maintained under negative pressure by an extraction system fitted with a special filter arrangement to prevent product dust release to atmosphere.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

In-process materials and solvents are handled in vessels fitted with water-cooled condensers operating on reflux.

Aqueous effluents from the plant and from captive drains are pumped to a holding tank where the pH is adjusted to greater than 12 with caustic soda, the liquors heated to 85 and recycled for 2 hours prior to analysis for residual ivermectin.

Liquors are discharged to the site effluent system at less than 2 ppm of ivermectin.

This is determined by techniques sensitive down to 1 ppm.

Average total effluent from the site is approximately 100 times greater than the discharge rate from this plant and all effluent passes through equalizing basins before discharge, thus ensuring a high degree of dilution of the already low concentration of ivermectin.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

Organic wash liquor residues are disposed of by incineration. Solid waste in the form of contaminated clothing and equipment is sealed in polyethylene plastic bags and similarly treated under direct supervision. Catalyst material recovered as a thiourea complex is collected in a bag filter, solvent washed, and then sealed in a polyethylene container for eventual transfer to the original supplier for metal recovery and refining, the first stage of which is controlled incineration.

The major pieces of legislation controlling environmental emissions in the United Kingdom are:

A) Control of Pollution Act 1974: This mainly deals with disposal of waste, pollution of water, pollution of the atmosphere and noise.

B) Health & Safety at Work Act 1974: This mainly deals with maintaining a safe working environment but also the prevention of emission into the atmosphere of noxious or offensive substances.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

C) Alkali etc., Works Regulation Act 1906 with orders of 1966 and 1971: This is a more specific Act designed to regulate production of certain substances but also to control the emission of specific fumes and gasses.

D) Clean Air Act & Public Health Act could also apply but are of more general application and more often than not utilized by local authorities to prevent nuisance from boiler smoke, etc.

1) Disposal of Solid or Sludge Wastes

This is normally entrusted to a licensed contractor who will analyze such waste and devise suitable means of disposal within the authority granted to him by the local and national inspectorates in the area of disposal. Means of destruction could be: land fill, disposal at sea, incineration with or without exhaust stack scrubbing. In the case of ivermectin all solid waste, including discarded protective clothing, cleaning cloths, etc., are collected in plastic sacks, sealed and periodically incinerated at a municipal

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

incinerator under our direct supervision. The incinerator administration have been fully informed of the nature of the material handled and have made no special requirements with respect to this waste.

2) Disposal of Liquid Effluent

All our liquid effluent streams from the ivermectin plant are neutralized where necessary with lime and discharged via settling tanks to the municipal sewer. Settled sludges are disposed of as in 1. above. Discharge to sewer is governed by consent agreements with the sewage treatment authority who regularly sample and analyze our effluent. In the event of violation of the agreed limits, the authority is empowered to revoke consent agreements or take other action against the offenders. We make no effluent discharge to canals or rivers. All liquid effluent streams from the ivermectin plant are collected separately from all other site waste streams. Aqueous waste including floor washings

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

and roof rain water collections as well as process streams, are filtered and then treated with sodium hydroxide to pH 12 at 80 until samples show an acceptable level of ivermectin has been achieved. At this point the effluent is discharged to the normal site effluent treatment plant where it is diluted with the much greater stream from the rest of the site.

Non-aqueous liquid waste consisting mainly of organic solvents is collected separately and periodically disposed of via a licensed contractor who will incinerate on duly approved premises.

3) Discharge to the Atmosphere

Local authorities are empowered to require the provision of estimates of emission of pollutants or other substances into the air but the major legislation requirement is embodied within the Alkali Act. The premises are subject to periodic visits by the Alkali

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

Inspectorate with specific reference to the emission of HCl, HNO_3, SO_2, NO_2, acetic acid, acetic anhydride and chlorine and its compounds.

No specific vapors are released from this plant to atmosphere which would come under the provision of the Alkali Act. All plant ventilation equipment discharges to atmosphere through hepa filter screens which are periodically replaced. The discarded screens are being incinerated with other solid waste.

4) Noise

Under the Control of Pollution Act, the local authority is empowered to inspect, and where satisfied that a nuisance exists, take such action as may be appropriate to require that noise abatement measures are taken.

Additionally, under the Health & Safety at Work Act, government inspectors may require compliance with a

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

published code of practice or, in the future, regulations which have not yet been published but which are now in the consultative stage.

Haarlem, Holland Plant

The following procedures are employed to monitor and control environmental emissions and occupational exposure to ivermectin.

1) Weekly monitoring of dust level in presolution room where ivermectin powder is handled.
2) Pending results of activated charcoal treatment, all waste water is temporarily being incinerated.
3) Blood tests of all employees working in ivermectin production every three months and later on every six months.
4) Swab tests every two weeks on equipment, floors and production bottles in production area.
5) Swab test every month from hands of Packaging personnel.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

MERCK SHARP & DOHME B.V. at Haarlem, the Netherlands operates regarding environmental matters within the Environmental Pollution Act.

1) Liquids from the ivermectin manufacturing processes are all collected and treated with a charcoal purification unit before entering the plant's general waste system, which also includes domestic sewage waste.
This goes via a neutralization tank (pH 6-8) and via the municipal sewage system to the Municipal Sewage Water Treatment Plant.

This plant operates under the control of the Hoogheemraadschap Rijnland. M.S.D. has a permit from the municipality for entering the sewage treatment plant with their plant-effluent.

2) Air emissions from the process fall under the State Rules and Regulations Act with regard to Environ-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(n) Impact of Manufacturing (Cont'd)

mental Pollution. The regulations are administered by the Haarlem Department of Environmental Control.

3) Charcoal from the filter system within the charcoal treatment system is collected in plastic bags, put into drums and shipped for incineration. All other collected waste from this factory is combined with plant trash and transferred by closed vehicle to the Rijnmond or Alkmaar incinerator. A yearly permit for transport and incineration, issued by the Provincial Environmental Control Agency, under the Law regulates transport and processing solid wastes.

4) With regard to noise, regulations require a working climate, in which 85 dB is maximal. In case the noise level exceeds 85 dB, protective measures have to be provided to all personnel.

3. Describe the Probable Adverse Environmental Effects that Cannot Be Avoided

Based on the discussion in Section D 2, it is not anticipated that any substantial adverse effect on the environment will occur when the new animal drug application for ivermectin is approved. Of course, any manufacturing process must make some contribution of products to the environment. However, as indicated in Section D 2, the liquid, solid and air disposal of by-products from the manufacturing process is done under the applicable environmental requirements of various laws. Furthermore, such wastes from the ivermectin process would make a negligible contribution to the waste problem of modern industrial society.

4. Evaluate Alternatives to the Proposed Action

There is no practical alternative to the use of chemotheraputic agents in controlling parasitism.

Prior to the development of effective therapeutic agents, the control of parasitic infections was limited to management systems of pasture rotation and the use of harsh and often injurious chemicals.

4. Evaluate Alternatives to the Proposed Action

Ivermectin is a substantial advance over currently-used products to control sheep parasites and good efficacy has been consistently demonstrated against all of the major gastrointestinal parasite species in sheep. Furthermore, Oestrus ovis, the sheep nasal bot, has been shown to be safely controlled at any stage of its life cycle. Clearly, the simultaneous control of both internal and external parasites with subsequent savings in time and labor reflect an unprecedented advance in sheep parasite control.

Ivermectin's potent broad-spectrum, parasiticidal activity establishes this animal drug as having the advantage of controlling a large number of species of parasites. The quantities of undesirable compounds reaching the environment following production and utilization of this new animal drug will be small and environmental effects are not expected to occur.

5. Describe the relationship between local short-term uses of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity

Short-term effects upon the environment, as discussed in Section D 2 (phytotoxicity to a variety of plants, hazard to fish, earthworms, aquatic organisms, etc.), are not expected due to the low levels of noxious compounds which will be present in the environment. Also, as discussed, there would be minimal short-term effect of the disposal of by-products from the manufacturing process upon the productivity of the environment.

These same factors also would mitigate against any long-term detrimental effects on the environment.

Short- and long-term beneficial effects from the use of ivermectin could be substantial in terms of producing healthier sheep, allowing sheep to realize their full genetic potential to utilize feed more efficiently, eliminate losses from morbidity and mortality from parasite infection. Taken together, this means that more food for

5. Describe the relationship between local short-term uses of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity (Cont'd)

man (lamb protein) can be produced per pound of feed without increasing the need for such feed and the resulting expenditure of energy.

6. Describe any irreversible and irretrievable commitment of resources

The raw materials used to manufacture ivermectin are common organic compounds -- all of which are in ample supply. Energy commitment would be nominal. Also, some of the raw materials used in the process are recycled or recovered for use. Though some of the raw materials are irretrievable, the proportion used in the ivermectin process compared to the total annual production of them would be minimal.

7. Discuss the objections raised by other agencies, organizations or individuals

We know of no agencies, organizations or individuals who have questioned the effect on the environment from the use of ivermectin to treat and control internal and external parasites in sheep.

8. If the proposed action should be taken prior to 90 days from the circulation of a draft environmental impact statement or 30 days from the filing of a final environmental impact statement, explain why

The information presented in this environmental impact analysis report amply documents the position that the approval of the new animal drug application for ivermectin by the Food and Drug Administration does not constitute a major agency action which would significantly affect the quality of the human environment. Thus, there is no reason for the Agency to prepare and circulate for comments a Draft Environmental Impact Statement.

9. Analyze whether the benefit to the public of the proposed action will outweigh the action's potential risk to the environment

The benefits to be obtained from the use of ivermectin as discussed in Sections 2 and 5 outweigh any potential risk to the environment.

The risk to the environment can scarcely be identified whereas the benefit in terms of savings from economic loss to the sheep producer and the consumer are substantial.

9. Analyze whether the benefit to the public of the proposed action will outweigh the action's potential risk to the environment (Cont'd)

In view of the severe worldwide shortage of protein food and animal feed, the benefits from the use of an agent such as ivermectin are critically needed. Any conceivable risk to the environment would be negligible in comparison.

E. Certification

The undersigned applicant/petitioner certifies the information furnished in this Environmental Impact Analysis Report is true, accurate and complete to the best of his knowledge.

Date: 11 February 1985

Kenneth G. Davis
(Signature)

Director, Regulatory Affairs
(Title)

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