AMENDMENT TO
ENVIRONMENTAL IMPACT ANALYSIS REPORT
IVOMEC INJECTION FOR CATTLE

Additional Label Claims:

1. Bunostomum phlebotomum
2. Solenoptes capillatus

Bunostomum phlebotomum is a common nematode parasite of cattle. This species causes loss of blood from the small intestine and usually occurs in relatively low numbers. However, parasitic gastroenteritis in cattle is almost always caused by concurrent infections by several nematode species, one of which is B. phlebotomum.

Solenoptes capillatus is a common species of lice affecting cattle. These lice are active during the winter months and nutrition is derived by ingestion of blood derived from bites in the skin. Other species of "sucking" lice (so-called because of their feeding habits) also are commonly encountered, frequently in mixed infestations. Distinctions between species usually are not made.

The addition of these two claims on the IVOMEC label will result in little or no change in the overall use pattern or the existing market for the product. Thus, there will be no appreciable change in the amount of ivermectin which is introduced into the environment.

Prepared by:

G.W. Benz, D.V.M., Ph.D.

Environmental Impact Analysis Report

IVOMEC® (Ivermectin) Injection for Cattle

A. October 25, 1983

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, MSD) Injection for Cattle to be administered by subcutaneous injection to cattle at a dose rate of 200 mcg/kg of body weight for the treatment and control of the following internal and external parasites:

Gastrointestinal nematodes (adults and fourth-stage larvae):

Haemonchus placei

Ostertagia ostertagi (including inhibited larvae)
O. lyrata

Trichostrongylus axei
T. colubriformis

Cooperia oncophora
C. punctata
C. pectinata

Oesophagostomum radiatum

1. Describe the Proposed Action

(a) (Cont'd)

Nematodirus helvetianus (adults only)
N. spathiger (adults only)

Lungworms (adults and fourth-stage larvae):

Dictyocaulus viviparus

Grubs (first, second and third instars):

Hypoderma bovis
H. lineatum

Lice:

Linognathus vituli
Haematopinus eurysternus

Mites:

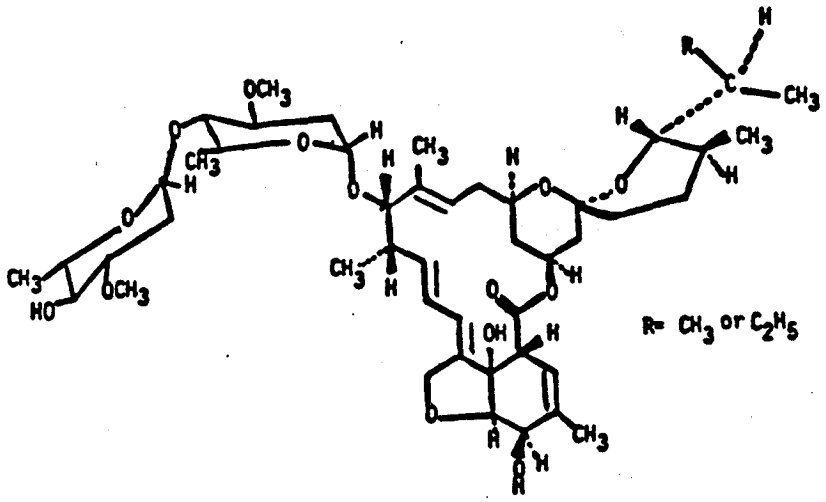
Psoroptes ovis (syn. P. communis var. bovis)
Sarcoptes scabiei var. bovis

A drug withdrawal period of 36 days prior to slaughter of cattle for food has been established.

Treatment may be repeated at intervals of not less than 36 days; however, rate of parasite reinfestation, prudent husbandry practices and economic considerations would dictate that most beef cattle would be treated four times or less during the life of the animal. Cattle 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, $[a]_D^{25°}C$, ~-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. In a solution, ivermectin is photolabile. 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.

1. Describe the Proposed Action

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

Ivermectin has been shown to be stable for at least six months when stored under ambient conditions. 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.

(c) Pharmacology

Ivermectin inactivates nematodes, arachnids and insects. Its action on the nematodes is by inhibiting signal transmission from the ventral cord interneurons

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

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.

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

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

avermectins had 2 and 3 worms each. In a simlar 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.

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 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 111

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 ^3H-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,

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

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 or teratogenic effect. The threshold for maternotoxicity 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

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

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 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 gastrointestinal hemorrhage and/or congestion was observed in 2 of the dogs. No other treatment-related histologic change was observed in any dogs.

Thirty-two cattle were used in 2 trials 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,

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

but no clinical signs of 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.

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.

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

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

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

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

(See table on following page -- 16.)

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 beef and dairy products. Thus, the increased significance of parasitism has led to the widespread use of antiparasitic drugs. Losses to the beef and dairy industry have been primarily attributed to the loss in feed efficiency, due to internal parasites and the interruption of feeding habits caused by external parasite infestation.

Ivermectin is an effective, new antiparasitic agent which is not chemically related nor paralleled in its spectrum of activity to any other drug now being marketed. In the proposed form, ivermectin provides the most convenient, ready-to-use method of control without leaving hazardous or potentially dangerous wastes which require careful handling, storage, transport and disposal. Since IVOMEC is an injectable product, the environmental concern of disposing of "spent" dips and sprays is obviated.

1. Describe the Proposed Action

(e) Purpose and Benefits (Cont'd)

The unique activity of this product also permits control of external parasites of significance at times of the year when currently available products, such as dips and sprays, cannot be used. Clearly beneficial effects with economic value would result from its use, such as decreased morbidity, resultant increase in feed efficiency and environmental protection.

(f) Potential Market Handling and Storage

The marketplace for IVOMEC injection is presently segmented into two distinct entities; namely, the endoparasite or anthelmintic market and the ectoparasite market. Presently, the parasiticide market is being served by a multitude of products designed for either endo- or ectoparasite control. Currently, two products dominate 95 percent of the estimated 60 million doses of anthelmintics administered to 111 million cattle in the United States. The ectoparasite market is characterized by many products and compounds of which there is no recognized market leader. IVOMEC

1. Describe the Proposed Action

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

injectable is expected to attain a significant market share within each market segment, due to its control of both ecto- and endoparasites.

There are no special handling or storage requirements for IVOMEC injection for cattle. Stability studies show that IVOMEC injection will be stable for two years when stored under normal conditions.

The proposed trade channels for distribution of IVOMEC injection will be similar to both anthelmintic and ectoparasiticide 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.

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides etc. (Cont'd)

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.

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

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides etc. (Cont'd)

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.

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 towards 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)

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides etc. (Cont'd)

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 dose, or via a bolus. 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 posttreatment. Oral doses of 1 mcg/kg/day killed all horn fly larvae in the manure, while a 5 mcg/kg/day 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

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides etc. (Cont'd)

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 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

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides etc. (Cont'd)

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 below. The primary impact from the use of ivermectin in cattle on the natural environment will be the excretion of the drug by treated cattle 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 (Cont'd)

the following properties and the drug sponsor has concluded that the use of ivermectin in cattle 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 cattle 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 cattle 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 five 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 (Cont'd)

2) Stability in Soil

Half-life of ivermectin and ivermectin in steer 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

Steer feces containing radiolabeled ivermectin and its metabolites was mixed into small amounts of four soil types and each sample was placed on top of a column of identical soil. About 2-7 column

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

volumes of water, depending on flow rates, were percolated through the soils during a period of about seven weeks. Radioactivity measurements and chemical assays of the leachates were made. At the end of the experiment, each soil column was divided into 7 segments and the radioactivity of each segment measured.

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,

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

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.

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.

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

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:

"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.

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

Do not permit water runoff from feedlots to enter lakes, streams, or ponds. Do not contaminate water by direct application or by the improper disposal of drug containers."

A secondary and minor potential adverse impact on the evnironment could occur in the manufacture of ivermectin and in formulating IVOMEC Injection for Cattle. 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 IVOMEC (ivermectin) in cattle involves the parenteral administration of the drug at a dose level of about 0.2 mg/kg body weight. The animals may be contained in a pasture, a small independent feedlot or a large commercial feedlot. Generally, the cattle 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.

Most cattle will be dosed with ivermectin in the cattle feedlots. The following calculations based on the U.S. Environmental Protection Agency publication (Development Document for Effluent Limitations Guidelines and New Source Performance Standards for the FEEDLOTS - Point Source Category, U.S. Environmental Protection Agency, Washington, D.C. 20460, January, 1974) 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 a fertilizer. These calculations

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

(a) Environmental Burden (Cont'd)

show that the concentration in the manure will be only 19 parts per billion and in the field, when spread as a fertilizer at a level of 5 tons per acre and mixed with the top 6 inches of soil, will be only 0.09 parts per billion.

Attached is a flow diagram from the reference (loc. cit.) showing the daily raw waste produced in a typical feedlot operation in which a 270 kg calf entered the operation and in 130-180 days reached a market animal weight of about 477 kg. During this period the animal would be treated once with ivermectin at a dose level of 0.2 mg/kg.

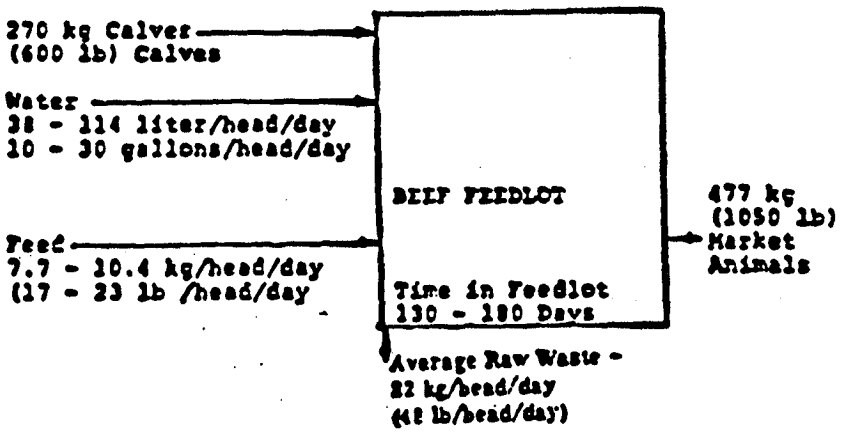


Figure 1
Typical Beef FeedLot Flow Diagram

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. This concentration, of course, would not change regardless of the actual number of cattle treated in the feedlot.

Weight of steer		270 kg
Dose of ivermectin	x	0.2 mg/kg
Weight of ivermectin dosed	=	54 mg
Waste produced per steer per day		22 kg
Total time in feedlot	x	130 days
Total waste produced per steer	=	2860 kg

Concentration of drug and metabolites in waste:

$$\frac{54 \text{ mg dose}}{2860 \text{ kg waste}} = \frac{0.019 \text{ mg}}{\text{kg}} = 19 \text{ ppb}$$

If the manure from the feedlot were spread on a field as fertilizer at a rate of 5 tons per acre, the total ivermectin plus metabolite would be 85 mg/acre or 2 micrograms per square foot:

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

(a) Environmental Burden (Cont'd)

Dose of ivermectin per steer	54 mg
Waste produced per steer	2860 kg

Concentration of ivermectin and metabolites in waste:

$$\frac{54 \text{ mg}}{2860 \text{ kg waste}} \times \frac{1 \text{ kg}}{2.2 \text{ lb}} \times \frac{2000 \text{ lb}}{1 \text{ ton}} = \frac{17 \text{ mg}}{\text{ton waste}}$$

at a rate of 5 tons/acre:

(5 tons/acre)(17 mg/ton) = 85 mg/acre

$$\frac{85 \text{ mg}}{\text{acre}} \times \frac{1 \text{ acre}}{43560 \text{ sq. ft.}} \times \frac{1000 \text{ mcg}}{\text{mg}} = 2 \text{ mcg/sq. ft.}$$

When mixed into soil at a depth of 6 inches the concentration of ivermectin plus metabolites would be 0.09 ppb.

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

$$(864 \text{ cu. in.}) \times \frac{16.4 \text{ cc}}{\text{cu. in.}} \times \frac{1.5 \text{ g}}{\text{cc soil}} = 21254 \text{ g soil}$$

$$\frac{2 \text{ mcg}}{21254 \text{ g}} \times \frac{1000 \text{ ng}}{1 \text{ mcg}} = 0.09 \text{ ng/g soil} = 0.09 \text{ ppb}$$

Thus under expected use conditions, the concentration of ivermectin and metabolites in the soil will be extremely low.

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

(a) Environmental Burden (Cont'd)

In the various experiments carried out with soil plus feces from steers dosed with ivermectin, the concentrations used were substantially higher than those calculated above (19 ppb in manure and 0.09 ppb when mixed with soil). The feces were collected for 2-5 days after dosing (when most of the drug and metabolites were excreted) rather than the 130 days used in the feedlot calculations and the initial dose was 0.3 mg/kg rather than 0.2 mg/kg, the dose level sought in the NADA. Consequently, the concentration of drug plus metabolites was actually 600 ppb rather than 19 ppb.

Since most of the drug and its metabolites were excreted within the first week, drug residue levels in the wastes during that period would be higher than 19 ppb, the average level for the whole 130 day period in the feedlot. The concentration of drug and metabolites during this first week would be 130/7 or 18.6 times higher than the values just discussed.

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

(a) Environmental Burden (Cont'd)

Thus, the concentration of drug and metabolites in the first week's waste would be:

19 ppb x 18.6 = 353 ppb

This would be true only if the 1-7 day collection was taken separately and not diluted in the 8-130 day collection, an unlikely possibility.(13)

Spreading this waste on a field as fertilizer at a rate of 5 tons/acre would mean a drug and metabolite level of 2 mcg/sq. ft. x 18.6 = 37 mcg/sq. ft. When mixed into the soil at a depth of 6 inches, the concentration of ivermectin plus metabolites would be:

0.09 ppb x 18.6 = 1.7 ppb

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

(a) Environmental Burden (Cont'd)

In the worst-case analysis in which the 7-day waste accumulation, containing essentially all the dose of drug plus metabolites, is spread on a field, the concentration of the drug and metabolites would be about 1581 mg/acre or 0.0035 lb/acre.

(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 1-2% of the dose is excreted in the urine. Analyses of a 2-5 day feces composite by the reverse isotope dilution assay or by the direct fluorescence assay accounts for about 40-50% of the total radioactivity in the feces as unaltered drug. The remaining 50-60% of the radioactivity consists of ivermectin metabolites. These compounds are soluble in methylene chloride but are generally more polar than ivermectin. Based on

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

(b) Metabolism of Ivermectin

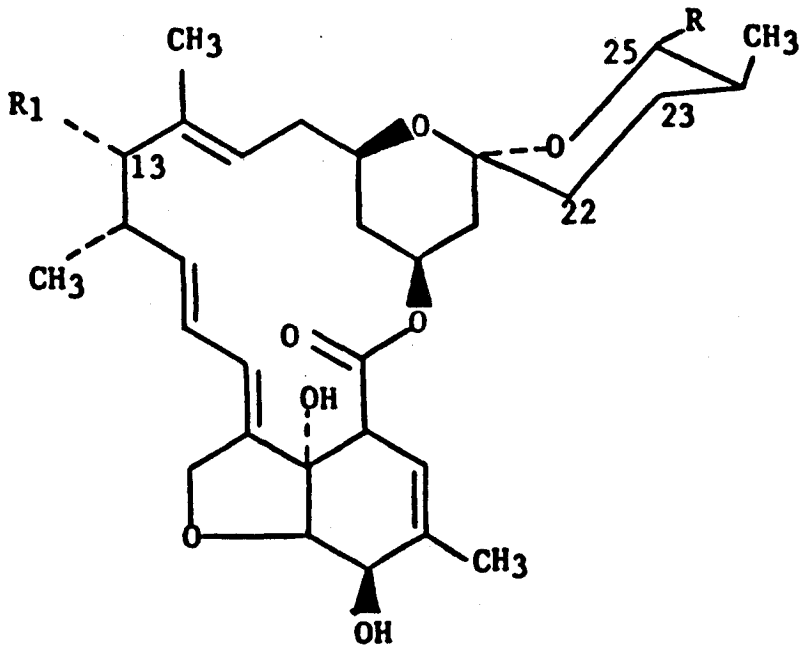
extensive analyses of the residues found in the liver and fat of steers dosed with ivermectin, one might expect that the metabolites present in feces consist of the hydroxylated ivermectin and also the monosaccharides and aglycones of ivermectin and the hydroxylated analogs (see Figure 2). Figure 2 also shows the structural similarities between ivermectin and one member of the milbemycin family, B41D.

As can readily be seen, milbemycin B41D represents the 13-deoxy aglycone of the minor component of the ivermectin mixture in which the 25-carbon position carries an isopropyl substituent. Although the milbemycins represent a family of natural products they differ most significantly from ivermectin by the absence of the disaccharide, α-L-oleandrose-α-L-oleandrose at the C-13 position of the macrocyclic lactone structure. Section D-2(i) summarizes the results of insecticide assays with ivermectin, ivermectin monosaccharide, ivermectin aglycone and avermectin B_1a.

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

(b) Metabolism of Ivermectin (Cont'd)

Figure 2. Structures of Ivermectin, its monosaccharide and aglycone and of milbemycin B41D.



NAME	R	R_1
MILBEMYCIN B41D	ISOPROPYL	H
IVERMECTIN AGLYCONE	a	OH
IVERMECTIN MONOSACCHARIDE	a	α-L-OLEANDROSE
IVERMECTIN	a	α-L-OLEANDROSE-α-L-OLEANDROSE

Where a = {R-Sec Butyl ≥80% / R-Isopropyl ≤20%}

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(h), 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.

Also, toxicity studies of ivermectin and its monosaccharide towards Daphnia magna showed these compounds to have 48-hour LC_{50} values of about 0.02 ppb and about 0.4 ppb, respectively. The 48-hour no-effect level of the aglycone of ivermectin appears to be above 10 ppb. Thus, results on the toxicity of the available metabolites of ivermectin, including the monosaccharide and aglycone, indicate that all are less toxic than the parent drug to Daphnia.

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

(c) Stability in Soil

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 168 days. The experimental set consisted of two types of samples: (1) feces from a steer 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 in Soil (Cont'd)

The detection limit for the radioactivity measurement was about 1 ppb and for the fluorescence assay about 10 ppb. The concentrations of ivermectin and metabolites in the stability, percolation and other soil experiments were set high enough to permit assay of the feces/soil samples.

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.

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

(c) Stability in Soil (Cont'd)

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

Most of the degradation products, which amounted to 30-50% of the drug in all samples at 168 days, were found to be extractable into non-polar solvents but did not respond to the chemical assay for ivermectin.

Although the stability studies were designed to yield information on the rate of decomposition, it is difficult to extract a definitive rate covering the entire 24-week period of the study. Considering the data for the B_1a component, the rate of disappearance appears much faster in the early periods. Application of a two-compartment model allows the estimation of an early period half-time (up to 14 days) and a terminal

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

(c) Stability in Soil (Cont'd)

half-life (28-168 days). By this procedure, the rate of disappearance in the early period corresponds to a half-life of 2.6-5.8 days. During the terminal part of the experiment, half-life estimates vary from about 139 to 365 days. This interpretation is consistent with the hypothesis that the rate of degradation by microorganisms is not the rate-limiting step in either of the two phases. The use of the two-compartment model is consistent with the observation that ivermectin is very strongly absorbed on soil. In the strongly adsorbed state, as represented by the terminal phase of the experiment, the rate of desorption from the soil is the rate-limiting step. The rapid half-life observed in the earlier period reflects the faster desorption from the fecal material. High concentrations of ivermectin are available for microbial action during the time that the ivermectin is equilibrating from the fecal matter to the higher-affinity sites in the soil.

At the end of the experiment, a small fraction (2.4-7.0%) of the initial radioactivity had been collected

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

(c) Stability in Soil (Cont'd)

in the ethanol used to trap volatile products. The formation of such products indicates that extensive degradation of the ivermectin and metabolites had occurred.

(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 19 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 radio-activity, 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%

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)

and 8.7% 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 the 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.

(e) Soil Translocation

A soil translocation (leaching) study with excreta from cattle fed radioactive ivermectin was conducted with four different soil types.

1) The soil leaching study was designed to determine the movement and distribution of a test material and metabolites through a 30 cm soil column.

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

(e) Soil Translocation (Cont'd)

Columns were prepared in triplicate for each soil type. The feces (0.5 g) were mixed with 1 g soil and applied to the top of the column. Water, corresponding to 10-20 acre inches, was applied and 180-600 ml of leachate was collected, depending on soil type. The columns were cut in seven segments, and radioactivity measured (after combustion) by liquid scintillation.

The mean radiolabel recovered in the leachates was:

	Volume ml ± Std. Dev.	Total % Eluted	% per ml of Leachate
Silt loam	295 ± 37	26.98 ± 5.56%	0.091
Clay loam	595 ± 5	48.07 ± 1.54	0.081
Loam	193 ± 14	9.83 ± 1.67	0.051
Sandy loam	591 ± 3	42.73 ± 10.0	0.072

The radioactivity in the effluent was subjected to extraction with methylene chloride, which was expected to remove ivermectin and metabolites. More than 87% of the radioactivity was extracted (the loam effluent was not processed), but HPLC analysis showed no unchanged ivermectin (detection

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

(e) Soil Translocation (Cont'd)

limit 13 ± 7 ng or 4% of the amount charged to the columns). Activity in the effluents is probably due to metabolites but is not unchanged ivermectin.

Analysis of segments of the soil column for total radioactivity showed that most of the remaining radioactivity was within the top 2.5 cm of the column and hence had not been present in the effluent.

2) To simulate pasture conditions, a similar soil leaching was run, using specimens prepared by mixing 5 grams of feces (in suspension) with 50 grams of soil in replicate, and storing the material aerobically at 22°C for 30 days. This aerobically aged feces/soil material was then added to the top of sandy loam columns and leached slowly (15 ml per day) for 45 days. The diffusion of activity in the soil was similar as for the unaged material: 46.83 ± 0.74% eluted, corresponding to 0.074% per ml.

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

(e) Soil Translocation (Cont'd)

In all cases, including the aged specimens, a large portion of the applied activity remained in the top segment of the soil columns. This gave additional evidence that the material is not likely to be readily translocated into ground water.

It is known from an isotherm experiment that soil contains high affinity sites for ivermectin and that soil has a relatively high capacity for ivermectin. Even in the presence of trace amounts of DMSO (used to dissolve ivermectin), soil has been shown to decrease the level of ivermectin below the LC_{50} of Daphnia (about 20 ppt).

3) As part of a study of avermectin B_1a as an insecticide for fire ant control, the carbon-14 labeled compound was applied in a bait formulation to duplicate plots of Bermuda grass. The application rates were 50, 150 and 500 mg of avermectin per acre, or 1, 3 and 10 times the recommended

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

(e) Soil Translocation (Cont'd)

application rate. Grass samples were harvested at 0, 1, 2, 4, 6 and 8 weeks posttreatment. At the conclusion of the experiment, two core samples of soil were taken from each of the duplicate subplots. Based on combustion radioanalyses, there was no significant radioactivity in any grass or soil sample, and therefore, no dose response correlation. These results indicated that avermectin B_1a, structurally related to ivermectin, was not taken up by the grass from a pregel defatted corn grit formulation.

(f) 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.

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

(f) 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.

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.

$$NH_4^+ \longrightarrow NO_2^- \longrightarrow NO_3^-$$

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

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

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.

The data, summarized in Table 1, 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.

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

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

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), would be about 0.1 ppb or a factor of 300-fold lower.

Table 1: 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)

(f) 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 2.

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 5 tons/acre) and plowed to a depth of 6 inches (a calculated concentration of about 0.1 ppb).

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

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

Table 2: Effect of Ivermectin and Metabolites in Feces on the Soil Respiration Process

Assay Days	Soil Type	Mean Accumulative % 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)

(g) Phytotoxicity of Ivermectin

Chlorella pyrenoidosa, a fresh water unicellular, nonmotile 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.

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, 1, 2, 3, 4, 7, 9, 11 and 14 test days. The following results were obtained:

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

(g) Phytotoxicity of Ivermectin (Cont'd)

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

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

Only a very limited number 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[14] follows:

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

(g) Phytotoxicity of Ivermectin (Cont'd)

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 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)

(g) 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.

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

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

(g) Phytotoxicity of Ivermectin (Cont'd)

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.

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.

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

(g) Phytotoxicity of Ivermectin (Cont'd)

Grapefruit: Field trials have been conducted with avermectin B_1 during the years 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 concentration rate on foliage or in the soil with any avermectin or avermectin formulation on this plant species.

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.

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

(g) Phytotoxicity of Ivermectin (Cont'd)

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

Pears: Three field trials were conducted on pears during 1980. Rates of avermectin B_1 as high as 16 ppm (approximately 0.10 lb B_1/acre) in dilute spray were nonphytotoxic 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.

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.

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

(g) Phytotoxicity of Ivermectin (Cont'd)

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.

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.

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

(g) Phytotoxicity of Ivermectin (Cont'd)

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 soil incorporated at 0.3 lb ai/acre.

It is believed that 85 milligrams/acre (the rate projected from applications of bovine manure) would have no phytotoxic effect on naturally occurring field species or cultivated plant species.

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

(h) Toxicity to Aquatic Organisms

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.

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 mcg/l. Pure dilution water and water

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

(h) Toxicity to Aquatic Organisms (Cont'd)

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) mcg/l.

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.

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

(h) Toxicity to Aquatic Organisms (Cont'd)

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) mcg/l.

3) Daphnia magna Straus

Daphnia magna Straus, 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 solutions were thoroughly mixed and divided equally in two replicate

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

(h) Toxicity to Aquatic Organisms (Cont'd)

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, 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.

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

(h) Toxicity to Aquatic Organisms (Cont'd)

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

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 jar containing 0.013, 0.022, 0.036, 0.060 and 0.10 mcg/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) mcg/l (ppb), while the no-discernible-effect concentration through 48 hours was 0.013 mcg/l.

To more nearly reproduce circumstances which will exist under actual field conditions, feces from steers administered ivermectin containing a total drug-equivalent level of 620 ppb (33-fold higher than the calculated feedlot concentration of 19

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

(h) Toxicity to Aquatic Organisms (Cont'd)

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 manga.

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 water indicated a 48-hour LC_{50} near 7 ppb of total

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

(h) Toxicity to Aquatic Organisms (Cont'd)

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)

(i) Toxicity to Nematodes, Arachnids and Insects

Data in our files 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.

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

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

Aphid contact and systemic poison tests were made on the black bean aphid while feeding on nasturtium plants grown in 2½ 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 2½ inch pots and infected with the two-spotted spider mite 24 hours previously. Plants

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

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

were dipped in a suspension of the test material at 250 ppm. Immediately afterward, 21 ml of a 250 ppm suspension was poured on the surface of the soil (rate equivalent to 25 lb/acre) to provide both a contact and 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 beetles and the extent of inhibition of feeding

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

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

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. Although 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.

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

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

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 seeds were sown in each cup and covered with 30 ml of sand to a depth of about ¼ 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

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

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

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. 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

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

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

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 5 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, 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

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

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

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 translocation 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

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

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

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.

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, but lesser activity at 0.19 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, L-638,723) was tested at an application rate of 0.5 ppm against the Mexican bean beetle, where it displayed a

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

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

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.

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

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

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

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 rootknot nematode at levels of 0.75 lb/acre and lesser activity at 0.19 lb/acre. These levels are 4000- and 1000-fold, respectively, the application levels expected in fields fertilized with feces from dosed steers (85 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)

(j) 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.

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

(j) Toxicity to Annelids (Cont'd)

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 other three replicates, all 10 worm 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 Wilcoxon[15] and found to be 315 mg/kg soil. However, the confidence

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

(j) Toxicity to Annelids (Cont'd)

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

A greater than 3 million-fold difference exists between the LC_{50} level for earthworms and the environmental burden expected to exist in the soil as a result of fertilization with manure from cattle treated with ivermectin at the intended use level.

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

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

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

(k) Impact of Manufacturing (Cont'd)

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.

The aqueous-based waste stream will consist of the spent fermentation broth and wash waters and will

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

(k) Impact of Manufacturing (Cont'd)

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 air emissions of the Pennsylvania

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

(k) Impact of Manufacturing (Cont'd)

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)

(k) 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)

(k) 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)

(k) 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

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

(k) Impact of Manufacturing (Cont'd)

air in the process 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.

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:

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

(k) Impact of Manufacturing (Cont'd)

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.

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

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

(k) Impact of Manufacturing (Cont'd)

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.

Organic wash liquor residues are disposed of by incineration. Solid waste in the form of contaminated clothing

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

(k) Impact of Manufacturing (Cont'd)

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)

(k) 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

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

(k) Impact of Manufacturing (Cont'd)

collected in plastic sacks, sealed and periodically incinerated at a municipal 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

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

(k) Impact of Manufacturing (Cont'd)

are collected separately from all other site waste streams. Aqueous waste including floor washings 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

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

(k) Impact of Manufacturing (Cont'd)

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 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.

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

(k) Impact of Manufacturing (Cont'd)

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 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.

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

(k) Impact of Manufacturing (Cont'd)

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.

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.

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

(k) Impact of Manufacturing (Cont'd)

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 Environmental 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

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

(k) Impact of Manufacturing (Cont'd)

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. Give Alternatives to the Proposed Action

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.

Ivermectin is a substantial advance over currently-used products to control cattle parasites. High efficacy has been consistently demonstrated against Psoroptes, the causative agent of scabies in cattle, and the hypobiotic stage of Ostertagia. Furthermore, Hypoderma, the cattle grub, 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 cattle 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 cattle, allowing cattle 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 man (beef protein) can be produced per pound of

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)

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 cattle.

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 cattle 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:

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(Signature)

Director, Regulatory Affairs
(Title)

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