IVOMEC® EPRINEX™ (eprinomectin) Pour-On for Beef and Dairy Cattle

ENVIRONMENTAL ASSESSMENT

November 4, 1996

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Pour-On for Beef and Dairy Cattle
Environmental Assessment

1. Date November 4, 1996

2. Name of applicant/petitioner: Merck & Co., Inc.

3. Address P. O. Box 2000
Rahway, NJ 07065-0900

4. Description of the proposed action:

A. Requested action

Merck & Co., Inc. is seeking approval for use of IVOMEC EPRINEX (eprinomectin) Pour-On at a dose rate of 500 mcg/kg of body weight (1 mL per 10 kg of body weight) for treatment and control of endo- and ectoparasites of beef and dairy cattle.

B. Need for the action

The beef and dairy industries suffer extensive economic losses due to both internal and external parasites. These losses have been attributed to clinical disease and loss of productivity due to reduction in feed efficiency. IVOMEC EPRINEX Pour-On provides for effective treatment and control of a broad spectrum of endo- and ectoparasites of cattle. Gastrointestinal roundworms, lungworms, lice, grubs and mange mites, especially *Chorioptes bovis,* are important parasites to control.

IVOMEC EPRINEX Pour-On is a ready-to-use formulation for application with commercially available equipment; it is applied along the backline from the shoulder to the tailhead at a dose volume of 1 mL per 10 kg of body weight.

C. Location where the product will be produced and the types of environments adjacent to those locations

The manufacture of the bulk drug substance, eprinomectin, begins with the fermentation of avermectin broth in the applicant's facilities in Elkton, Virginia and in Danville, Pennsylvania. The avermectin broth manufactured at the Elkton facility will be shipped to the Danville facility for isolation to abamectin and conversion from abamectin to eprinomectin.

Formulation and packaging of the drug product (IVOMEC EPRINEX Pour-On) will take place at the applicant's facilities in Barceloneta, Puerto Rico and Haarlem, Holland for the U.S. market.

The types of environments present at the locations mentioned above, specific to the vicinity of product manufacture and formulation, are described in the following sections.

i. The type of environment at Elkton, Virginia.

Geographic Conditions - The Elkton plant is located on the south fork of the Shenandoah River approximately three miles south of Elkton, Virginia in Rockingham County. Coordinates of the plant's location are latitude 38° 23' N and longitude 78° 39' W. The town of Elkton is located approximately 3 miles northeast of the plant, has a population of less than 1,935 people according to the 1990 U.S. Census Bureau.

The site is approximately 58 acres and employs greater than 800 people. The surrounding neighborhood includes Merck's chemical operations, farmland, wooded acres, and residential homes.

Air Resources - The plant is located in Virginia's Air Quality Control Region II which is in attainment with the National Ambient Air Quality Standards (NAAQS) for sulfur oxides, nitrogen oxides, total suspended particles and ozone. State air regulations generally incorporate standards and procedures required by the United States Environmental Protection Agency (USEPA). The state has incorporated into its regulations the new source performance standards (NSPS), the National Emission Standard for Hazardous Air Pollutants (NESHAPS), and the National Ambient Air Quality Standards (NAAQS). The program for prevention of significant deterioration (PDS) has been delegated to the State of Virginia under 40 CFR Part 51. The plant is approximately two kilometers from a Class I Area (Shenandoah National Park). Prevailing winds near the plant are from the south-southwest.

The mean summer temperature is 23°C (73°F) and the mean winter temperature is 1°C (33°F). Annual rainfall is about 34 inches.

Water Resources - Separate sanitary, process and storm water sewer systems are maintained by the plant. The sanitary wastes, after solids separation and chlorination, are mixed with the process waste for additional treatment in the plant's waste water treatment facility. Water from the storm water system and non-contact cooling water is mixed with the waste water treatment plant effluent and discharged to

the South Fork of the Shenandoah River through the plant's VPDES outfall. There are no injection wells on the plant's property, and the only surface waters within 1000 feet of the plant is the South Fork of the Shenandoah River. The 100-year flood plain elevation at the plant is approximately 973 feet above mean sea level. One well supplies the plant's potable water needs with an additional well as backup.

Land Resources - The terrain surrounding the plant is valley flatland. The Elkton plant is underlain by carbonate rocks of the Rome and Elbrook formations, surficial deposits consist of fluvial sand and gravel, and regolith of residual clays. The bedrock strata beneath the plant are tilted and strike north 57° and dip to the northwest 45°. Handling and disposal of solid waste streams at the Elkton plant is subject to, and in compliance with, the Federal Resource Conservation and Recovery Act (RCRA), the Virginia Solid Waste Management Regulations and the Virginia Hazardous Waste Management Regulations, which are administered by the Department of Environmental Quality.

ii. The type of environment at Danville, Pennsylvania.

Geographic Conditions - The Danville plant is located on a 180 acre site in the Susquehanna River Valley approximately 70 miles north of Harrisburg, Pennsylvania in the borough of Riverside. The plant is located adjacent to the south bank of the North Branch of the Susquehanna River. Coordinates of the plant's location are latitude 40° 57' N and longitude 76° 38' W.

Air Resources - Annual rainfall at the Williamsport Airport (approximately 30 miles from the plant) is 41 inches. The mean summer temperature is 22°C (72°F), while the mean winter temperature is -2°C (28°F). The entire state of Pennsylvania has no significant nitrogen dioxide pollution. The entire state of Pennsylvania is included in the Northeast Transport Region. The Danville plant is located in Northumberland County which is in attainment with the standards for the National Ambient Air Quality Standards (NAAQS) for all criteria pollutants except ozone. The state has incorporated into its regulations the new source performance standards (NSPS), the National Emission Standards for Hazardous Air Pollutants (NESHAPS), and the National Ambient Air Quality Standards (NAAQS). There are no Class I Areas within 50 km of the plant. Prevailing winds near the plant are from the west-northwest direction.

Water Resources - Separate sanitary, process, and storm sewers are maintained at the plant. The sanitary sewer flows to the Borough of

Danville's waste water treatment plant, while the process sewer flows to the plant's waste water treatment facility. Water from the storm sewer merges with the effluent from the plant's waste water treatment system, and the combined streams are discharged to the Susquehanna River through the plant's National Pollutant Discharge Elimination System (NPDES) outfall. The only surface water within 1000 feet of the plant is the North Branch of the Susquehanna River. There are no injection wells on the plant property, and the 100-year flood plain elevation at the plant is approximately 460 feet above mean sea level. The plant derives its potable water entirely from an on-site treatment plant which uses the North Branch of the Susquehanna River as its source. The plant potable water quality meets all requirements of the Federal Safe Drinking Water Act and the Pennsylvania Safe Drinking Water Act.

Land Resources - The Danville Site is located within the Appalachian Mountain Section of the Valley and Ridge Physiographic Province. General topographic trends of the region include long, continuous ridges separated by valleys of varying width. The Danville Site lies on a fairly flat region around which the North Branch of the Susquehanna River flows. Montour Ridge is located directly across the river from the Danville Site, and rises to an elevation above 1000 feet above mean sea level. Elevations on the Danville Site range from approximately 450 to 470 feet above mean sea level, with the steepest slopes occurring along the banks of the river.

iii. The type of environment at Barceloneta, Puerto Rico

Geographic Conditions - The Merck Sharp & Dohme Quimica de Puerto Rico, Inc. (MSDQ) facility is located on a 166 acre site in Barceloneta, Puerto Rico. The city of Barceloneta contains a population of approximately 20,000 people and is located 38 miles due west of San Juan and three miles south of the Atlantic Ocean. The MSDQ plant is located at km 56.7 along state Highway 2. Coordinates of the plant's location are latitude 18° 25' N and longitude 66° 32' W.

Weather/Air Resources - Puerto Rico generally has attained National Ambient Air Quality Standards (NAAQS) although there are problems with particulates in the Cataño air basin. The Barceloneta plant is located in the Barceloneta air basin. The state requires new source permits and operating permits for all point sources. Puerto Rico has been delegated authority over the National Emission Standards for Hazardous Air Pollutants Program (NESHAPS).

Meteorological data for the area is collected at the Isla Verde Airport in San Juan (about 47 miles east of Barceloneta). Annual rainfall is near 60 inches and the mean ambient temperature varies between 24 and 28° C (76 and 82°F). An easterly trade wind is the predominant wind pattern.

Water Resources - The entire fresh water requirements for the plant are supplied by one pumped well and two artesian wells. The artestian wells are used as the primary source of plant water. No other well, or surface water bodies, are located within 1000 feet of the facility. The plant potable water quality meets all requirements of the federal Safe Drinking Water Act. Separate sewer systems exist for sanitary, process and storm water runoff. Process waste water flows into the plant's pretreatment system and then to the Barceloneta Regional Waste Water Treatment Plant (BRWTP). Sanitary waste from the plant joins the effluent from the pretreatment system and the combined streams flow to the BRWTP.

Storm water from the plant is collected in independent trench system, consisting of concrete dikes and swales and directed away from the facility. Surface water runoff from portions of the plant discharge to the sinkhole system which is described in the land resources section below. The MSDQ plant is located approximately 1.25 miles west of the Manati River and 70 meters (230 feet) above mean sea level. The plant is located well above the 100-year flood plain.

Land Resources - The plant is located in an inter-mogote depression. The depression is elongated east-west over a distance of 2 km. The mogotes are asymmetrical hills that are built of massive thick-bedded members of the Aymamon Limestone. A series of sink holes and secondary depressions are located at east and tend in a northwesterly direction from the site. Bedrock beneath the plant site consists primarily of moderately solutioned, recrystallized limestone of the Aymanmon Formation. In depressions between mogotes and ridges, the limestone is overlain by the quaternary blanket sands. The blanket deposits consist mostly of silty or sandy clay which underwent rapid deposition in a subaerial fluvial plain environment. Based on soil borings from the site, 20 percent of the soil is sand. Red-brown to yellow silty clay comprises the dominant soil found in the borings. Land use surrounding the plant includes industrial and mixed industrial. Other industries lie north and west of the facility, the community of Trinidad lies north of the facility, and the rest of the surrounding area is undeveloped.

iv. The type of environment at Haarlem, Holland

Geographic Conditions - The MSD plant in Haarlem, Holland is located in the municipality of Haarlem, near the North Sea coast and approximately 20 km (13 miles) from the city of Amsterdam. The plant is located east of the city of Haarlem on 18 hectare (45 acres) of land near the river Spaarne. The plant is located in the area of Waarderpolder, which is dedicated to industrial activity only. The population of Haarlem is approximately 150,000 people.

Weather/Air Resources - Dutch government laws prescribe emission standards for hazardous air pollutants. No significant air pollution generating industries are located in the vicinity. Annual rainfall is 0.75 meter (30 inches). Mean January temperature is 5-8°C (40-45°F). Prevailing wind directions are west and southwest (sea wind) at a windforce of 3 to 8 Beaufort.

Water Resources - All water used for consumption, process, and sanitary equipment is obtained from the official county supplier. Water quality meets standards of potable water. Water for firefighting can be withdrawn from the River Spaarne. There are no injection wells on the plant property. The sanitary and storm sewer system are directly coupled to the municipal sewer system, while the process effluents are treated before discharge into the municipal sewer. The discharge of wastewater into the municipal sewer is covered by an official permit from the municipality. All wastewater from the municipal sewer is treated in the municipal wastewater treatment plant. The effluent from the treatment plant is discharged into the River Spaarne.

Land Resources - The land of the industrialized zone where the plant is located is reclaimed ("polder"). The soil is composed of layers of clay, sand, and peat.

D. The location where the product will be used and disposed of

IVOMEC EPRINEX Pour-On is a clear, slightly yellow-colored, ready-to-use solution that will be used for treatment of replacement heifers, lactating and non-lactating dairy cows, and for all classes of beef cattle (cows, bulls, heifers and steers) in feedlots or on pasture.

The states with the highest dairy cow populations are Wisconsin, California, New York, Minnesota and Pennsylvania.[1] There were roughly 9.5 million dairy cows and 4.1 million milk cow replacements in the USA as of January 1, 1995.[1] In 1995, there were roughly 2.28

million anthelmintic treatments administered to dairy cows.[2] IVOMEC EPRINEX Pour-On is expected to expand that market due to its use for the treatment of lactating dairy cows. The increase in total doses of avermectins sold is expected to be small, leading to only a minor increase in the total environmental introduction of avermectins.

Cattle feedlots and pastures are located throughout the United States in many different types of environments. The states with the highest beef cattle populations are found in the southwestern, midwestern and western United States, including Texas, Kansas, Nebraska, Oklahoma, California, Missouri, Iowa and South Dakota. [1]

The use of avermectin antiparasitics in beef cattle is an established practice. The overall use of all avermectin antiparasitic products (including IVOMEC EPRINEX Pour-On) on beef cattle is projected to increase by approximately 2-3% per year. Since IVOMEC EPRINEX Pour-On is an alternative for the already established topical and injectable formulations of commercially available avermectins, its use on beef cattle will not result in an incremental increase in environmental introduction of avermectins.

Beef cattle will not be treated with other antiparasitics in the avermectin family concurrently with IVOMEC EPRINEX Pour-On, as the spectra of activity of these compounds are very similar.

Use of IVOMEC EPRINEX Pour-On in dairy husbandry will include dairy cows and replacement heifers. Manure from dairy cows is frequently used directly as fertilizer in accordance with local practices. In a dry lot, excreta is accumulated over time, i.e., similar to a beef cattle feedlot. In the free-stall barns, some bedding is mixed in with excreta. Manure from concrete areas, such as around the milking facilities (or parlor) or around feeding areas, is flushed off several times a day and is usually collected in settling ponds.

Uses of IVOMEC EPRINEX Pour-On in beef cattle husbandry will be similar to those of other avermectins approved for treatment of beef cattle. Most (approximately 58%) of the treatments of beef cattle with avermectins are for cattle in the cow/calf sector. The rest of the treatments of beef cattle with avermectins are for animals in the commercial feedlots (approximately 22%), stockers on pasture (approximately 8%) and cattle in the farmer/feeder market (approximately 4%). Most of the sales of avermectins occur in the fall, which coincides with movement of large numbers of cattle into feedlots,

the weaning of calves and the movement onto winter grazing.[3] Manure from feedlots is frequently used directly as fertilizer in accordance with local practices.

Manure from facilities where cattle are treated with IVOMEC EPRINEX Pour-On would not be spread on fields concurrently treated with abamectin pesticide formulations. EPA-approved uses (cotton, citrus, ornamental crops, tomatoes, peppers, eggplants, head lettuce, celery, pears, strawberries, almonds and walnuts) are for foliar application to growing plants, not for preemergence applications directly to soil. Similarly, applications of abamectin for control of fireants are not applied to freshly plowed fields used for crop production. Thus, there would be no concurrent applications of manure containing eprinomectin on fields also undergoing treatment with any other avermectin products.

Empty applicators will be disposed of in household trash. Negligible quantities of eprinomectin and other ingredients used to formulate the IVOMEC EPRINEX Pour-On product will enter the environment primarily through landfill. Environmental concentrations of eprinomectin resulting from manufacture and disposal of the product will be many orders of magnitude below the levels entering the environment from use of the product to treat cattle.

Merck has a return goods policy to handle outdated, off-spec, and damaged product. In the U.S. market, outdated material or otherwise unsellable goods are returned by the customer to our St. Louis distribution center. The goods are collected in St. Louis and sent to either: Merck West Point facility in Pennsylvania or to a permitted facility for incineration. At Merck's West Point facility, the air emission controls for the disposal of this product meet the requirements of the Pennsylvania Air Pollution Control Regulations under Title 25 of the Pennsylvania Code, Article III- Department of Environmental Protection (PA DEP), Chapters 121-143. Ash generated from the Merck West Point facility incineration process is disposed of at a permitted facility and is monitored to conform its acceptability with prevailing solid waste regulations.

5. Identification of chemical substances that are the subject of the proposed action

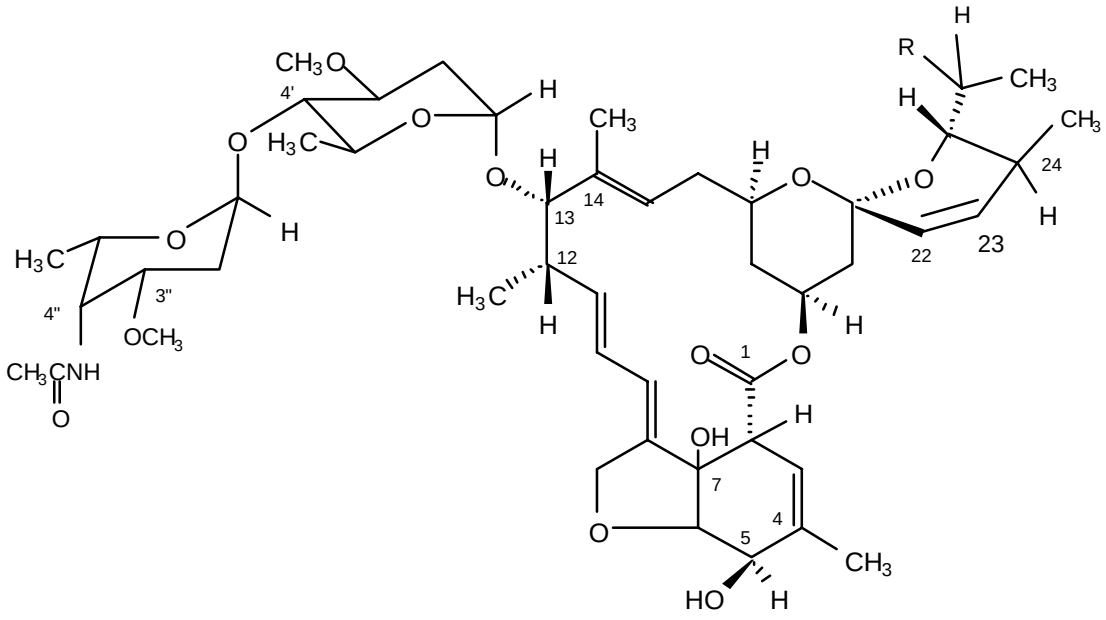
A. IVOMEC EPRINEX (eprinomectin) Pour-On for Beef and Dairy Cattle:

The active ingredient which is the subject of this document:

- Eprinomectin (MK-397; L-653,648)
- Chemical name: Eprinomectin, CAS-159628-36-1, is a mixture of two components having a ratio of 90% or more of eprinomectin component B_{1a} and 10% or less of eprinomectin component B_{1b}; CAS-133305-88-1 [component B_{1a}]; CAS-13305-89-2 [component B_{1b}]. The chemical names of the two major components are, (4"R)-4"-(acetylamino)-5-O-demethyl-4"-deoxyavermectin A_{1a} (R=C_2H_5 in Figure 1) and (4"R)-4"-(acetylamino)-5-O-demethyl-25-de(1-methylpropyl)-4"-deoxy-25-(1-methylethyl)avermectin A_{1a} (R=CH_3 in Figure 1). The former is also known as 4"-epiacetylamino-4"-deoxyavermectin B_{1a} and the latter is also known as 4"-epiacetylamino-4"-deoxyavermectin B_{1b}.

B. The structure and properties of eprinomectin

FIGURE 1



		Molecular Weight
B_{1a} Component	R = C_2H_5	914
B_{1b} Component	R = CH_3	900

Eprinomectin is produced by chemical modification of the fermentation product abamectin (avermectin B_1) and is a mixture of two closely related homologues belonging to a class of compounds known as avermectins.[4] Eprinomectin contains at least 90% of the compound in which R in the above structure is the ethyl group and less than 10% of the compound in which R is the methyl group.

Eprinomectin is freely soluble in polar organic solvents. The infrared, mass and nuclear magnetic resonance spectra are consistent with the proposed structures. [4]

Physical properties of eprinomectin are listed below (see Appendixes C-1 and C-2 for details):

Physical Properties of Eprinomectin

Sublimation (Vapor) Pressure, torr[a]	$4\pm1 \times 10^{-6}$
Log(Octanol/Buffer Partition Coef.)[b]	5.4 ± 0.3
K_{oc}[c]	≥3000
Aqueous Solubility[d], ppm	3.5(±0.2)
A1% 1 cm, 244 nm[e]	343
Density, g/cm^3	1.23 ± 0.04
Melting Point, °C[f]	163-166
Dissociation Constant (pKa)	No pKa between 3 and 10

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[a] 22.5 ± 0.9°
[b] pH 6.8
[c] three soils used
[d] pH 7.26 ± 0.09
[e] 50:50 water:acetonitrile
[f] differential scanning calorimetry at 2°C/min under nitrogen

6. Introduction of substances into the environment

The introduction of substances into the environment can occur from the manufacture of the drug substance (abamectin to eprinomectin) manufacturing facilities, drug product (IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle) manufacturing and packaging facilities and the sites of intended use of this product in cattle.

A. Elkton, Virginia

The following summarizes the environmental aspects of manufacture of abamectin at the Elkton plant.

i. Liquid Waste

The manufacturing process generates aqueous waste streams from fermentor vents, fermentor sample funnels, equipment washes and floor drains. All aqueous waste is collected via piping or collection sump in a 20,000 gallon collection tank or directly transferred to either holding tanks or tank trucks. From the collection tank, the waste can be transferred either to an evaporator system to concentrate the liquid waste prior to shipment off-site or directly to a tank truck. The liquid waste is then sent to the applicant's Danville facility in Pennsylvania for treatment and disposal. The specifics of waste water treatment

employed at the Danville facility are described in the section (B) below. On a limited case-by-case basis, liquid wastes that have been determined through process knowledge and detailed analysis to contain less than a threshold concentration of avermectins will be sewered to the site's advanced activated sludge system (wastewater treatment plant).

Effluent from the facility's wastewater treatment plant is discharged directly to Shenandoah River under the Virginia Pollutant Discharge Elimination System (VPDES) Permit #VA0002178. The VPDES permit is administered by the Virginia Department of Environmental Quality. The effluent currently has maximum daily limits of TSS ≤ 5,338 kg/d and COD ≤ 17,246 kg/d and pH limits between 6.5 and 9.5. No new permit limits are anticipated as a result of the proposed action and approval will not impact the facility's ability to comply with all applicable permit conditions.

ii. Air Emissions

The fermentation step generates fermentation off-gases that contain typical respiration byproducts, including carbon dioxide (CO_2). The on-site incinerator emissions consist of typical combustion products.

Air emissions are subject to and in compliance with the Virginia Regulations for the Control and Abatement of Air Pollution. The on-site trash incinerator is in compliance with the Commonwealth of Virginia Regulations for the Control and Abatement of Air Pollution. No new permit limits are anticipated as a result of the proposed action and approval will not impact the facility's ability to comply with all applicable permit conditions.

iii. Solid Waste

Burnable, non-hazardous, solid wastes containing a "de minimis" amount of avermectins may consist of paper, aluminum, plastic, and drums. Such wastes are incinerated on-site or sent to a permitted incineration facility able to accept such waste streams. Other non-hazardous wastes which cannot be recycled are disposed of at a state licensed landfill.

Disposal of non-hazardous solid waste is subject to and in compliance with Permit #183 issued under the Virginia Solid Waste Management Regulations. There are no numerical permit limits on solid waste generation and no additional permit conditions are anticipated as a result of the proposed action.

iv. Employee Protection

Material Safety Data Sheets are available on site for all chemicals as required by the Occupational Safety & Health Act of 1971, the Hazards Communication Act of 1985 and Title 29 Code of Federal Regulations (CFR) Part 1910. Employees associated with the manufacture of drug product have appropriate MSDSs available for their review. Employee protective clothing, such as gloves, uniforms and safety glasses are used during the packaging process to assure compliance with the Occupational Safety & Health Act of 1971 and the Hazard Communication Act of 1985 and Title 29 CFR Subpart I.

v. Environmental Exposures

Quantities of substances that enter environmental media (i.e. soil, water and air) as a result of use and/or disposal of products related to the manufacturing of abamectin are expected to be inconsequential.

B. Danville, Pennsylvania

The following summarizes the environmental aspects of manufacture of abamectin pure and eprinomectin at the Danville plant.

i. Liquid Waste

The manufacturing processes for abamectin and its conversion to eprinomectin generate two types of liquid-waste streams: one, a combination of solvent-based waste streams, the other, a combination of aqueous waste streams.

Solvent-Based Liquid Wastes - The solvent-based waste streams from the abamectin manufacturing process are generated in the isolation step and in the recovery of solvents used for the isolation. They contain discarded organic compounds (e.g., avermectins) in a solution of solvents such as toluene, methanol, ethanol, hexane.

Solvent-based waste streams are also generated during the conversion of abamectin to eprinomectin. They contain discarded organic compounds (e.g., avermectins) in a solution of solvents such as toluene, methanol, isopropyl acetate, heptane, and acetonitrile.

Solvent-based wastes will either be sent-off site for disposal to a permitted facility, disposed of in an on-site permitted incinerator, or processed so as to recover the major portions of the organic solvents to

the extent feasible to minimize any potential release of organic compounds to the environment. Residues from the solvent recovery operations are destroyed by incineration or sent off-site for disposal or beneficial reuse. The incineration process is subject to and in compliance with the Pennsylvania Rules and Regulations for the protection of Environmental Resources, Title 25, Part I, Subpart C, Article I, Land Resources, Chapter 75, Solid Waste Management and Article III, Air Resources and 40 CFR Parts 264 and 265, Standards Applicable to Owners and Operators of Hazardous Waste Treatment, Storage and Disposal Facilities.

Aqueous-Based Wastes - The aqueous-based waste streams from abamectin manufacturing consist of spent fermentation broth and wash waters that contain unconsumed fermentation nutrients, unrecovered by-products and traces of avermectins and dissolved solvents such as hexane, methanol, ethanol, and toluene. The aqueous-based waste streams from the conversion of abamectin to eprinomectin consist of traces of avermectins, organic salts and dissolved solvents such as methanol, ethanol and tetrahydrofuran.

Aqueous waste streams will either be sent off-site to a permitted facility for disposal, or treated in an on-site high pressure, high temperature reactor using caustic designed to destroy residual avermectins. The effluent from the high pressure reactor is further treated in an on-site two-stage biological waste water treatment plant before being discharged into the Susquehanna River. The final plant effluent is discharged under the requirements of and in compliance with NPDES Permit No. PA 0008419 which is administered by the Pennsylvania Department of Environmental Resources. The amount of avermectins released into the Susquehanna River is below levels of environmental concern based on toxicity testing.

ii. Air Emissions

The fermentation step generates fermentation off-gases that contain typical respiration by-products, including carbon dioxide. Air emissions generated from the abamectin production consist of volatile organic compounds (such as hexane, methanol, ethanol, and toluene) and dust. Volatile organic emissions from the abamectin production process are controlled by condensers and a fume incinerator. Dust in the process building will be filtered with HEPA-type filters to control the introduction of avermectins and dust into the ambient air with an efficiency greater than 99.9%.

Air emissions generated from the conversion of abamectin to eprinomectin consist of volatile organic compounds (such as toluene, methanol, isopropyl acetate, heptane, and acetonitrile). Volatile organic emissions from the conversion process are controlled by condensers, a vent scrubber, or a fume incinerator.

Air emissions are in compliance with the regulations of the Pennsylvania Department of Environmental Resources (Title 25, Part I, Subpart C, Article III, Air Resources) and operating Permit No. 49-313-032.

iii. Solid Waste

Dry solid waste (such as paper, trash, and HEPA-type filters) from the abamectin production process and conversion process is disposed of by off-site incineration.

iv. Employee Protection

Material Safety Data Sheets (MSDS) are available on-site for all chemicals required by the Occupational Safety & Health Act of 1971 and the Hazards Communication Act of 1985. Employees associated with the manufacturing of abamectin have appropriate MSDSs available for their review. Employee protective clothing, such as gloves, uniforms, and safety shoes, and protective equipment, such as safety glasses, are used during the manufacturing process to assure compliance with the Occupational Safety & Health Act (OSHA) of 1971 and the Hazards Communication Act of 1985.

To minimize worker exposure to avermectins, the following monitoring activities are conducted:

a. At least bi-annual monitoring of dust levels for abamectin where abamectin powder is handled; and

b. At least monthly wipe test on equipment, floors and production bottles in the production area.

Air, liquid, and solid waste emissions are in compliance with the environmental control regulations mentioned above. The Danville plant is also in compliance with all applicable OSHA requirements.

v. Environmental Exposure

Quantities of substances that enter the environmental media (i.e. soil, water and air) as a result of use and/or disposal of products related to the manufacturing of abamectin are inconsequential.

HEPA-type filters control the introduction of avermectin dust into the ambient air with an efficiency greater than 99.9%.

Solvents are recovered for reuse, incinerated on-site or sent off-site to a permitted facility for disposal/incineration, and aqueous waste are either sent off-site to a permitted facility for disposal/incineration or treated on-site.

Wastewaters containing residual avermectins are treated to destroy the avermectins in a high pressure reactor using caustic. Effluent from the high pressure reactor is further treated in the on-site wastewater treatment plant before being discharged into the Susquehanna River. The traces of avermectins allowed into the Susquehanna River are determined by the Pennsylvania Department of Natural Resources. The amount of avermectins released into the Susquehanna River is below levels of environmental concern.

C. Barceloneta, Puerto Rico

The following summarizes the environmental aspects of formulating and packaging IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle at the applicant's facility in Barceloneta.

i. Liquid Waste

A small organic waste stream containing solvents may be generated from equipment cleanouts and wipedowns. In such cases, the solvent stream is incinerated.

The on-site incineration process will be subject to and in compliance with the Puerto Rico Environmental Quality Board (EQB) Regulations for the Control of Atmospheric Pollution and the U.S. EPA regulations for the control of hazardous waste, 40 CFR Parts 264 and 265. Currently, the solvent incinerator operates under a permit issued by the EQB Hazardous Waste Program and under EQB Permit NO. PFE-09-12911668-I-III-0 issued by the EQB Air Program. The USEPA hazardous waste identification for the site is PRD090028101.

ii. Air Emissions

Air emissions generated during the formulation of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle consist of organic compounds (such as propylene glycol octanoate deacanoate) and dust. Air from the process building, formulation area sterile facility is exhausted through HEPA-type filters prior to discharge to the atmosphere to control particulate emissions of eprinomectin (drug substance). Air emissions are subject to and in compliance with the Puerto Rico Environmental Quality Board under the "Regulations for the Control of Atmospheric Pollution." Manufacture of drug product is also in compliance with conditions under permit number PFE-09-1289-1089-I-III-0.

iii. Solid Waste

Dry solid waste generated in the production of drug product such as paper, trash, and HEPA-type filters etc., will be disposed of in an incinerator on-site. The incinerator is in compliance with air & solid waste disposal regulations of the Puerto Rico Environmental Quality Board (EQB).

iv. Employee Protection

Material Safety Data Sheets are available on-site for all chemicals required by the Occupational Safety & Health Act of 1971 and the Hazards Communications Act of 1985. Employees associated with the manufacturing of drug product have appropriate MSDSs available for their review. Employee protective clothing (such as gloves, uniforms, safety glasses, safety shoes, and protective equipment) is used during the manufacturing process of drug product to assure compliance with the Occupational Safety & Health Act of 1971 and the Hazards Communication Act of 1985. To minimize worker exposure to eprinomectin, the following monitoring activities will be conducted:

a. At least semi-annual monitoring of dust levels where eprinomectin powder is handled;

b. Wipe tests are performed to verify the cleanup of spills in the formulation area.

v. Environmental Exposure

Quantities of substances that enter environmental media (i.e., soil, water, air) as a result of formulation of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle are inconsequential.

HEPA-type filters control the introduction of eprinomectin into the ambient air with an efficiency greater than 99.9%.

As per the MSDS for eprinomectin, any solid waste containing the substance is incinerated at a temperature greater than 600°C.

D. Haarlem, Holland

The following summarizes the environmental aspects of formulating and packaging IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle at the applicant's facility in Haarlem.

i. Liquid Waste

Waste streams containing eprinomectin are generated in the formulation and packaging of the drug product. Small quantities of organic solvents, such as Miglyol and water, from equipment cleaning and wipedowns are generated. Waste organic solvents are collected and sent to the Rotterdam incinerator. The disposition of organic solvents is in compliance with the Hazardous Waste Act and the Waste Act.

Any aqueous waste resulting from manufacturing the drug product will be collected and treated with an activated carbon purification unit to remove the eprinomectin. The wastes will then enter the plant's general waste system which includes domestic sewerage and will go via a neutralization pit (pH >6.5) to the municipal sewerage treatment plant. This plant operates under the control of the Hoogheemraadschap van Rijnland. Merck has a permit from the municipality for entering the sewerage treatment plant with their plant effluent. The waste water discharge is regulated by, and in compliance with, the "Wet Verontreiniging Oppervlaktewateren" which includes the Waste Water Regulations. Spent activated carbon from the filter system will be collected in plastic bags, put into drums, and handled as a hazardous waste as described below.

ii. Air Emissions

Air-borne particulates and dust are controlled by HEPA-type filters. Any air emissions from the plant are regulated by, and in compliance

with, the State Rules and Regulations Act with regard to environmental pollution. These regulations are administered by the Haarlem Department of Environmental Control.

iii. Solid Waste

Solid waste resulting from production and packaging of the drug product, such as HEPA-type filters, will be combined with other plant trash and transferred via closed vehicle to the Rotterdam incinerator. A permit for transport and incineration is issued by the provincial authorities under the laws regulating transport and processing of solid wastes.

iv. Employee Protection

Material Safety Data Sheets (MSDS) are available for all chemicals required by the Dutch Safety Law (Arbo Law) and the Dutch Safety Rules for Industry and Workshops. Employees associated with the formulation and packaging of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle have appropriate MSDSs available for their review. As additional worker protection, monthly swab tests are performed for eprinomectin on equipment, floors, and production bottles in the production area.

The manufacturing is regulated by, and in compliance, with the Dutch Safety Law (Arbo Law) and the Dutch Safety Rules for Industry and Workshops. The manufacturing is also regulated, and in compliance with, the "Wet Milieubeheer" which includes the Air Pollution Act, the Noise Abatement Act, the Hazardous Waste Act, the Waste Act and the Waste Regulation, and in compliance with the “Wet Verontreiniging Oppervlaktewateren” which includes the Waste Water Regulations.

v. Environmental Exposure

Quantities of substances that enter environmental media (i.e., soil, water and air) as a result of the formulation and packaging of the IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle are inconsequential. HEPA-type filters control the introduction of eprinomectin dust into the ambient air with an efficiency greater than 99.9%.

E. Effect of Application Approval on Compliance with Current Emissions Requirements

Merck & Co., Inc. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of drug product at its facilities in Elkton, Virginia; Danville, Pennsylvania; Barceloneta, Puerto Rico; and Haarlem, Holland as well as emission requirements set forth in applicable federal, state, and local statutes and regulations applicable to the production of eprinomectin and IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle at its facilities in Elkton, Virginia; Danville, Pennsylvania; Barceloneta, Puerto Rico; and Haarlem, Holland.

Approval of the use of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle will have no significant effect upon compliance with current emissions requirements at either Elkton, Virginia; Danville, Pennsylvania; Barceloneta, Puerto Rico or Haarlem, Holland.

F. Introduction through use in the target animal

i. Dosing

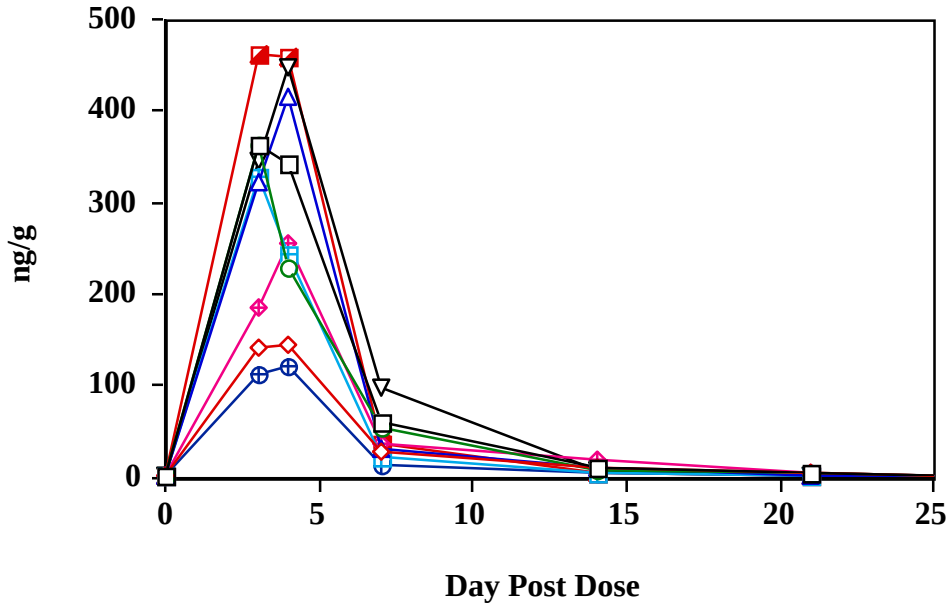
IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle is applied topically at a dose level of 500 mcg/kg body weight, i.e., 1 mL of 0.5% (w/v) eprinomectin applied per 10 kg of body weight. Treated dairy cows are generally housed in free-stall barns or maintained in open barn and dry lot conditions. Generally, the dairy cows will be given only one dose of the drug annually; however, year-round parasite control programs could involve 2 treatments per year, including use in young replacement heifers on pasture. Beef cattle which would be treated with IVOMEC EPRINEX Pour-On in a feedlot would be treated once upon entering the feedlot; beef cattle on pasture could be treated early in the grazing season to prevent acquisition of parasites or late in the grazing season to remove acquired burdens.[3] The average number of all anthelmintic treatments given to cattle in the US is between 1 and 1.3, with younger animals tending to be treated more frequently.[3]

ii. Drug residue in excreta

In a radioresidue trial, Study CA-368, samples of feces from two steers dosed topically with [^{3}H]eprinomectin at 500 mcg/kg body weight (the proposed dose rate) were collected daily through day 14 and on days 21 and 28 post dose (APPENDIX C-3). A total of 19% and 16% of the administered dose was excreted in feces of the two steers in the 28-day slaughter group. Only 0.45% of the administered dose was excreted in urine of either steer, for a total of about 17 - 20% of the dose in excreta through 28 days. Excreta was collected as a mixture of feces and urine from the heifer in the 28-day slaughter group and 18% of the dose was excreted. Eprinomectin accounted for 85.9% of the total radioresidues in feces/excreta with the B_{1a} component representing 78.3% of the total residues (APPENDIX C-4). The levels of the B_{1a} component in feces in a non-radiolabeled trial, conducted under commercial field conditions with nine cattle, peaked on days 3 - 4 post dose at between 122 and 462 ng/g on a wet-weight basis, Figure 2 (APPENDIX C-5). Since the B_{1a} component comprises approximately 78% of the total eprinomectin-related residue in feces, the peak eprinomectin-related levels would be between 150 to 600 ng/g on a wet-weight basis in feces. The mean peak level of total eprinomectin-related residues in feces, on a dry weight basis, was 2372 ng/g on days 3-4 after dosing. The moisture content in wet feces was about 87.6% on days 3 - 4 post dose. Based on the eprinomectin B_{1a} content per gram of dry matter, no depletion of residues occurred over 126 days in pats formed from bulk collections on Day 3/4 and deposited on pasture on Day 4. However, the dry weight of the pats decreased over time and the amount of eprinomectin B_{1a} per pat decreased from 246 mcg at deposition to 137 mcg at 126 days after deposition. Thus, the half-life of degradation of both pat and eprinomectin was about 150 days. Also, pats from treated and control animals degraded at the same rate based on a comparison of the pat dry weights on days 105 and 126. Inner and crust layers of pats were assayed for up to 63 days after deposition. Based on the eprinomectin B_{1a} content per gram of dry matter, there were no marked differences between eprinomectin B_{1a} residue levels in inner and crust layers.

FIGURE 2.

Residue levels (B_{1a} component) in feces in a nonradiolabeled trial, conducted under commercial conditions.



iii. Metabolism

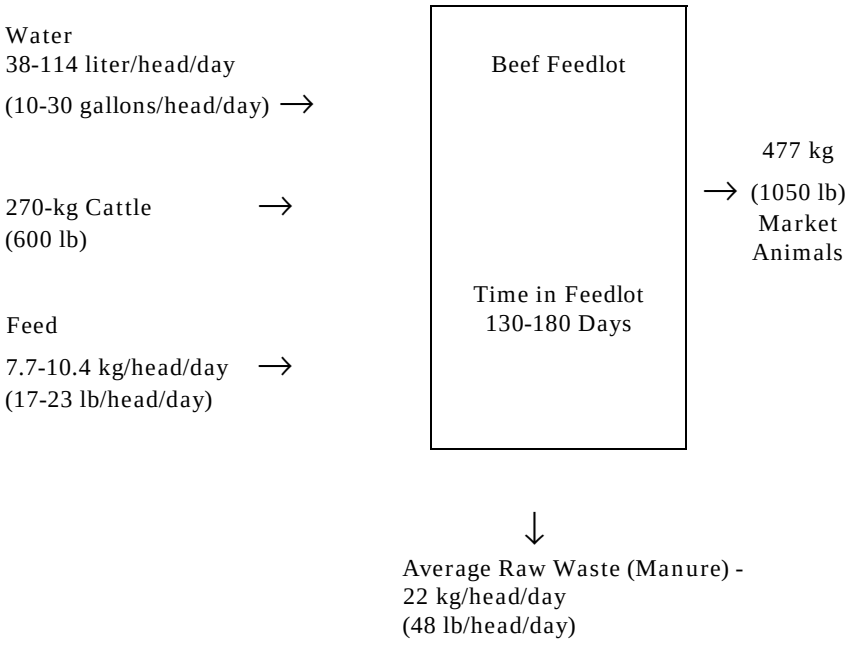
Extracts from liver and composite feces from cattle in Study CA-368 were subjected to HPLC using reverse phase conditions to determine the extent of metabolism (APPENDIX C-4). The major radiolabeled component in liver and feces of cattle dosed topically with eprinomectin was unchanged drug. Eprinomectin comprised about 86% of the total residue in a composited sample of feces excreted on days 1 - 14 post dose. There was only one major, more-polar metabolite in the feces which accounted for 7.4% of the total drug residues. Since the drug-residue in the feces of cows dosed with eprinomectin is mostly unchanged drug, the effects scenarios in this EA are developed using toxicity data for eprinomectin.

iv. Use of manure as fertilizer

In the case of beef cattle dosed with IVOMEC EPRINEX Pour-On in feedlots (the worst-case situation with respect to the concentration of cattle in an area), the following calculations, based on a U.S. Environmental Protection Agency publication,[5] show the expected concentration of eprinomectin and metabolites in the raw waste (manure) and the concentration in a field when the manure is spread as a fertilizer.

Below is a flow diagram from the above reference showing the daily raw waste produced in a typical feedlot operation in which a 270 kg animal enters the operation and in 130-180 days reaches market weight of about 477 kg. During this period, the animal would be treated once with IVOMEC EPRINEX Pour-On at a dose level of 0.5 mg/kg.

Typical Beef Feedlot Flow Diagram



The following calculations (based on 100% of drug and metabolites excreted or shed) show the average concentration of eprinomectin and its metabolites in the waste produced by a single animal over a 130-day period.

Weight of animal	270 kg
Dose rate	500 mcg/kg
Dose of eprinomectin	135 mg
Amount of eprinomectin or metabolites excreted (assume 20% of dose) or shed (80% of the dose)	135 mg
Waste produced per animal per day	22 kg
Total time in feedlot	130 days
Total waste produced per animal	2860 kg

Concentration of drug and metabolites in waste (130 days):

$$\frac{\text{135 mg excreted}}{\text{2860 kg waste}} = \frac{\text{0.047 mg}}{\text{kg}} = 47 \text{ ppb}$$

In the case of mature dairy cows dosed twice per year with IVOMEC EPRINEX Pour-On the following calculations (based on 100% of dose excreted or shed as drug and metabolites with hair or skin) show the expected concentration of eprinomectin and metabolites in the raw waste (manure) over 1 year.

Weight of animal	500 kg
Dose rate	500 mcg/kg
Dose of eprinomectin (2 applications/year)	500 mg
Amount of eprinomectin and metabolites excreted (20% of dose) or shed (80% of dose)	500 mg
Excreta produced per animal per day[a]	50 kg
Total excreta produced over 365 days	18,250 kg

[a] estimated to be 10% of body weight/day.

Concentration of drug and metabolites in excreta (365 days):

$$\frac{\text{500 mg excreted}}{\text{18,250 kg waste}} = \frac{\text{0.027 mg}}{\text{kg}} = 27 \text{ ppb}$$

The above "worst-case" calculation would be for a dairy cow dosed twice per year. Manure might be composted, hauled and spread on fields only once or twice a year to minimize labor costs and because of the lack of available land for spreading during the growing season. Or, manure and bedding might be scraped, hauled and spread daily or periodically on fields as weather permits.[6] The scenario assumes all the applied drug would be absorbed and excreted or shed with sloughed hair and skin. The sloughed hair and skin would be a component of the bedding which would be cleaned from the barns with the excreta as manure. Thus, the scenario assumes 100% of the dose will be in the manure and will be applied to a field in one application.

If the manure (feces and urine) collected over a period of 130 days from beef cattle dosed once were spread on a field as fertilizer at a rate of 15 tons per acre and plowed to a depth of 6 inches (see APPENDIX A for calculation), the total concentration of eprinomectin-related residues in the soil would be 0.69 ppb (Table 1).

If the manure (feces and urine) collected over a period of 1 year from dairy cows dosed twice a year were spread on a field as fertilizer at a rate of 15 tons per acre and plowed to a depth of 6 inches (see APPENDIX A for calculation), the total concentration of eprinomectin-related residues in the soil would be 0.40 ppb (Table 1).

TABLE 1 **Environmental Burden** **for Eprinomectin dosed at 500 mcg/kg**	
Beef Cattle:	
Total Drug Dosed Per 270-kg Animal (1 application/130 days)	135 mg
Amount of Drug Residues Entering Environment[a]	135 mg
Level of Drug Residues in Waste[b]	47 ppb
Level of Drug Residues in Plowed Field (15 tons waste per acre plowed 6 inches deep)	0.69 ppb
Dairy Cows:	
Total Drug Dosed Per 500-kg Dairy Cow (2 applications/year)	500 mg
Amount of Drug Residues Entering Environment[a]	500 mg
Level of Drug Residues in Waste[c]	27 ppb
Level of Drug Residues in Plowed Field (15 tons waste per acre plowed 6 inches deep)	0.40 ppb

a Assumes 20% of applied drug will be absorbed and excreted and 80% will be shed with hair or skin
b Waste collected for 130 days
c Waste collected for 365 days

v. Introduction of Drug Residues into Pastures

a) Cow/calf unit scenario

A scenario has been developed to address a 180-day pasture situation involving a cow/calf unit on one acre in which both animals receive IVOMEC EPRINEX Pour-On. The scenario assumes that all the administered eprinomectin is excreted as parent drug and metabolites via the feces.

With respect to the environmental burden on pasture resulting from the use of IVOMEC EPRINEX Pour-On, a cow/calf unit (both animals treated; 200-kg calf at 500 mcg/kg, and 500-kg cow also at 500 mcg/kg) would excrete 350 mg of drug-related residue.

At a cow/calf stocking rate of one unit per acre and with uniform distribution of dung pats across a pasture, the environmental burden would be 350 mg/acre. This may, in fact, not accurately reflect the distribution of residues in a pasture. Cattle are gregarious, and on pasture aggregate in herds. Hence, eprinomectin residues in pats from treated animals would tend to be localized in those areas traversed by the herd during the first several weeks post application.

Cattle are highly mobile, and the distance traveled by them during their grazing on pastures is generally several miles per day.[7-11] According to Hart *et al.*, the distance to water appears to be the major factor controlling the distances traveled by cattle; these authors found that grazing was heavier near water than at distances greater than 3 km away from a water source.[11] In another study, Hart and associates found that on a moderately stocked pasture (72 cow/calf pairs and 108 yearlings on 612 ha) animals grazed an average of 0.53 km from water.[12] Sixteen percent of the total area was within 300 m of water, with 47% of animal use therein. Based on counts of fecal droppings, Wilkinson *et al.* reported that feces frequency (number of pats/steer/day/m^2) was three times greater near water than away from it, and Seman *et al.* also found that steers spend more time grazing at sites close to water source.[13,14] Pinchak *et al.* reported that 77% of observed use by cattle was within 366 m of water during a 3-7 week grazing season on ~600 ha pastures. [15]

Cattle, individually and presumably as components of herds, thus move considerably large distances each day during grazing, with their focus on water supplies. As forage is consumed the cattle move from cropped to fresh areas within a pasture, and this movement will result in the deposition of dung pats across wide areas of pastures. Havstad *et al.* reported that on the average only 35% of a 708-acre pasture (2.4 acre per cow/calf pair) was utilized by beef cows during a summer grazing season.[7] If 100 cow/calf pairs treated with IVOMEC EPRINEX Pour-On were present on a 100-acre pasture, and if it were conservatively assumed that only 20% of the pasture was grazed by the animals during the first four weeks post application, nearly all of the 350 mg of drug residue per cow/calf pair would be present in pats on 20 of the 100 acres for a concentration of 1750 mg/acre. This calculated value is ~2.7 times

greater than the amount of eprinomectin residue resulting from use of manure from treated beef cattle as fertilizer (15 tons/acre x 47 mcg/kg x 0.453 kg/lb x 2000 lb/ton x 10^{-3} mg/mcg = 639 mg/acre).

b) Seasonal treatment of pastured cattle scenario

This assessment is based upon the projected seasonal treatment of pastured cattle with endo- and ectoparasiticides.

Information supporting this section was provided to the CVM in detailed confidential reports. Information from these reports has been incorporated into this Environmental Assessment. Regional specialists in the United States listed in Section 12 of this Environmental Assessment contributed to the reports.

First, anthelmintic usage, including endectocides, in beef cattle was assessed. Second, since IVOMEC EPRINEX Pour-On will be marketed for use on lactating dairy cows, use of anthelmintics and ectoparasiticides was also assessed for dairy cows. To assess anthelmintic usage in beef cattle by season, the United States was partitioned into regions that could be rationalized based upon the nature of the cattle industry, seasonal availability of pasture and husbandry practices. The cattle industry varies from the northern dairy states, where there are important dairy and cow/calf operations, to the southeast, which is primarily cow/calf, to the western range where cow/calf operations are managed on arid pasture with stocking rates often as low as one cow/calf unit per 50 acres. In these arid areas, parasitological challenge is less of a concern than in areas of lush grasses.

Ten regions of the United States and the states in each region subjected to analysis are as follows:

Region	States In Region
Upper Southeast	AR, DE, KY, MD, MO, NC, TN, VA, WV
Lower Southeast	AL, FL, GA, LA, MS, SC
South Central	KS, OK, TX
Southwest	AZ, CO, NM, NV, UT
Pacific States	CA, OR, WA
Hawaii	HI
Big Sky	ID, MT, WY
Plains	NE, ND, SD
Northern Dairy	IA, IL, IN, MI, MN, NY, OH, PA, WI
New England	CT, MA, ME, NH, NJ, RI, VT

Alaska was not included in the analysis because the cattle population is small.

For each region, cattle specialists gathered information about beef cattle management practices and anthelmintic use. The specific goal was to determine the estimated actual seasonal use of anthelmintics in pastured cattle by class. The estimated actual represents the experts' assessments regarding the percent of cattle actually being treated with anthelmintics. To project the estimated actual use, each regional specialist obtained and provided expert opinion regarding the uses of all anthelmintics, the percent of pastured cattle treated by class and the months of the year when treatments occur.

The regional experts described cattle management practices, including breeding schedules and weaning time, and percent of calves that are born in the fall and spring within the assigned regions. Differentiation of spring and fall calvings is necessary; spring-born calves and fall-born calves are treated at different times of the year. Pasture types/management, grazing season and environmental conditions within each assigned region were also defined.

Several assumptions were made to determine the seasonal use of anthelmintics by class. USDA cattle statistics were used as an accurate estimate of cattle numbers by class.[16,17] To estimate the number of beef and dairy calves, the calf crop was partitioned according to the ratio of beef to dairy cows. It was assumed that the cattle industry and husbandry practices are similar across each region since this was the basis for the regional assessment. Where this was not the case, the region was subdivided based upon the expert opinion of the regional specialists. The estimated actual treatment represents the experts' assessment regarding the percent of cattle actually being treated by class and the frequency and timing of treatment. Sales estimates of anthelmintics agree with and support the estimated actual usage values. The market analysis was conducted by the Merck AgVet marketing organization and verified by an independent organization.

Dairy calves are reared in confinement until they are weaned. Thereafter, they enter other categories in the USDA statistics, i.e., calves <500 pounds, or dairy replacements. Consequently, dairy calves are not considered among pastured cattle for purposes of this assessment.

Classes of cattle that are considered in this assessment include beef cows, beef replacements, beef calves, milk replacements, other heifers >500 pounds, steers >500 pounds, bulls >500 pounds and calves <500 pounds. USDA statistics for classes of cattle commonly found in feedlots (other heifers >500 pounds, steers >500 pounds and calves <500 pounds) were adjusted to remove cattle in feedlots by subtracting USDA statistics of feedlot cattle.

Numbers of pastured cattle, by class, were used with the projected seasonal use and the frequency of use to derive the estimated actual number of beef cattle being treated with anthelmintics by class at any given time of the year. Regional assessments were prepared using information derived for individual states.

Table 2 presents the estimated actual use of all anthelmintics for each of the regions by month.

TABLE 2
ESTIMATED ACTUAL PERCENTAGES OF PASTURED BEEF CATTLE
DOSED WITH ANTHELMINTICS BY REGION AND MONTH

	JAN	FEB	MARCH	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
Upper Southeast			4	21	1		15	4		13	9	
Lower Southeast		3	15	13			13		3	15	7	
South Central			5	9					13	21	9	
Southwest				7	13					4	4	1
New England			6	12	9	3				33	41	8
Hawaii	16	2	8	7	1			3	11	2		2
Big Sky				3	3					17	7	
Plains (North Central)		1	2	3	8	1			5	18	14	3
Northern Dairy			4	24	12	1	3	1		5	8	
Pacific Eastern				9	2							
Pacific Coastal	9											37

To assess anthelmintic and ectoparasiticide usage in dairy cows, four regions were selected: Northern, New England, South Central and Pacific. The states encompassed in each region were the same as in the assessment of anthelmintic use in beef cattle with the following exception. In that assessment, Missouri was classified in the upper Southeast region of the United States. Because dairy management practices in Missouri are similar to dairy management practices in Kansas, Oklahoma and Texas, Missouri was classified in the South Central Region in this assessment.

These four regions were selected because 1) dairy management practices were thought to be somewhat homogenous within each region, 2) the regions contain the ten largest dairy producing states in the United States, 3) over 75% of all lactating dairy cows in the United States are represented within the four regions and 4) these regions contain states in which a high percentage of their total cattle populations are dairy cows.[18]

The management of lactating dairy cows in the United States was categorized into four systems by the regional specialists. Based on regional research, field surveys, and personal experience, the percent of lactating dairy cows managed under each management system was estimated for each region. The dairy management systems as defined in this assessment are as follows:

Managed pasture. A dairy management system where a major portion (>50%) of forage requirements are derived from grazing (lactating dairy cows) for a minimum of three months.

Exercise/pasture. A dairy management system where some forage requirements (<50%) are derived from grazing. The grazing area is also used as a resting/exercise area for lactating dairy cows. The grazing area contains permanent vegetation.

Dry Lot. Lactating dairy cows are confined to a permanent earthen area lacking vegetation. Portions of the housing area may be concrete.

Confinement. Lactating dairy cows are confined within a permanent shelter. Permanent shelter includes free stall barns, stanchion barns, or open concrete lots. Cows do not have general access to an earthen area. Free stall or stanchion barns with small earthen mound (areas) to which cows have limited access are considered as confinement housing.

Experts (veterinarians, consultants, feed dealers, and parasitologists) were contacted within each of the regions to assess anthelmintic and ectoparasiticide use in lactating dairy cows. The experts were asked to also define rationale for anthelmintic and ectoparasiticide use in each region. Insecticides used to control flies were not considered in this assessment and the term ectoparasiticide when used hereafter shall only refer to products used to control mange, lice, grubs, ticks, and horn flies.

Individual treatments with anthelmintics were determined to be a routine management practice for dry cows 0 to 30 days prepartum and thus co-dependent with seasonal calving patterns. Whole herd anthelmintic treatment of lactating cows was determined to occur one time per year. This determination is supported by market surveys.[19]

Lactating cows were designated as being under managed or exercise pasture systems only during months when pasture is available in the region. When pasture is unavailable, lactating cows under managed or exercise pasture systems were considered to be in confinement. For lactating cows in managed or exercise pasture systems, monthly proration of whole herd anthelmintic treatment was based on a calendar year and not a pasture season. This was done because an accurate assessment of anthelmintic use could not be obtained for lactating cows while only on pasture. Accurate information on anthelmintic use on managed or exercise pasture systems could only be obtained by identifying the months cows could be on pasture and identifying the months whole herd anthelmintics were used. Therefore, anthelmintic use in managed or exercise pasture systems was often indicated in months when cows were not on pasture. This is logical as the majority of managers utilizing managed or exercise pasture systems indicated whole herd treatment after cows were removed from pasture. These treatments were considered as treatments occurring in confinement because cows were in confinement and not on pasture.

Seasonal patterns of ectoparasiticide use for each dairy management system in each region were estimated using methods identical to those used to estimate seasonal patterns of anthelmintic use.

The results from the assessment of estimated anthelmintic and ectoparasiticide uses for dairy cows under managed pasture and exercise pasture systems were converted to the numbers of cows treated per month in the four regions. However, for a product such as IVOMEC EPRINEX Pour-On, which has both anthelmintic and ectoparasiticide claims, summing estimated anthelmintic and ectoparasiticide treatments is overly conservative. Nevertheless, the sums of cows treated under these two management systems were added to the numbers of beef cattle treated in the same regions, i.e., to the data used to generate Table 2. The resulting sums were divided by the total beef and dairy cattle on pasture in the corresponding month. Table 3 compares the estimated actual percentages of beef cattle on pasture and treated with an anthelmintic with the estimated actual percentages of all cattle, dairy and beef, on pasture and treated with an anthelmintic or ectoparasiticide. The data labeled as excluding cows is from, or is derived from, Table 2 while the data labeled as including cows represents the estimated actual percentages of treatments for all classes of cattle on pasture. To make this comparison, the Pacific region data in Table 3 combines the coastal and eastern range numbers while the South Central data includes Missouri, as previously discussed.

TABLE 3

Estimated Actual Percent of Cattle On Pasture Treated with an Anthelmintic or Ectoparasiticide

Region		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Northern Dairy	excl. cows			4	24	12	1	3	1		5	8	
	incl. cows			4	22	11	1	2	1		5	7	
New England	excl. cows			6	12	9	3				33	41	8
	incl. cows			6	12	8	2				24	31	8
Pacific (overall Eastern	excl. cows	5			5	1							19
& Coastal treatments)	incl. cows	5			5	1							19
South Central &	excl. cows			5	9					13	21	9	
Missouri	incl. cows			5	9					13	21	8	

7. Fate in the environment

A. Key fate studies

i. Solubility

The water solubility in unbuffered water at pH 7.26 ± 0.09 was determined to be 0.0035 ± 0.0002 mg/mL (3.5 ± 0.2 ppm) at 25.0°C (See APPENDIX C-1).

ii. Octanol/water partition coefficient

The partition (distribution) coefficient between octanol and pH 6.8 phosphate buffer was determined by the shake-flask method. The average Log P was 5.4 ± 0.3 (See APPENDIX C-1).

iii. Dissociation constant

The dissociation constant was determined by potentiometric titration in 50% aqueous methanol with standardized solutions of sodium hydroxide and hydrochloric acid. No dissociation constant (pKa) was found between 3 and 10, consistent with the molecular structure (See APPENDIX C-1).

iv. Photodegradation

The photodegradation of the B_{1a} component of eprinomectin exposed to summer sunlight in New Jersey, U.S.A. was studied (see APPENDIX C-6). Based on the degradation of the B_{1a} component of eprinomectin under these conditions, it was calculated that eprinomectin would photodegrade near the surface of open, flat bodies of water under clear skies in summer and winter sunlight with minimum half-lives of 0.29 and 1.10 days, respectively. This rapid photodegradation in water should effect swift elimination of eprinomectin from the aquatic environment.

v. Mobility in soil

Compounds possessing K_{OC} values greater than 1000 are tightly bound to soil organic matter, and as such can be considered to be immobile in soil, Technical Assistance Document 3.08.[20] As the B_{1a} component of eprinomectin has K_{OC} values of 3231 to 9208 for sorption and desorption with three soils, this drug has been classified as tightly bound to soil and hence immobile (APPENDIX C-2). Consequently, the possibility of

translocation of eprinomectin through soil from one site to another in the environment is remote. When the B_{1a} component of eprinomectin was partitioned between water and loam, loam/sandy loam and clay loam soils, soil to water distributions (K_d) were 88.2, 53.1 and 133.5, respectively, averaged for sorption and desorption (APPENDIX C-2). Thus, in a 1:1 mixture of soil and water, ~98% of the drug would be bound, with only ~2% or less in the solution in equilibrium with the soil.

vi. Aerobic degradation in soil

Under aerobic conditions at 22 ± 3°C over 64 days in three soil types (sandy loam, loam and silt loam) in triplicate, [^{14}C]eprinomectin mineralizes to $^{14}CO_2$ to an average of about 3-4% (APPENDIX C-7). After 64 days, parent compound (eprinomectin) accounted for 47-50% by HPLC analysis and 51-55% by TLC analysis of the applied radioactivity, as determined by chromatography of soil extracts. Degradation products were more polar (based on reverse phase HPLC and normal phase TLC elution characteristics) than eprinomectin, but were not further identified. Thus, the half-life for aerobic biodegradation of eprinomectin in soil at ~22°C is about 64 days.

vii. Hydrolytic Stability

The half-lives of eprinomectin at pH 4, 5, 7, and 9 were estimated to be 622, 614, 2026, and 414 days, respectively (APPENDIX C-8). A chemical with a half-life of greater than 1 year at 25°C is considered to be hydrolytically stable.

B. Fate Summary

Given the tight binding of eprinomectin to soil, which greatly reduces its effective concentration, significant transport of eprinomectin residues from fields fertilized with cattle manure to bodies of water in the vicinity is highly unlikely. Both oxidative degradation in soil under aerobic conditions and photodegradation (on soil surfaces and in water) will diminish the environmental concentration of eprinomectin. Based on the discussion of soil binding, degradation via aerobic soil metabolism and photodegradation, it can be reasonably predicted that eprinomectin present in the environment would not be expected to undergo significant movement or translocation, and would not accumulate or persist. Given its environmental fate characteristics, eprinomectin will be readily eliminated from the aquatic and terrestrial environments.

8. Environmental effects of released substances

A. Environmental effects studies

i. Toxicity toward *Daphnia magna*

The acute toxicity of eprinomectin to the cladoceran, *Daphnia magna*, was determined under flow-through test conditions (see APPENDIX D-1). Based on the mortality/immobility data for 24 and 48 hours of exposure of daphnids to eprinomectin, the 48-hour EC_{50} value (95% confidence limits) was 0.45 (0.37-0.64) mcg a.i./L (ppb) while the 48-h no-mortality concentration was less than 0.37 mcg a.i./L, the lowest concentration tested (see Table 4).

ii. Toxicity toward fish

The acute toxicity of eprinomectin to the rainbow trout, *Oncorhynchus mykiss* (APPENDIX D-2), and the bluegill sunfish, *Lepomis macrochirus* (APPENDIX D-3), were determined during a 96-hour exposure period under flow-through test conditions. The LD_{50} values were 1.2 and 0.37 mg a.i./L (ppm), respectively (see Table 4). The 96-hour no-observed-effect concentrations, determined by visual examination of the mortality and observations data, were 0.37 and 0.14 mg a.i./L, respectively.

TABLE 4
RESULTS OF EFFECTS STUDIES WITH EPRINOMECTIN

Species	Study	Result	95% C.I.	No-Mortality Level	No-Effect Level
Cladoceran, *Daphnia magna*	48-h LC_{50}	0.45 ppb	0.37-0.64	<0.37 ppb	<0.37 ppb[a]
Algae, *Selenastrum capricornutum*	14-d MIC	15 ppm	---	---	7 ppm
Earthworm, *Lumbricus terrestris*	28-d LC_{50}	>951 ppm dry soil	---	295 ppm dry soil	<90.8 ppm dry soil[a]
Rainbow Trout, *Oncorhynchus mykiss*	96-h LC_{50}	1.2 ppm	0.99-1.4	0.37 ppm	0.37 ppm
Bluegill, *Lepomis macrochirus*	96-h LC_{50}	0.37 ppm	0.33-0.42	0.14 ppm	0.14 ppm
Northern bobwhite, *Colinus virginianus*	acute oral LD_{50}	272 mg/kg body wt.	203-364	125 mg/kg	<62.5 mg/kg[a]
Northern bobwhite, *Colinus virginianus*	8-day dietary LC_{50}	1813 ppm in feed	1420-2312	1000 ppm in feed	<316 ppm in feed[a]
Mallard, *Anas platyrhynchos*	acute oral LD_{50}	24 mg/kg body wt.	18-32	7.8 mg/kg	<7.8 mg/kg[a]
Mallard, *Anas platyrhynchos*	8-day dietary LC_{50}	447 ppm in feed	357-558	178 ppm in feed	<100 ppm in feed[a]

[a] Lowest Level Tested

iii. Toxicity toward avians

The acute toxicity of eprinomectin, when administered as a single oral dose in a capsule, was determined for the northern bobwhite, *Colinus virginianus*, (APPENDIX D-4) and the mallard, *Anas platyrhynchos* (APPENDIX D-5). The LD_{50} values were 272 mg/kg and 24 mg/kg, respectively (see Table 4). With respect to sublethal effects, at

the lowest dosage level employed in the mallard LD_{50} test (7.8 mg/kg), slight lower limb weakness and loss of coordination occurred within 3 hours after dosing and lasted through the afternoon of day two. From day 3 until the end of the test, the birds appeared normal. The subacute LC_{50} values for eprinomectin, when administered via the feed in an eight-day dietary study, 5 days on medicated feed followed by 3 days on eprinomectin-free diet, were 1813 ppm for the northern bobwhite and 447 ppm for the mallard duck, respectively (Table 4 and Appendixes D-6 and D-7). At the lowest concentration studied (100 ppm eprinomectin) in the mallard, lethargy, reduced reaction to external stimuli, loss of coordination and lower limb weakness were observed as sublethal effects one day after exposure to the eprinomectin-containing diet. These effects lasted only during the on-drug phase of the study, and all birds appeared normal 24 hours following their return to the basal diet.

iv. Antimicrobial activity

Using a standard, antimicrobial screen, eprinomectin was shown to have no significant antimicrobial effects *vs* 26 microbial species (including bacteria and fungi) at concentrations as high as 1000 ppm (APPENDIX D-8). [21] In all, 52 tests were performed; some species were incubated at both 25º and 37º C, some species were incubated in the presence and absence of lactamases, and both normal and antibiotic-resistant strains of some species were included in the screen.

v. Earthworm toxicity

A study to determine the toxicity (LC_{50}) of eprinomectin to the earthworm, *Lumbricus terrestris*, was conducted in artificial soil for 28 days (see Table 4). All worms used in the test were mature with clitellum and were acclimated 14 days prior to the initiation of the test (APPENDIX D-9). The LC_{50} value for earthworms exposed to eprinomectin in an artificial soil was determined to be greater than 951 mg a.i./kg dry soil, the highest concentration tested. The no-mortality concentration was 295 mg a.i./kg dry soil. The no-observed-effect concentration was less than 90.8 mg a.i./kg dry soil, the lowest concentration tested, based on a treatment-related loss in body weight among worms in this treatment group.

vi. Phytotoxicity

a) Algae

The phytotoxicity of eprinomectin to the fresh water unicellular green alga *Selenastrum capricornutum* was determined under static conditions for 14 days (APPENDIX D-10). Cell densities were determined at approximately 48-hr intervals during the 14-day study. Analyses of cell density and maximum growth rate data included the t-test and a dose-response trend test (APPENDIX D-11). Based on the mean log cell densities on day 14 and the maximum mean specific growth rates, the minimum inhibitory concentration (MIC) for *Selenastrum capricornutum* exposed to eprinomectin for 14 days was determined to be 15 mg a.i./L (see Table 4). The 14-day no-observed-adverse-effect concentration was 7.0 mg a.i./L.

b) Terrestrial Plants

The lack of phytotoxicity toward six plant species (cucumber, lettuce, soybean, perennial ryegrass, tomato, and wheat) has been demonstrated with eprinomectin in both a seed germination and root elongation study (APPENDIX D-12) and a seedling growth study in sand (APPENDIX D-13). The results (NOEC values) from the studies are in Tables 5 and 6. All NOEC values were based on mean measured concentrations.

TABLE 5 RESULTS FROM THE SEED GERMINATION AND ROOT ELONGATION PHYTOTOXICITY STUDY WITH EPRINOMECTIN		
SPECIES	NOEC, ppm	
	GERMINATION	ROOT ELONGATION
Cucumber	1300	95
Lettuce	1300	8.5
Soybean	1300	95
Perennial Ryegrass	1300	8.5
Tomato	1300	8.5
Wheat	1300	8.5

TABLE 6 RESULTS FROM THE SEEDLING GROWTH PHYTOTOXICITY STUDY WITH EPRINOMECTIN IN SAND			
SPECIES	NOEC, ppm		
	SHOOT LENGTH	SHOOT WEIGHT	ROOT WEIGHT
Cucumber	0.47	0.47	0.47
Lettuce	6.5	6.5	6.5
Soybean	6.5	6.5	6.5
Perennial Ryegrass	0.47	0.47	0.47
Tomato	0.47	0.47	0.47
Wheat	0.47	0.47	0.47

vii. Dung Beetles

The toxicity of eprinomectin was determined towards two species of dung beetles, *Onthophagus gazella* and *Euoniticellus intermedius* (APPENDIX D-14). Control feces was homogenized and divided into 5-kg aliquots. One aliquot served as a non-treated control. To the remainder, eprinomectin was added in 5 mL of dimethylformamide. Treated fecal samples contained eprinomectin B_{1a} at 0.0 (vehicle-treated control), 7.0, 24, 64.7, 166 and 590 ppb on a wet-weight basis. Fecal pats were placed on top of soil in plastic pails and three male-female pairs of *O. gazella* or *E. intermedius* beetles were placed in each of 6 pails per treatment for each species. There were no effects on adult beetles, as measured by lethality, i.e. number of live adults recovered or numbers of brood balls formed over the range of eprinomectin tested. No live progeny were recovered at the 166 or 590 ppb levels. The NOEC, based on numbers of emerged progeny relative to pooled controls (untreated and solvent controls), was 64.7 ppb for both species. An LC_{50}, based on the number of brood balls formed by the adults, could not be calculated.

B. Environmental Hazard Assessment

i. Hazard assessment in aquatic ecosystem

As *Daphnia magna* is the freshwater aquatic species found to be most sensitive to other avermectins, it is employed for assessing the possible hazard to aquatic ecosystems resulting from use of eprinomectin as a topically applied endectocide on cattle.

Two scenarios were considered to evaluate the introduction of eprinomectin into the aquatic environment and the resulting impact. The scenarios include the translocation of eprinomectin from fields fertilized with manure from cows treated with eprinomectin to a nearby body of water and the potential wash off of eprinomectin from the backs of treated cows which are standing in a pond.

a) Use of manure containing eprinomectin residues as fertilizer

An initial concentration of eprinomectin in soil of 0.69 ppb, arising from use of excreta directly as fertilizer (see Table 1), is considered in Table 7. It can be reasonably assumed that the initial concentration would be reduced (~98%), by soil binding, to an

estimated concentration of unbound eprinomectin residues of 0.014-ppb in water in direct contact with the fertilized soil. The eprinomectin residue concentration in water *en route* to a nearby body of water (e.g., pond) would be decreased by multiples of ~98% because of adsorption to soil. Just one adsorption/desorption equilibration would reduce the above concentration by a factor of ~98, to 0.28 part per trillion (ppt). A greater distance between the fertilized field and the body of water would require more extensive movement, through unfertilized soil, of water carrying dissolved unbound eprinomectin, resulting in further binding and greater reduction in available drug residue. Even if traces of eprinomectin were ultimately to reach a body of water, as it entered, up to 98% would be bound by suspended soil particulates and sediment (to give an estimated concentration of 0.0056 ppt). These concentration changes are summarized in Table 7, and do not include dilution effects. Neither do they include any degradation of eprinomectin resulting from aerobic soil metabolism (See 7.A.vi.) or photodegradation on the surface of soil (See 7.A.iv.). Even if cows on pasture were dosed several times with eprinomectin, the resulting modest increase in drug residue concentration in manure (and hence soil fertilized with the manure) would have no meaningful impact on the concentration of eprinomectin in pond water. Further, the unbound eprinomectin in the pond would undergo rapid photodegradation (calculated summertime and wintertime minimum half-lives of approximately 0.29 and 1.10 days, respectively; APPENDIX C-6) and the initial concentrations would decrease (see Figure 3) in 4 days by factors of ~14,000 (summer) and ~12 (winter).

The concentration in the pond following binding to soil sediment, 0.0056 ppt, is far below (factor of about 80,000) the LC_{50} for eprinomectin toward *Daphnia* (0.45 ppb). Further, rapid photodegradation would diminish this already exceedingly low concentration (Figure 3), increasing an already more than adequate margin of safety for aquatic organisms.

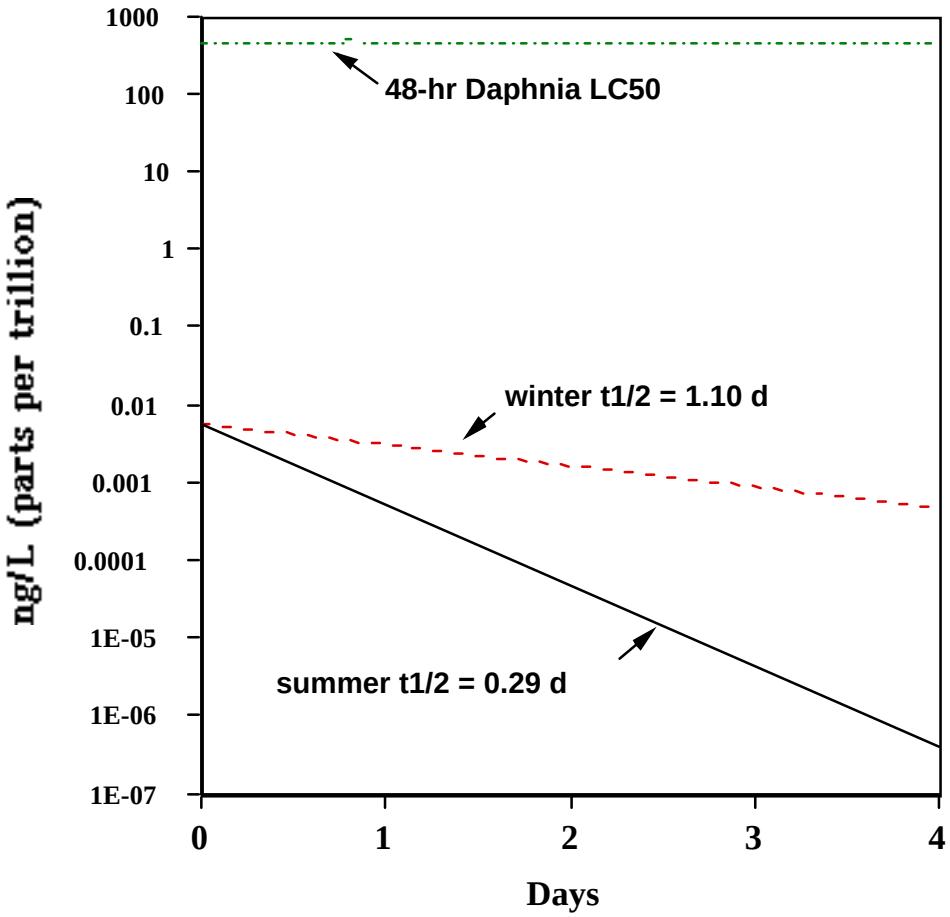
TABLE 7
EFFECT OF SOIL BINDING UPON EPRINOMECTIN CONCENTRATIONS ARISING FROM USE OF MANURE CONTAINING DRUG RESIDUES AS FERTILIZER

Initial Conc in Soil	Conc in Water in Contact With Soil[a]	Conc Entering Pond Following Movement Through Unfertilized Soil[b]	Conc in Pond Following Binding to Soil Sediment[d]
0.69 ppb	0.014 ppb	0.28 ppt[c]	0.0056 ppt

[a] Assumes 98% remains bound to soil.
[b] Assumes only a single loss of 98% (i.e., fertilized soil adjacent to pond).
[c] ppt = parts per trillion
[d] Assumes a second loss of 98%.

FIGURE 3.

Comparison of 48-h LC_{50} of eprinomectin towards *Daphnia* with effective eprinomectin concentration in a pond as impacted by photodegradation. Scenario involves introduction of eprinomectin from use of manure from beef cattle as fertilizer.



b) Impact from dung pats deposited on pastures by treated cattle

The environmental burden of eprinomectin residues from a single cow/calf unit pastured on an acre of land would be 350 mg. The drug residue in the dung pat will bind tightly to organic matter in the feces and not leach into soil. Any minute amounts of drug not confined to the pat would not move far because of tight binding to soil and would be subject to photodegradation on the soil surface.

Since use of manure as fertilizer would result in concentrations of eprinomectin in a nearby pond far below levels of concern with respect to toxicity to *Daphnia*, the even lower concentrations resulting from deposited dung pats would also be of no concern with respect to aquatic organisms.

c) Washoff by rain of eprinomectin from backs of treated cattle directly into a pond.

In clinical trials, there was no loss of efficacy when cattle treated with IVOMEC EPRINEX Pour-On were wet or were exposed to rain within one hour post-dosing. IVOMEC Pour-On for Beef and Dairy Cattle contains a related avermectin formulated with different excipients. A washoff study with IVOMEC Pour-On for Beef and Dairy Cattle determined that an average of 0.6% (upper 95% one-sided confidence limit = 1.3%) of topically-applied ivermectin washed off three 250-kg cattle exposed to 0.5 inch of rain over 10 minutes (the volume of water applied was 6 L) at 6 hours post-treatment.[22]

For IVOMEC EPRINEX Pour-On, calculations of washoff amounts (below) use the maximum environmental burden. For a cow/calf pair (500 kg, 200 kg) of animals held on one acre of pasture, the maximum environmental burden is the total dose of 350 mg (250 mg plus 100 mg). Since 1) the water solubility of eprinomectin is low, 3.5 ppm, and similar to that of ivermectin, 3.5 vs. 4 ppm, as are the other physical/chemical properties (Section 5.B.), 2) Miglyol 840 is highly lipophilic and "insoluble" in water, and 3) the formulation of IVOMEC EPRINEX Pour-On is 100% Miglyol, the amount of eprinomectin expected to wash off of cattle during rain and the resulting concentration of eprinomectin in water would be very low and similar to or less than the corresponding values determined in the wash-off study with IVOMEC Pour-On. As there is no isopropyl alcohol component in the IVOMEC EPRINEX Pour-On formulation to act as a water-miscible cosolvent, the possibility of washoff is even less for eprinomectin than for IVOMEC Pour-On. However, this scenario uses the worst-case assumption of 100% (350 mg per cow/calf pair) wash off. In order for the effective (in solution) concentration of eprinomectin in a 4.9 x 10^6 L (1 acre in area and 4 feet deep) pond surrounded by many acres of pasture to reach 0.45 ppb, the LC_{50} toward *Daphnia*, 2205 mg of drug from the animals would have to wash off their backs in rain and then enter the pond. Most (98%) of the drug removed from the animals by rain would bind to soil beneath the animals,

and most (98%) of the remaining unbound eprinomectin would bind to soil as the flowing rainwater moved toward the pond (See Section 8.B.i.a.). These two losses would require that drug be removed from 15,750 cow/calf units (15,750 pairs x 350 mg/pair x 0.02 x 0.02 = 2205 mg). Considerable dilution would result from the flow of rainwater into the pond which would further reduce the concentration of eprinomectin, and input of drug would cease soon after the rain stopped falling.

Because of tight binding of eprinomectin to soil, even in the extremely unlikely event that the entire dose were to wash off the animals during a rain event, 15,750 cow/calf pairs on 15,750 acres of pasture surrounding a 1-acre, 4-ft deep pond would be required for the effective concentration in the pond to reach the LC_{50} toward *Daphnia*, 0.45 ppb. This scenario demonstrates that introduction of eprinomectin into a pond by washoff onto surrounding pastures sufficient to result in concentrations toxic to *Daphnia* is remote.

One can also calculate the minimum pond area which would accommodate 100 cattle standing in a 4-ft deep pond before an effective concentration of 0.45 ppb would result from a single rainfall-induced runoff and assuming 100% of the applied dose washed off the backs of the cows [100 animals x 250 mg eprinomectin washed off per animal x 2% unbound to sediment (See Section 8.B.i.a.) = 500 mg effective mass in the pond]. A pond containing 500 mg (5.0×10^8 ng) of eprinomectin requires a volume of 1.11×10^6 L to result in a concentration of 450 ng/L (0.45 ppb). This is 22% of the volume (and thus 22% of the area) of the one-acre pond. This area of only 0.22 acre allows only 97 sq ft (9.8 ft by 9.8 ft) per animal.

As indicated above, all the eprinomectin applied to the backs of 100 cattle crowded into a 0.22-acre, 4-ft deep pond would have to wash off and directly enter the pond water for the effective concentration of drug to reach 0.45 ppb, the LC_{50} for *Daphnia*. Such an event would not occur.

d) Summary of hazard assessment in aquatic ecosystems

The MIC and NOAEC for eprinomectin towards the fresh water unicellular green alga *Selenastrum capricornutum* are greater than 4 times and 2 times, respectively, the aqueous solubility of eprinomectin (3.5 ppm, Section 5.B.). The concentration of eprinomectin in bodies of water adjacent to fields fertilized with manure containing eprinomectin residues or in ponds resulting from direct wash-off from cattle will be far below the LC_{50} for *Daphnia*. Although eprinomectin hydrolyzes very slowly in the dark, it photodegrades rapidly and binds tightly to soil, where it degrades aerobically. Therefore, it will neither persist nor accumulate in aquatic ecosystems.

Thus, the above assessments demonstrate that there will be very little risk to the aquatic environment resulting from the use of eprinomectin as a topically applied endectocide on cattle.

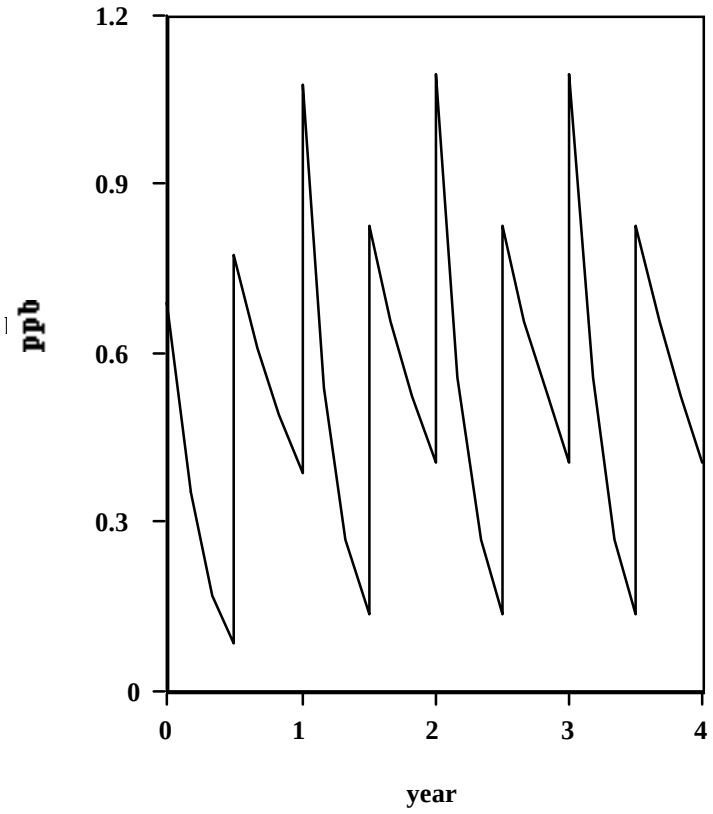
ii. Hazard assessment in terrestrial ecosystems

a) Residue levels in fields fertilized with manure from cattle dosed with eprinomectin

With respect to the possible accumulation of eprinomectin residues in fields fertilized with manure collected for 365 days from animals treated with eprinomectin, data presented below demonstrate that accumulation is highly unlikely. A conservative scenario involves application to a field of 15 tons of manure plowed to a depth of 6 inches. As shown in Table 1, the resulting concentration of eprinomectin-related compounds in the soil would be only 0.69 ppb. Using the aerobic degradation half-life of eprinomectin of approximately 60 days for the spring to autumn period, conservatively based on a half-life of approximately 64 days in three soils in the laboratory at 22±3°C (see Section 7.A.vi.) and assuming 180 days for autumn to spring, there will be no accumulation of drug residues with time, even if this eprinomectin-containing manure were applied twice in a year following semiannual cleanout of a dairy barn or feedlot. The maximum soil concentration following the second year's springtime application would be only 1.1 ppb, dropping to 0.13 ppb just prior to the next semiannual (autumn) application (see Figure 4). Clearly, buildup of drug residues in fertilized soil will not occur, nor will there be persistence of residues when fertilization is discontinued.

FIGURE 4

Levels of Total Residues in Soils Treated Twice Yearly with Feces from Cattle Treated with Eprinomectin Using Spring-to-Autumn and Autumn-to-Spring Half-lives of 60 and 180 days, Respectively



b) Hazard assessment for avians

(1) Avian toxicity data for eprinomectin and abamectin

Comparing the avian toxicity data for eprinomectin with the corresponding data for abamectin, Table 8, LC_{50} values for the acute and dietary studies with the northern bobwhite were lower for eprinomectin. However, the acute and dietary LC_{50} values were similar in studies with the mallard (considering

that the mallards regurgitated in the oral acute study with abamectin, the values in Table 8 for the acute oral study are likely high). Additionally, the no-mortality levels are similar for both acute and dietary routes of exposure for both bird species (NOEL values were not determined as they were all below the lowest levels tested). Therefore, since the LD_{50} and no-mortality levels for eprinomectin and abamectin were comparable by both routes of exposure to the mallard, the toxicity of these two compounds appears to be similar. Based on that similarity, the data from the eighteen-week, mallard reproduction study with abamectin should also be applicable for eprinomectin.

In the definitive eighteen-week avian reproduction study with abamectin, male and female mallard ducks were exposed at levels of 3, 6 and 12 ppm in the diet for approximately 10 weeks prior to and continuing through egg laying.[22] The mallard duck was chosen as the test species as it is at least one order of magnitude more sensitive to the toxicity of abamectin than is the northern bobwhite. The ducks showed no treatment-related mortality, overt signs of toxicity or effects upon body weight or feed consumption. No statistically significant differences, compared to control birds, were noted in the number of eggs laid or in the number of hatchlings from live 3-week embryos. Thus, chronic exposure of mallard ducks to abamectin at concentrations of 3, 6 and 12 ppm in the feed did not affect overall reproductive success. This demonstrates a NOEL of 12 ppm. It was observed in a range-finding study that, at a concentration of 64 ppm of abamectin in the diet (6-week feeding), mallard ducks laid fewer eggs and the hatchability of the eggs laid was reduced.[22] No other signs of sublethal toxic effects were observed, however, even at this high level. The NOEL is, therefore, 64 ppm for all except reproductive effects.

TABLE 8
AVIAN TOXICITY

	Northern Bobwhite		Mallard Duck	
	Eprinomectin	Abamectin[a]	Eprinomectin	Abamectin[a]
Acute, oral, mg/kg				
LD50	272	>2000	24	85 [b]
95% C.I.	203-364	---	18-32	67-120
NOEL	<62.5	<62.5	<7.8	<10 [b]
No-mortality level	125	125	7.8	31.6[b]
Subacute 8-d Dietary, ppm				
LC50	1813	3102	447	383
95% C.I.	1420-2312	2338-4393	357-558	302-487
NOEL	<316	<288	<100	<162
No-mortality level	1000	910	178	162
Eighteen-wk Reproduction, ppm				
LC50				--
NOEL				12
NOEL (all effects except for reproduction)				64

[a] Data from Environmental Assessment for IVOMEC Pour-On. [22]

[b] Regurgitation occurred.

This low level of toxicity toward birds for a compound highly active against insects is not surprising, for the avermectins act within the peripheral nervous system of lower animals, by stimulating the release of the inhibitory neurotransmitter GABA from the presynaptic nerve terminals as well as by potentiating GABA binding to the post-synaptic receptors. With higher animals (e.g., birds), in which GABA serves as a neurotransmitter within the central nervous system (CNS), the blood-brain barrier is relatively impervious to avermectins, attenuating any toxic effect these compounds might have upon the CNS.

(2) Impact of the pour-on formulation of famphur on magpies

Henny *et al.,* found evidence that ingested famphur-containing hair from cattle treated topically with this organophosphate caused the deaths of magpies.[23] The cause of death was held to be depression of brain cholinesterase activity.[23] Henny and coworkers estimated that as little as 8 - 19 mg of hair could have contained a dose of famphur lethal to magpies. The possibility of secondary poisoning of raptors, occurring through the eating of disabled or dead magpies containing toxic concentrations of organophosphate insecticides, appears to be at least as much of an environmental concern as poisoning of the magpies. [23] Concern has also been raised that eagles and other raptors are poisoned by eating carrion arising from livestock dosed topically with organophosphate insecticides. [24] Magpies are not found on any endangered species list. *Pica pica,* the black-billed magpie, is found in the U.S. on the western Great Plains, in the Great Basin and Columbia Plateau regions and also in a narrow strip of eastern California. [25-27]

(3) Feeding habits of magpies

It appears, based on the writings of Kalmbach and Bent, that ingestion of cattle hair by magpies is probably not deliberate, but rather, incidental to the birds picking insects from the backs of cattle, eating flesh at wound sites or eating carrion. [26,28]

Kalmbach examined the stomach contents of 313 adult and 234 nestling magpies to determine food preferences.[26] The diet varied according to the season, but overall, 60% was from animal sources and 40% from vegetable sources. The animal sources averaged 36% insects (weevils, ground beetles, caterpillars, bees, ants, other hymenopterans, grasshoppers, carrion beetles, etc.), 14% carrion, 8% small mammals and the remainder, small amounts of mollusks (mostly land snails), fish, reptiles, amphibians, wild birds and their eggs and domestic fowls and their eggs. The vegetable sources averaged 13% grain, 21% wild fruit, 3% cultivated fruit and 3% vegetable rubbish. Flesh or hair were frequently present with insects associated with carrion.

The propensity of magpies for attacking sickly, newly branded, young or even healthy adult livestock, and the eating of carrion, caused the bird to be considered a pest by many ranchers and livestock producers in the early 1900's.[26] Hair of horses and cattle, wool of sheep and bristles of hogs, as well as the remains of some smaller mammals and even birds, have been found in magpie stomachs. Kalmbach concluded from field observation and reports from livestock raisers that comparatively few birds indulge in carnivorous behavior to excess and recommended that ranchers eliminate those birds at the first indication of such behavior, lest other birds learn by example.[26] The last section of Kalmbach's bulletin, a publication from the U.S.D.A., discusses measures to control magpie populations.

Bent cites reports that magpies pick insects off of the heads and backs of deer, wapiti, and bighorn sheep, especially in the spring when the animals are infested with ticks.[28] The only other cited interactions between magpies and large herbivores concern magpies pecking at sores or at carcasses of horses, elk and buffaloes.

Linsdale cites observations of magpies picking grubs from the backs of cattle and spending time on the backs of cattle and mules, especially in cold weather.[29] Linsdale also recounts numerous observations of magpies attracted to sores on sheep, cattle, horses and mules. In some cases, the magpies were attracted to maggots in the wounds, but most observations were of magpies attracted to and feeding from the wounds. In many cases, the magpies persisted in attacking the wounds until the animals died. One citation was of magpies sitting on the backs of sheep to watch for and feed on grasshoppers which became disturbed by the flock as it fed.

Henny and coworkers studied a magpie population in Washington that lived on or near ranches which used famphur as a pour-on insecticide.[23] Gizzard contents from 13 magpies that died during their study consisted of 51% vegetable matter, 37% animal matter and 12% cattle hair. Cattle hair was found in all gizzards (range of <1 - 50% of contents) except one that was empty. Few magpies in the treatment areas consumed famphur, based on plasma cholinesterase activity of 47, live-trapped birds and only one magpie was sighted on the back of a bovine during the 2-month field-observation period. Henny

and coworkers speculated that the hair might have been purposely ingested as an aid to eliminate indigestible materials, although Linsdale observed the ejection of pellets only by captive magpie. [23,29] Total weights of gizzard contents or of ingested hair were not reported, but Henny and coworkers indicated that as little as 8 - 19 mg of cattle hair could have contained lethal doses of famphur.

Birkhead summarized the feeding habits of magpies by supplementing the observations of Kalmbach and Linsdale with more recent citations.[26,29-30] The newer citations underscored the previous ones with the conclusion that adult magpies feed mainly on plant material during the autumn and winter and animal material, mainly invertebrates, during the summer. Small birds and vertebrates also were noted in their diet. Birkhead included no new citations on interactions of magpies with large herbivores other than to refer to magpies that are seen "these days" using domesticated herbivores as perches while foraging, or removing ticks or other parasites. [30]

(4) Exposure of magpies to Eprinomectin Pour-On

IVOMEC EPRINEX Pour-On is poured, not sprayed, from applicators onto the backs of cattle at only 1 ml per 10 kg body weight, i.e., only about 1 fluid ounce for a 275-kg calf and about 2 fluid ounces for a 600-kg cow. IVOMEC EPRINEX Pour-On is thus applied to a narrow strip, probably less than 1 or 2 inches wide, along the backline and, based on the formulation, is not expected to spread. It is therefore reasonable to assume that most of the plucked hair is from areas not dosed with eprinomectin. If we assume that a magpie will pluck hair from a l-ft wide zone along the back of a steer, less than 20% of the plucked hairs will be coated with eprinomectin. Further, under outdoor conditions some of the eprinomectin could undergo photodegradation (APPENDIX C-6).

Extensive and prolonged exposure of magpies to treated hair is unlikely. Plucking and ingestion of cattle hair by magpies, except incidental to their eating of cattle-borne insects, flesh at wound sites and carrion, is not discussed in the definitive works on this avian species by Kalmbach, Bent or Linsdale. [26,28-29] Further, Henny and coworkers reported only one sighting of a magpie perched on the back of a bovine during their study. [23]

The average value of 12% hair for magpie gizzard contents reported by Henny *et al.* is unexpectedly high in view of the articles cited above. [23] The 12% value probably represents an accumulation of hair ingested over a number of days. This seems reasonable, for Henny and coworkers reported that in one magpie gizzard content was 50% cattle hair. [23] It appears highly unlikely that on a continuous basis half of a bird's diet would be cattle hair. If hair accumulation occurs, a daily diet consisting of 12% hair would therefore be too high, but could represent a "worst-case" for daily intake. The 8 - 19 mg of hair containing a lethal dose of famphur represents only 0.04 - 0.1% of a 20-g diet but might be a reasonable daily intake.

To estimate the concentration of eprinomectin on ingested hair, data from IVOMEC Pour-On for Beef and Dairy Cattle is used. In a study carried out indoors with steers dosed percutaneously with [^{3}H]ivermectin applied, in a different formulation, but at the same dose of 500 mcg/kg, approximately 774 mcg of drug-related residue was present per gram of hair from the dosed area 7 days post-administration; at 42 days post-dose the residue was 522 mcg/g of hair from the dosed area. [22]

In a scenario, less extreme than the worst-case, but still conservative, if:

- a 200-g magpie consumes one-tenth of its body weight, 20 g, of matter in a day;
- 6% of the diet, or half of the "worst-case" assumption, is hair;
- 20% of the ingested hair is from the dose site; and
- 774 mcg/g is the concentration of eprinomectin on hair;

then, the bird would ingest 1.2 g of hair and thus 0.185 mg of eprinomectin residue. Since the exposure is dietary, this value should be compared to the 8-day dietary LC_{50}. An intake of 0.185 mg of eprinomectin per 20 g of diet is 9.3 ppm. This value is about 2% of the subacute 8-day dietary LC_{50} for eprinomectin in the mallard (447 ppm, Table 8) and about 10% of the lowest level tested in the mallard (100 ppm), at which eprinomectin caused modest sublethal effects. This intake also amounts to a dose of 0.93 mg of eprinomectin/kg body weight, well below levels causing mortality and well below the lowest

level tested in the mallard, 7.8 mg/kg, where lower limb weakness and loss of coordination were observed. Therefore, primary poisoning of magpies, who would be exposed through their diet, is highly unlikely.

An intake of 9.3 ppm is also below the no-effect level of 64 ppm for effects of abamectin to the mallard and less than the overall NOEL including reproductive effects of 12 ppm, so reproductive effects would not occur even if eprinomectin treatments coincided with the magpie breeding season. According to Kalmbach, magpies lay eggs before the middle of April in Colorado, Utah, California and southern Oregon.[26] In Washington and Montana, the northern part of its range in the U.S., egg laying by the magpie begins about two weeks later. In these areas of the U.S., the most likely periods for use of anthelmintics are late summer through the fall, the time of the year range cattle are accessible for dosing. Use of eprinomectin topical is expected to follow the same pattern. Thus, the magpie mating season (spring) does not coincide with the most likely period of eprinomectin use in that part of the U.S. where magpies are commonly found. Problems with magpie reproduction are, therefore, not expected from use of this product.

For the purpose of a "worst-case" calculation, the following "extreme" assumptions can be made:

- all ingested hair is from the dose site, i.e., from a zone 1 - 2 inches wide along the midline;
- hair accounts for 12% of the daily dietary intake, based solely on gizzard contents;
- and, all of the residue is eprinomectin.

A 200-g magpie, consuming 20 g of matter in a day, and assuming that the concentration of eprinomectin on hair is 774 mcg/g, would ingest 2.4 g of hair and thus 1.86 mg of eprinomectin. Since the exposure is dietary, this value should be compared to the 8-day dietary LC_{50}. An intake of 1.86 mg of eprinomectin per 20 g of diet is 93 ppm. This value is about one-fifth that of the subacute 8-day dietary LC_{50} for eprinomectin in the mallard (447 ppm, Table 8), and slightly below the lowest level tested (100 ppm) at which eprinomectin caused modest sublethal effects. Thus, under "worst-case" assumptions, some sublethal effects might occur. This intake

is above the 64 ppm level where treatment-related effects of orally dosed abamectin were observed upon reproductive performance in the mallard during a 6-week feeding period. However, as already discussed, asynchrony exists between the magpie breeding season (spring in NW US) and major treatments of cattle (fall) and it is highly unlikely that "worst-case" daily dietary exposure would persist, especially not for 6-weeks. This amounts to a dose of 9.3 mg/kg, slightly above the lowest level tested (7.8 mg/kg) in the acute study and a dose at which lower limb weakness and loss of coordination might be expected. Therefore, under "extreme" or "worst-case" scenarios, some sublethal effects might occur and effects on reproduction are possible, but unlikely. The likelihood of any toxic effects would also be lower if magpie sensitivity were closer to that of the northern bobwhite than to that of the mallard (Table 8).

(5) Secondary poisoning of raptors and exposure to carrion-feeders

If a magpie exposed to eprinomectin were eaten by a raptor, the latter's secondary exposure to eprinomectin would be minimal. In a conservative scenario, if a 600-g raptor (e.g., red-tailed hawk) were to obtain its entire day's food intake (60 g) from a magpie (200 g) contaminated with 0.185 mg of eprinomectin, on the average a raptor would ingest 0.06 mg of eprinomectin. A daily dietary intake of 0.06 mg eprinomectin per 60 g feed is equivalent to only 1 ppm, well below the LC_{50} of 447 ppm and no-mortality level of 178 ppm for eprinomectin in the mallard and the NOEL of 64 ppm for non-reproductive effects and the NOEL for reproductive effects of 12 ppm for abamectin in the mallard. On an acute basis, the dose to the raptor would be 0.06 mg of eprinomectin per 600 g of body weight, or 0.1 mg/kg, well below the 7.8 mg/kg of eprinomectin found to cause lower limb weakness and loss of coordination in the mallard, and far below the LD_{50} of 24 mg/kg for the mallard. Therefore, secondary poisoning of raptors would be most unlikely. This conclusion is also true under the "worst-case" scenario, where the magpie would contain 1.86 mg of eprinomectin and the raptor would ingest 0.56 mg of eprinomectin. A daily dietary intake of 0.56 mg eprinomectin per 60 g feed is equivalent to only 9 ppm, well below the LC_{50}

of 447 ppm and no-mortality level of 178 ppm for eprinomectin in the mallard and below the NOEL of 12 ppm for abamectin in the mallard. The dose would be only 0.93 mg/kg, well below the 7.8 mg/kg of eprinomectin found to cause lower limb weakness and loss of coordination in the mallard, and far below the LD_{50} of 24 mg/kg for the mallard. Even under the "worst-case" scenario, secondary poisoning of raptors is most unlikely.

Eagles and other raptors have died from exposure to organophosphate pour-on insecticides as a result of eating of carrion arising from dosed cattle, but lethal doses could have been ingested by a 5-kg eagle from intake of 0.5 g or less of hair, based on 8 - 19 mg of hair containing a lethal dose to a 200-g magpie.[24] However, this is highly unlikely to occur with IVOMEC EPRINEX Pour-On. At 7- and 28-days post dose, total eprinomectin residues in cattle liver, the tissue of highest residue, are 977 and 185 ppb, respectively, and the corresponding values for muscle are 8 and 2 ppb (APPENDIX C-3). The following exposure assessment will use the "extreme" scenario of a 5-kg eagle consuming 500 g of a 7-day post-dose liver containing 977 ppb eprinomectin as its entire day's intake. This is an oral dose of 0.489 mg of eprinomectin, or 0.098 mg/kg body weight, which is far below the LD_{50} for eprinomectin in the mallard, 24 mg/kg. Consumption of the 500 g of liver would result in a daily dietary intake of 0.98 ppm eprinomectin, far below the abamectin dietary NOEL of 12 ppm in the mallard.

It can be reasonably assumed that a carrion-eating eagle would preferentially take tissue from the underbelly of a carcass, rather than from the narrow-band along the dorsal dose site. The dorsal approach is made difficult by the presence of the spine and ribs. The ventral approach allows access to the internal organs and other easily obtained meat. With regard to the consumption of carrion hair by a 5-kg eagle, a 500 g dietary intake which includes 6% hair, where l% of the ingested hair is from the dose site since the dose site is ~l% of the surface area of a steer [surface area (m^2) = (0.13 x wt in kg)$^{0.56}$ (0.13 m^2/kg x 365 kg)$^{0.56}$ = 8.7 m^2; 2" (0.051 m) wide x 1.5 m length of dose site = 0.08 m^2; 0.08 m^2/8.7 m^2 • 1% of surface area] and 50% of the total residue on the hair (774 ppm) is eprinomectin leads to an intake of 0.116 mg

eprinomectin.[115] The amount via this route would be far below any level of toxic concern. This intake yields 0.023 mg/kg body weight and 0.23 ppm in the diet. Combined with the exposure resulting from the intake of liver tissue (0.98 ppm), total dietary exposure would be 1.2 ppm, only 10% of the NOEL observed for abamectin in an 18-week mallard reproduction study. Therefore, poisoning of carrion-feeders is unlikely even under an "extreme scenario".

(6) Summary of hazard assessment for avians

No effects should occur to magpies from the ingestion of hair from the backs of treated cattle under a less than worst-case, but still conservative, scenario. Under an "extreme" or "worst-case" scenario, some sublethal effects might occur to magpies and effects on reproduction are possible, but this scenario is unlikely because it assumes that:

- all ingested hair is from the dose site, i.e., from a zone 1 - 2 inches wide along the midline of the back which is <20% of the width of the back and <1% of the body surface area;
- hair accounts for 12% of the daily dietary intake, based solely on gizzard contents, which is too high to be sustained based on the findings of Kalmbach;[26]
- all of the residue is eprinomectin, which ignores any effects of photodegradation; and
- periods of reproduction and dosing are synchronous, whereas the magpie mating season (spring) does not coincide with the most likely period of eprinomectin use in the part of the U.S. where magpies are commonly found.

Secondary or direct toxicity to raptors and carrion-eaters is not likely even under the worst-case assumptions. Therefore no effects on avians are expected from the use of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle.

c) Hazard assessment for insects

The avermectins are toxic toward a wide variety of agricultural pests including the Mexican bean beetle, Southern army worm, aphids and mites. The effect of ivermectin upon animal ectoparasites including flies, fleas, lice, ticks and mites has also been determined. Review articles by Strong and Brown and Dybas discuss the avermectins in insect control.[31-33]

(1) Effects on insects other than dung beetles

Drug residues in the manure of animals treated with avermectins can affect insects, including pests, associated with fecal pats. In general, the toxicity of avermectins in dung toward insects is species dependent, and larvae of flies and beetles are more sensitive to avermectins in dung than are adult insects. This view is supported by results published by a number of investigators. For example, Miller *et al.* reported that drug residues in the feces (day 9 of treatment) from steers given ivermectin daily at 5 µg/kg (oral capsules) were lethal to all horn fly and face fly larvae, but only to approximately 60% of stable fly larvae.[34] Even at a dose rate of 1 µg/kg/day, all horn fly larvae were killed by the manure. These authors observed that a single subcutaneous dose of ivermectin at 0.2 mg/kg prevented development (>93% mortality) of horn flies in steer manure collected for up to four weeks post dose, whereas with stable flies over the same period, mortality averaged less than 40%. This observation was confirmed by Schmidt, who reported that adult horn flies failed to emerge from manure produced by cattle on day 1 to 28 following treatment with ivermectin (0.2 mg/kg intramuscular injection).[35] In contrast, adult stable flies emerged from all manure samples.

With respect to non-Diptera species, Schmidt found that emergence of several insects (e.g., sphaerocerids and sepsids) from manure containing ivermectin residues was greatly reduced; however, the manure did not kill all dung-dwelling insect species, for the populations of both gnats and staphylinids in dung from cattle were found to be unrelated to treatment.[35]

(2) Effects of avermectins upon dung beetles

The life cycle of nearly all species of dung beetles (Coleoptera; Scarabaeidae*)* is intimately associated with dung. Characteristics of these dung-associated insects which are most responsible for the successful utilization of dung for feeding and reproduction include mobility, and feeding and reproduction patterns. These will be discussed in relation to the sensitivity of the dung beetles to avermectins in dung and the usage pattern of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle.

Information supporting this section was provided to the CVM in detailed confidential hazard assessment reports on the characteristics of dung beetles and use scenarios of avermectins and their effects on dung beetles. Information from these reports has been incorporated into this section of the Environmental Assessment. Recognized experts listed in Section 12 of this Environmental Assessment contributed to and critiqued the reports.

(a) Mobility of dung beetles

Dung beetles are highly mobile. Mobility is crucial for insects which use fresh dung to feed and reproduce. Hanski described this habitat as "patchy and ephemeral".[36] Dung is distributed discretely throughout the range of the animal which produces it, and each dung pat may only be attractive and suitable for dung beetles for a limited period of time. Thus, it is essential that dung beetles are able to move readily from pat to pat and from pasture to pasture in pursuit of dung in suitable condition for feeding and/or ovipositing.

The evolutionary success of dung beetles, given the nature of the macro-and micro-environments in which they live, is evidence for their robustness and ability to adapt to the many and varied perturbations which might temporarily affect them. From an evolutionary perspective, some dung beetles evolved to follow migratory sources of dung and became associated with large grazing mammals such as the species which now inhabit the grasslands and plains of Africa. Mammals and dung fauna experienced cycles of expansion and contraction during their evolution, as the

herds of mammals and beetles migrated across available regions. [37] Grazing cattle are highly mobile and generally travel several miles per day, with proximity to water supplies controlling to a great extent the distances traversed. [7-15] As forage is consumed the cattle move from cropped to fresh areas within a pasture. This movement results in the deposition of dung pats across wide expanses of pastures.

Not only do cattle move around a pasture in their quest for forage, but it is common practice for cattle to be regularly rotated from pasture to pasture around a farm in order to optimize utilization of grass. It is important, therefore, that local populations of dung beetles, in order to survive, have the capacity to seek out cattle dung on pasture. This must occur regularly and would apply particularly when over-wintering stages emerge as adult beetles the next year. When these beetles emerge, cattle may be grazing in a part of the region distant from their location. Clearly, one key facet of dung beetle behavior which enables them to survive is their mobility.

Dung beetles have been called "proficient", “strong and swift" and "excellent" fliers, with speeds of 5-6 m/sec and 30 km/hour. [38-41] Further, migration of adult dung beetles over long distances has been well documented. [38,42-45] Some adult beetles have flown up to 30 km across seas to colonize off-shore islands, demonstrating their ability to make long-distance flights. [38,45] In Australia, *O. gazella* colonized areas several hundred kilometers from their release site within two years with spread rates of up to 80 km per season. [38,45] Blume and Aga reported that *O. gazella* released in Kleberg County, Texas in 1972 spread 32 km in 1974 and an additional 32 km in 1975.[42] According to Fincher *et al., O. taurus*, a common European dung beetle which entered the U.S. in approximately 1970 or 1971 in the vicinity of Pensacola, Florida, dispersed 300 km across southern Georgia into South Carolina in about 13 months or less during the mid 1970s.[43] These observations support the conclusion that dung beetles move readily, even through cattle-producing areas in which adequate food is available locally. High mobility of these insects and their strong dispersal instincts insure

movement of dung beetles among pastures and across regions.

With respect to movements over smaller distances and shorter periods of time (e.g., hours), two papers are relevant. Eschle *et al.* studied the suppression of horn fly populations using cattle at a site on a West Texas ranch. [46] No cattle had been on the ranch for one year. The herd of cattle nearest to the study site was 1.5 miles away, but numerous deer were present. Dung pats from the cattle involved in the study were often partially or wholly destroyed by dung beetles (mainly *Canthon* spp.) and raccoons. As cattle had been absent from the ranch for a year, the dung beetles attracted to the pats either migrated from at least 1.5 miles away and/or had been sustained by the fecal excreta of the deer. The former is in line with the available evidence that dung beetles are highly mobile and are attracted to dung from considerable distances. If the dung beetles which destroyed the cattle pats were from a local population, it follows that these beetles are not restricted to using cattle dung for food and reproduction. Further support concerning the mobility of dung beetles comes from work by Hanski who discussed "long-distance" movements (0.5 up to at least 1.5 km) observed in England for *Aphodius* spp. [47] To this author, long-distance movements are within an "ecological range" (an area of some tens of square kilometers), whereas within a "behavioral range" (the area of a pasture, i.e., about one hectare) movements are assumed to represent facultative migration between dung pats.

Hanski reported that mature female dung beetles may leave a dung pat if it is too crowded or otherwise unsuitable and seek out a more suitable pat for the laying of eggs, with the result that the new generations of beetles will arise across a wide area. [36,48]

In conclusion, mobility of dung beetles is well documented; their migration between pastures and immigration from the refugia are certain, and this assures that a reservoir of dung beetles for colonization of pats will be maintained and available.

(b) Use of dung for feeding and reproduction

Dung beetles which use cattle dung on open pastures in the U.S. are dung generalists. The large majority of these dung beetles use dung of mammals as the source of food for both adults and larvae. Hence, their reproductive success depends upon the availability of this excrement. *Aphodius* species are the dominant dung beetles in northern temperate regions.[48] Cervenka and Moon recorded 11 species of *Aphodius* in Minnesota, compared to 3 species of *Onthophagus* dung beetles and one *Geotrupe*.[49] Kessler and associates reported that seven species of *Aphodius* and two of *Onthophagus* were among the most prevalent species of dung beetles in both cattle and sheep manure in east-central South Dakota. [50] Of the 26 *Aphodius* species listed by Blume as being associated with bovine dung in American pastures (north of Mexico), most are dung generalists, rather than bovine dung specialists, which also utilize dung of other domestic mammals, especially horses and sheep. [48,51] Among the ten species of *Aphodius* which are European imports and which prefer open pastures and bovine dung, four were observed in both cattle and sheep manure in east-central South Dakota.[50,52] In coastal California areas, the introduced dung beetle *Aph. fimetarius* inhabits the small, soft dung pats (non-pellets) typical of deer and sheep from about February through April (Anderson, J. R., personal communication). In eastern Washington and northwestern Idaho, several *Aphodius* spp. were found in cow, horse and sheep dung.[53] Native American dung beetles which are found in open pastures and bovine dung, such as *Onthophagus* spp. (mostly *O. hecate*) and *Phanaeus vindex*, are attracted in greater numbers to swine feces than to cattle feces even on open pasture and are also attracted to dung from other sources, including opossum, fox, human, rat, raccoon, horse and sheep.[54] *O. hecate,* one of the most widely distributed and most common of North American species, utilizes droppings from dogs, rabbits and woodrats in addition to the sources cited above.[55] Various species of native ball-rolling dung beetles, including *Canthon pilularius* (L.), *C. vigilans* and *C. chalcites* (Halderman), utilize either cattle or horse/mule dung while *Boreocanthon praticola* (LeConte) utilizes cattle and prairie dog dung.[56] The dung of deer, elk, moose and sheep, mostly excreted as small,

hard pellets, can serve as a restricted seasonal resource for such introduced dung beetles as *Aph. fimetarius* (Anderson, J. R., personal communication). The intake of fresh green forage, along with early spring worm infections, results in these animals producing soft, mushy dung pats that resemble miniature cattle dung pats; such dung pats are used by *Aph. fimetarius*. Thus, dung beetle species commonly found in cattle dung and open pastures in the U.S. tend to be dung generalists and will utilize dung from other species besides cattle.

Deer dung can also be used by some of the native *Aphodius* species inhabiting the forested areas of the eastern U.S.[48,57] In contrast to the situation in Europe, large-scale deforestation in the U.S. did not take place until the westward expansion began in the late eighteenth century. Native American peoples, who did not have any domesticated mammals except dogs, did increase the sizes of old fields and of early successional forests, and this led to increases in the deer population.

Conditions until the fairly recent past thus favored forest-dwelling *Aphodius* species specializing in deer dung, and these native *Aphodius* dung beetles have not colonized the recent pasture ecosystems, probably because of their adaptations to forest habitats.[48]

Gordon reported that about 210 species of *Aphodius* are described for North America north of Mexico.[52] Of these, 17 species of eastern *Aphodius* are associated with deer dung (in an obligate fashion or strong preference) and the obligate deer dung species will not use bovine dung. About 60 species of *Aphodius* are associated with dung in rodent or tortoise burrows. Eight native generalist species are not known to have dung preferences (other than rarely using deer droppings); their main sources of dung do not include cattle. In the U.S., *Aphodius* species of European origin are mostly generalists, preferring open pastures and bovine dung. In contrast, the native species tend to occupy non-pasture areas and utilize dung of native wildlife rather than that of recent arrivals, i.e., cattle. It is unlikely, then, that native American *Aphodius* dung beetles will be much exposed to eprinomectin residues in dung.

Little is known concerning the native insect fauna associated with bison dung on the Great Plains, although there is evidence that three dung beetle species became extinct long ago.[48] In this part of the U.S., as pointed out by Gordon in Hanski, the climatic conditions, i.e., low precipitation and humidity, leading to rapid desiccation of pats, do not favor dung beetles which use bovine dung.[48]

Blume listed 450 species of insects in the U. S. and Canada associated with bovine droppings on pasture; none of these is listed as endangered or threatened (Blume, R. R., personal communication). [51,58]

Matthews indicated that six species of Canthonines are the only known representatives of Scarabaeine dung beetles in Puerto Rico.[59] The introduction of dung beetles (including *C. pilularius*, the "tumblebug" commonly found in the U.S.) was attempted in connection with horn fly control, but none of the beetles were ever seen following release. Puerto Rican canthonines are not found in open (un-wooded) areas, nor in cattle pastures. These forest-dwelling dung beetles apparently are not exploiting the cattle dung now present in Puerto Rico, and "cow dung remains virtually untouched in Puerto Rico".[59] Hence, it is unlikely that dung beetles in Puerto Rico will be exposed to eprinomectin residues.

According to Nealis and Lumaret *et al.* most species of dung beetles use a wide variety of fecal matter.[60,61] Fincher *et al.* reported that *Onthophagus* species (and other dung beetles) are attracted to feces from a wide variety of mammals in addition to cattle, including horses, sheep, and especially swine.[54] Kirk and Ridsdill-Smith reported that numerous species of dung beetles, candidates for introduction into southwestern Australia for fly control in cattle dung pats, including *Onthophagus* species such as *O. taurus* (also found throughout much of the southeastern U.S.), are attracted to sheep, goat, horse and mule dung as well as cattle dung.[62] Although *Phanaeus* spp. of dung beetles are most commonly found on cattle dung pats, they are also strongly attracted to the feces of swine, horses and humans.[63-64] *Canthon* species (also of the subfamily Scarabaeinae) of dung beetles including *C. pilularius* (the "tumblebug" commonly found in the eastern half of the

U.S., which rolls dung away for burial and use for food and reproduction) utilize cow, horse and sheep dung.[54,56] In general most of the *Canthon* species exhibit a wide geographical distribution and are often present in high abundance.[56] The success of these beetles may lie in the fact that most of the *Canthonini* species are not restricted to one type of dung and in the absence of preferred food they will accept a reasonable substitute.[56] Supporting the premise that many species of dung beetles are dung generalists, Halffter and Matthews and references cited their report that coprophagous Scarabaeinae are less concerned about the kind of excrement they utilize than about where it occurs (e.g., pasture or woodland).[41] Stewart has commented that, "The fact that most citations to feces attraction of dung beetles in the literature refer to cattle droppings may reflect only the particular investigator's interest or a more or less monofaunal locale where observations were made."[64]

In an area in which numerous cattle are pastured, it is their dung, and not that of other species, which undoubtedly accounts for most of the fecal excrement available for use by dung beetles. Dung of other mammals, both domestic and wild, will likely be found in the peripheral regions of the pasture area and on ungrazed land, and will serve as a source of dung when cattle have moved away from the area or prior to their movement into an area. Some of this non-bovine dung will be used for food by many types of dung beetles. It will also be used for egg laying by beetles which bury dung (e.g., *C. pilularius*), but less often by species which normally oviposit in or nest below a bovine dung pat (e.g., *Aph. fimetarius* and *O. gazella*, respectively). A moist clump of dung from sheep, deer, other cervid or equid may, however, substitute for a bovine dung pat.

(c) Dung beetle activity and reproduction

Cycles of temperature and precipitation strongly influence the activity of dung beetles at a given site. Dung beetles will seek out and utilize fresh dung for reproductive purposes as long as the environmental conditions (e.g., temperature and moisture content of pats and soil) are conducive to such activity. Oviposition is generally not a

one-time effort for Scarabaeinae, but rather occurs with spatial and temporal variation over the lifetime of a mature adult female; this can be several months or more for a female *O. gazella*.[40] In the southern U.S., beetles such as *O. gazella* can reproduce more or less continuously from spring through summer and into the autumn. A period of feeding and sexual maturation of several weeks to a month or more, subsequent to the emergence of the new generation of adults and prior to their reproducing, is necessary and common for many teneral adults of Scarabaeinae species. This period, known as Reifungsfrass, results in a delayed period of ovipositing.[40] However, some of these Scarabaeinae species, e.g., *O. gazella*, begin reproductive activity soon after emergence. According to Halffter and Edmonds, under optimum conditions the life cycle (egg to egg) of *O. gazella* may be completed in 30 days.[40]

Fecundity of dung beetles is, to a first approximation, inversely proportional to the efforts adults of a species expend in the preparation of nests. Most species of Scarabaeinae, which put their maximum reproductive effort in nesting behavior, have generally low fecundity. Fairly high levels of fecundity are found, however, with some Scarabaeinae species, several of which have been introduced into the U.S. and Australia for dung control programs.[40] For example, adult female *O. gazella* (one of the species introduced into Australia and the southern U.S.) can produce up to 200 eggs in a lifetime.[38,40,65,66] *Aphodius* species (dominant dung beetles in north temperate regions such as the U.S.) possess high fecundity, as their maximum reproductive output is invested in egg production and not nesting.[40,48]

Aph. fimetarius accounted for a major portion of the biomass in cattle dung pats studied by Merritt and Merritt and Anderson in the western foothills of the California Sierra Nevada Mountains at a research site about 97 km north of Sacramento.[67,68] Teneral adults begin to emerge in April and reach peak numbers from May to mid-June when they feed in fresh dung pats. Following this, they burrow into the soil and undergo a 5-7 month period of aestivation until the fall rains begin. At that time (October, November) the adults emerge, inhabit fresh dung

pats (causing a second population peak), and feed, mate and oviposit. Nulliparous females continue to appear in small numbers into December, and declining numbers of older, parous females continue to feed and oviposit until early spring. The newly laid eggs hatch and larvae spend the winter and early spring undergoing slow development, with pupation occurring in March/April and a new generation of teneral adults emerges from April to mid-June (Anderson, J. R., personal communication). Seasonal activity (e.g., inhabitation, oviposition and larval development) and the number of generations per year for *Aphodius* species will likely vary considerably depending upon geographical region (including latitude and elevation) and species.

A behavior characteristic of dung beetles that aids in maintaining their population level is density-dependent reproduction. Over-population and underpopulation are kept in check by a change in the number of eggs laid per female in pats (Moon, R. D., personal communication). Thus, in a crowded pat there will tend to be fewer eggs laid per female than in a less-crowded one.[69] Ridsdill-Smith *et al.* have reported that brood ball production per female with *O. binodis* is inversely related to the number of beetles per pat.[70] Intraspecific competition among an excessively large population of larvae in a pat will result in a diminished number of larvae developing to a large size. However, if undercrowding or lowered density of ovipositing beetles occurs because of a reduced population, the number of eggs laid per pat per female can increase. Such an increase can compensate for the decrease in the number of adult beetles per pat (Anderson, J. R., personal communication).[69] This phenomenon, plus the fact that females lay multiple clutches of eggs (Anderson, J. R., personal communication), may allow the population of the next generation to reach a level approaching normal, and will serve to maintain the population of dung beetles in areas that may, for whatever reason, have had a lowered density of adults. Also, based on data reported by Holter, fewer eggs per pat does not necessarily result in a proportional decrease in the number of large larvae per pat.[69] This effect presumably results from an increased opportunity for development of larvae in less-densely populated pats, as there would be diminished competition

for food and habitat in the pats among the larval progeny of the fewer females (Anderson, J. R., personal communication). With respect to their observations on dung beetles which produce brood balls, Ridsdill-Smith and associates state that "the ecological implications of the results are that maximum rate of increase of dung beetle populations will occur when low densities are present in the pat."[70]

In conclusion, the mobility of dung beetles has been demonstrated by numerous observations of their migratory propensity and ability, and their success in colonizing dung significant distances (kilometers/miles) from their starting point. Most species of dung beetles are dung generalists rather than bovine dung specialists, and will thus use dung of other domestic mammals as well as that of wild herbivores, for food and reproduction. There is no evidence that dung beetles native to the U.S. prefer bovine dung. Density-dependent reproduction (egg laying by females, and development of larvae in pats) among dung beetles is a compensatory mechanism which can mitigate against the possibility of population decreases caused by a lower than normal number of ovipositing females. These dung beetle characteristics can mitigate against an adverse impact upon dung beetle numbers caused by a variety of factors, by permitting the succeeding generation to rebound to former densities (Anderson, J.R., personal communication).

(d) Role of dung beetles in degradation of cattle dung and in its removal from pastures

In the U.S. dung beetles play, at most, a minor role in the degradation of cattle dung pats. Dung beetles can be classified into three distinct groups according to their habits of food manipulation: the "tumble-bugs" (or telecoprids), which form feces into balls and roll them away for burial; the dung-burying beetles or paracoprids, which bury feces under or beside the deposit; and dung feeders or endocoprids, which feed on dung and nest inside the dung deposit.[38,71] Cervenka and Moon concluded that dung feeders such as *Aphodius* spp. and other large beetles failed to achieve sufficient densities to disrupt cattle dung pats during May through October in Minnesota or to affect survival and size of large dung-feeding Diptera, which

included *Haematobia irritans*.[49] Fincher *et al.* also found that although many species of scarabs, including dung-burying species of *Onthophagus* and *Phanaeus*, ball rollers from the genera of *Canthon* and *Boreocanthon*, and dung feeders from the genera of *Aphodius* and *Ataenius*, were present on open pastures from March through November in east-central Texas, their populations were not great enough to bury a significant amount of dung.[72] In that study, maximum dung burial usually occurred in August, which coincided with yearly population peaks of the main species of dung-burying beetles. In two studies which examined the contribution of dung beetles to dung degradation, the conclusion was that dung beetles in temperate climates directly contribute little to overall degradation. Putman estimated that dung beetles in the U.K. in autumn contributed up to 13% to pat degradation while Holter estimated dung beetle larvae (mostly *Aph. rufipes)* were responsible for 14-20% of dung disappearance from August to October in Denmark.[73,74] Holter suggested that mechanisms regulating the population density of *Aphodius* larvae in dung pats might have evolved to protect the larvae from loss of both their food and habitat.[74] The development of *Aph. rufipes* larvae takes 5-8 weeks in dung, so that rapid breakdown of the pat by the larvae might lead to fatal exposure to predators or desiccation. The minor role beetles play in the degradation of cattle dung pats in the U.S. led to Federal and state applied research programs focused on the introduction of exotic dung beetles, largely as part of programs to control populations of pestiferous flies.[42,43,75] A few exotic beetles have become established in some southern states and in California, but these programs for beetle importation have been largely discontinued and resources have been shifted to other pestiferous-fly control programs (Blume, R. R., personal communication).[76] Exotic dung beetles established in the U.S. are capable of dispersing or burying considerable portions (>50%) of cattle dung in those regions where they have become established, but only when optimum soil and climatic conditions coincide with times of peak activity of adult beetles (Blume, R. R., personal communication).

Merritt and Anderson found that pats treated with insecticide to exclude insects degraded approximately as fast as naturally dropped pats in the fall in northern California.[68] In natural pats at that site, *Aph. fimetarius* created a frass-like material by larval ingestion of and larval growth within the pat and by adults foraging in new pats; these activities aided dung pat breakdown. However, Merritt and Anderson concluded that pasture management systems and seasonality had greater effects on pat degradation than did insects in that region.[68] Stevenson and Dindal, on the other hand, found that adult *Aphodius* spp. increased pat degradation in microcosms containing artificial cattle dung pats in a glasshouse in upstate New York, but did not increase drying or oxidation of the dung.[77]

Relative populations of dung beetles differ by regions in the U.S. In general, beetles which do not bury dung, such as *Aphodius* spp. and *Ataenius* spp., comprise the most numerous dung beetles in cattle dung pats in the northern sections of the U.S., while beetles which bury dung, such as *Onthophagus* spp. and *Phanaeus* spp., and ball rollers, such as *Canthon* spp. and *Boreocanthon* spp., predominate in the southern sections of the U.S.[49,50,60,64,68,72] In states such as Missouri and Nebraska, members of no one genus predominate, and *Aphodius* spp., *Ataenius* spp. and *Onthophagus* spp. are well represented.[78,79]

Time of year also influences the importance of dung beetles in degrading dung or removing it from pastures. Fincher estimated that 82-88% of artificially deposited 2.5-kg cow pats were buried within 1 week in Texas during the months of July through September, which coincided with the peak activity of *O. gazella*, an exotic, introduced species, while only 0-4% of feces deposited March through June were buried after 1 month.[71] Between 75-90% of the feces deposited during the winter were still present 9 months later.

Thus, differing beetle populations, food-manipulation habits and seasonally dependent population densities affect the contribution of dung beetles in degrading dung or in removing it from pasture surfaces in the U.S., but,

overall, dung beetles play, at most, a minor role in dung pat degradation in the U.S.

(e) Widespread distribution of beetles associated with cattle dung and open pastures

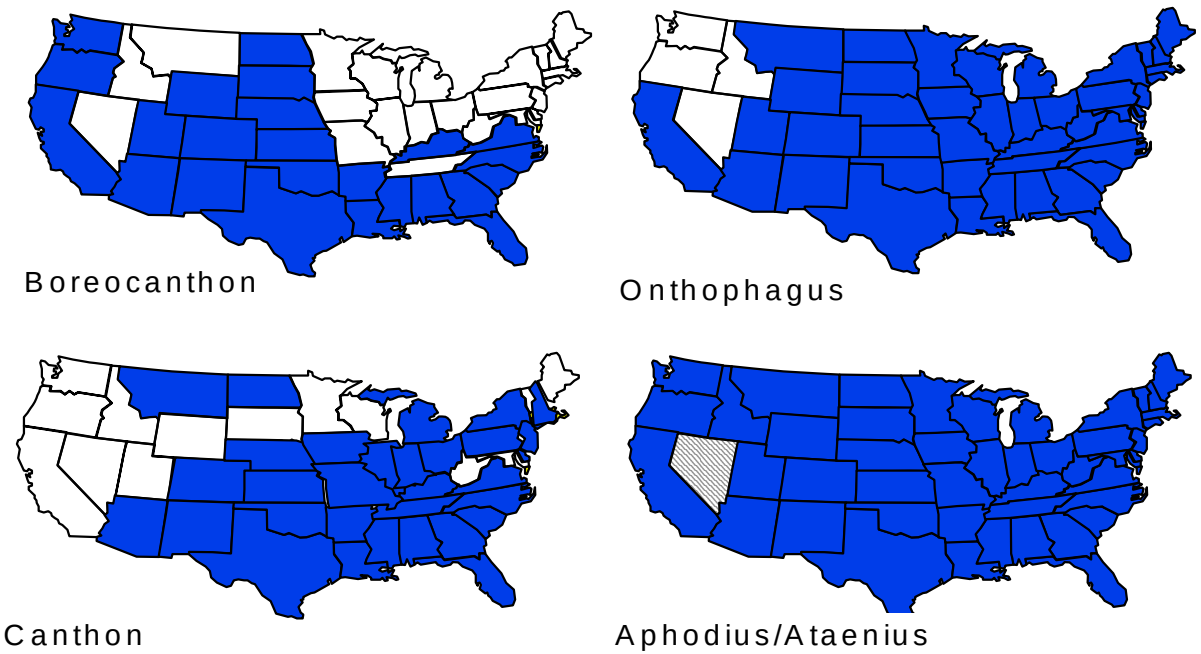
Dung beetle species associated with cattle dung and open pastures are widespread across the U.S. Readers are referred to Blume for details of the distribution of dung beetles by species.[51] Because of this widespread distribution, a localized elimination of beetles, for whatever reason, would not threaten the survival of a species. The tumble-bugs, which include the genera *Canthon* and *Boreocanthon*, and the dung-burying beetles, which include the genus *Onthophagus*, are distributed throughout much of the U.S.[51] Some *Onthophagus* species, e.g., *O. hecate*, are distributed as far west as the Rocky Mountains and north into Canada, but the introduced species *O. gazella* had spread only throughout the south and into California by 1985.[51] There are two species of the dung-burying beetle *Phanaeus* which are widely distributed in the U.S.[63] *P. difformis* is found primarily in and around Texas, Oklahoma and Kansas, while *P. vindex* occurs from the Atlantic coast to the Rocky Mountains and as far north as the Ohio and Missouri River valleys and northeast to Cape Cod.[63] The dung feeders, which include *Aphodius*, the dominant genus in the north temperate zone, and *Ataenius*, are distributed widely across the U.S.[48] *Aph. fimetarius*, a European species, has been reported in virtually all states.[51] It is the most abundant species in cattle droppings in northern California and one of the most numerous species in Minnesota.[49,68] *Aph. fimetarius* is also the dominant dung beetle, in terms of numbers of adults per pat, in central Texas during the late fall, November and December, and early spring, March and April (Blume, R.R., personal communication).

Figure 5 shows the distribution of some of the major genera of dung beetles associated with cattle dung on pastures in the U.S., based on data from Blume and on the observation and collection of *Aph. fimetarius* in northwestern and north-central Nevada, in counties along the California border (Anderson, J. R., personal communication).[51] States which are not shaded do not

necessarily indicate the absence of dung beetles, but rather, a lack of published sightings in that state (Blume, R. R., personal communication). Comparing the species of

FIGURE 5

Distribution of Some Major Genera of dung Beetles Associated with Cattle Dung on Pastures in the U.S.



dung beetles which are found in cattle dung pats in open pastures[48-50,60,64,66,72,79] with their distributional records it is clear that these species are widespread across the U.S.[48,51] Because of this widespread distribution, a localized elimination of beetles, for whatever reason, would not threaten the survival of the species.

(f) Effect of residues of avermectins on dung beetles

Drug residues in the manure of cattle treated with avermectins can affect dung beetles. The impact on dung beetles reported most frequently in studies on the effects of residues of avermectins in cattle dung is inhibition of larval development/adult emergence.[32,76,80] The results of the toxicity of eprinomectin towards 2 species of dung beetles is described in Section 8.A.vii. Studies reporting effects of residues of other avermectins besides eprinomectin on dung beetles are outlined below.

A study to ascertain the effect of ivermectin in dung of cattle treated with a bolus (~12 mg/day for approximately 120 days) upon dung fauna was carried out in Lauterbach, Germany.[81] One-quarter segments of pats and the underlying soil (8 cm deep) were collected 3, 7, 14 and 28 days post deposition and examined for adult and larval (immature) beetles, Diptera larvae and nematodes. Subject pats were deposited 21/22, 70 and 119 days after initiation of treatment. Compared to dung pats from control calves, those from the ivermectin-treated calves contained fewer beetle and Diptera larvae, but no treatment-related effects were observed in numbers of adult beetles.

In a trial conducted in Missouri, dung pats from control calves and those receiving ivermectin (~12 mg/day) via a bolus designed to deliver drug for 90 days were examined for Diptera larvae and adult insects. [82] The percent of dung pats containing fly larvae was treatment-related. Excluding day 7 post dose, none of the pats from calves given a bolus contained fly larvae until 112 days after the bolus was administered (three weeks after the designed shut off point of the bolus). These results demonstrate that

ivermectin residues effectively control emergence of flies from dung pats, and verify the functionality of boluses used in this trial. In contrast to the treatment-related effect with fly larvae, the percentage of dung pats containing adult insects (including dung beetles) was not treatment-related. Dung beetle activity was comparable for pats from treated and control calves. Insect tunneling was observed in all pats.

Wall and Strong reported that ivermectin residues in the feces of calves (200 kg) receiving drug (40 mcg/kg/day) via an experimental ruminal bolus had an insecticidal effect upon the dung pat insect community (including Coleoptera and Diptera).[83] Fresh dung, 0 - 12 hours old, was collected 11 - 17 days after treatment of the cattle and from control cattle. Artificial, 2-kg pats were evenly spaced at 1-m intervals in an enclosure in a dairy pasture. Pats were collected 20, 30, 40, 50, 60, 80 or 100 days later. Coleoptera, mostly *Aphodius* spp., were far more abundant in control pats than in pats from treated cattle. Strong and Wall later estimated a steady state fecal concentration of approximately 400 ppb of ivermectin-related residue.[84] These authors titrated the toxicity of ivermectin toward dung-breeding insects by adding ivermectin to control manure to achieve a concentration range of 0 to 500 ppb. They found that a concentration of 125 ppb was not toxic to larval Scarabaeidae (mainly *Aphodius* species), but toxicity was present at 250 and 500 ppb. Concentrations of ivermectin at 125 to 500 ppb did not repel dung insects nor affect adult Scarabaeidae during the 6-hour observation period.

Onthophagus gazella failed to develop from dung excreted up to 21 days after treatment of cattle subcutaneously at 0.3 mg/kg.[76] However, viability of adults and production of brood balls were not affected by ivermectin residues, even in dung collected during the first week after dosing.

Onthophagus gazella and *Euoniticellus intermedius* (exotic species introduced into the U.S.) were used by Fincher to study the effect of ivermectin residues on the emergence of the dung burying beetles.[85] The capacity of these beetles to reproduce in dung collected at weekly intervals was evaluated under laboratory conditions. The dung was

produced by cattle dosed subcutaneously with ivermectin at either 0.02 or 0.2 mg/kg. There was no apparent effect on brood ball production by either species from the two batches of dung. Emergence of *E. intermedius* was inhibited in dung collected from cattle at one week after treatment with 0.2 mg/kg but not later, and *O. gazella* development was inhibited in dung excreted at one and two weeks, but not three weeks, later. At no time post dose did the dung from the cattle given ivermectin subcutaneously at 0.02 mg/kg cause reduction in the emergence of adults of either beetle species. When confined on control dung, the progeny of those *O. gazella* reared on the 3-week post treatment dung from the higher-dose cattle constructed the same number of brood balls as beetles never exposed to ivermectin.

Sommer and Overgaard Nielsen and Sommer *et al.* reported that dung from ivermectin-treated cattle collected at 2 and 7 days post a 0.2 mg/kg subcutaneous dose was lethal to *O. gazella* larvae, but dung collected on day 17 did not affect larval mortality. [66,86]

Development of the larval stage of *Aphodius* species of dung beetles (widely found in the U.S.) was inhibited in dung collected from cattle one day after 0.2 mg/kg subcutaneous treatment with ivermectin, but dung collected 10, 20 or 30 days post dosing was without effect.[87] Similarly, numbers of *Aphodius* larvae were reduced in dung collected 1-2 days after subcutaneous or topical (0.5 mg/kg) dosing with ivermectin, but not on day 13 or later. [88]

Studying *Diastellopalpus quinquedens*, Sommer *et al.* found that the dung-burying capability of this African dung beetle was not affected by the presence of ivermectin residues in the dung of cattle 2, 8 and 16 days post a 0.2 mg/kg subcutaneous dose.[66,89] However, there was some reduction in the numbers of developing larvae in brood masses. Twenty-eight percent of the brood masses made from dung excreted 2 days post dose contained live larvae; nearly all of the masses made from dung collected on day 8 and day 16 (90 and 94%, respectively) contained live larvae (compared to 100% for masses made with control dung).

Development of *E. fulvus* larvae was totally inhibited in dung collected 1 day after ivermectin treatment of steers 0.2 mg/kg subcutaneously. [90] However, in dung collected 10 days post-dose only a slight delay in development was observed with no effect in dung collected 29 days post-dose. All adult dung beetles fed dung from treated steers survived. Ivermectin did not increase attraction of beetles to dung. However, dung from treated animals was more attractive to beetles on days 5 through 17 post-dose. A modification in the gut flora of treated cattle, rather than the presence of ivermectin, was hypothesized for the increased attractiveness of the beetles to dung.

Strong and Wall investigated effects that ivermectin residues in cattle dung had on colonization, survival and development of insects in June and July in the U.K.[91] Artificial, 2-kg pats were formed from dung collected 2, 7, 14 and 21 days after 0.2 mg/kg subcutaneous treatment of the cattle with ivermectin and from control dung. Eight pats from each group were randomly allocated to sites in a field and were protected from birds. On days 7, 14, 21 and 42 following placement, two entire pats from each group were removed, weighed and assayed for invertebrates. Dung beetles were predominately *Aphodius* spp. and numbers of adults were not different between pats from control and treated cattle. This indicates no difference in attraction to pats containing ivermectin residues relative to control pats or toxicity to adults. Larval *Aphodius* spp. were unable to survive in 7-day post dose pats but there were no differences between numbers or dry weights of *Aphodius* spp. larvae in control pats and pats collected 14 days after ivermectin treatment.

When ovipositing *Copris hispanus* females (not found in the U.S.) were fed for 43 days on dung collected from calves three days following intramuscular administration of ivermectin at 0.2 mg/kg, a reduced rate of oviposition and a lack of survival of immatures were reported.[92] No adult mortality was observed. Larvae did not survive in brood balls made from dung excreted on days 3 and 8 post-dose but survival in dung collected 16 days post-dose was approximately equal to that found for controls. Mortality for newly emerged beetles feeding for 43 days on dung from days 2 and 3 post-dose was 90%; it decreased to 27%

(about twice that of the controls) with dung deposited on day 16, and equal to that of controls (day 0 dung) with dung deposited on day 32 post-dose. *C. hispanus* that survived the lengthy exposure to dung collected up to 16 days post-dose showed atypical reproductive development. When fed for five weeks on dung collected on days 0, 16 and 32 post-dose, there was no mortality of sexually mature *Bubas bubalus* (another dung beetle not found in the U.S.), and there were no suggestions of deleterious effects due to exposure to ivermectin residues in day-32 feces. Following exposure for 32 days to day-32 post-dose dung, the population of newly emerged *Onitis belial* exhibited 22% accumulated mortality.

Ivermectin had no effect on the rate of dung beetle colonization in Denmark, Tanzania or Zimbabwe.[93,94] Dung was collected from cattle at intervals from 2 to 30 days after treatment at 0.2 mg/kg subcutaneously and from control cattle. Powdered ivermectin (source not indicated) was also mixed into some control dung at concentrations from 0.015 to 0.42 ppm on a wet weight basis. Pitfall traps were baited with dung and arriving beetles were counted and identified. A lack of a preference for dung containing ivermectin was observed, consistent with results from studies by Strong and Wall and Lumaret *et al.* [84,90]

McCracken and Foster used multivariate analysis to examine the effects of ivermectin on invertebrates in artificial 1-kg cattle dung pats in the U.K.[95] The injectable formulation of ivermectin was diluted with water and mixed with control dung to produce levels of 0, 0.5, 1 and 2 mg of ivermectin per kilogram of dung. The pats were placed in stratified random block plots on pastures adjacent to fields containing cattle. Pats, and the soil beneath (4 cm depth), were taken at 15, 30, 45, 60 or 90 days after placement. Placement dates were in May, June, August and September. Initially, there were 60 pats per collection group, but 73 of the original 228 pats were not visible on the day of sampling and another 21 were excluded from further analysis because they contained less than 3 taxa. Data from soil samples from beneath 57 pats were used for analysis. The study concentrated on differences between pats with regard to the numbers and types of Diptera and Coleoptera present and the numbers

of earthworms. Few differences were detected between the three levels of ivermectin used in the study, so experimental pats were regarded as either treated or controls. Eight distinct assemblages of taxa were found in the pats. Most (54%) of the between-pat variation in the invertebrate communities was attributed to duration of exposure after placement, while 30% was attributed to time of year of placement and only 16% to the presence or absence of ivermectin. The greatest (42%) variation in the invertebrate communities in the soil beneath the pats was again attributed to the duration of exposure, with 35% attributed to the presence or absence of ivermectin in the pat and 23% attributed to the seasonality of placement of the pat. As expected, earthworms were more prevalent in groups containing mostly older pats (45 and 60 days post deposition) than in groups containing mostly younger pats (15 and 30 days) regardless of the presence or absence of ivermectin. The authors concluded that ivermectin particularly affected cyclorrhaphan fly larvae. However, the groups of pats where these larvae were found were mostly groups comprised of both treated and control pats. Consequently, it is not possible to identify species-specific effects from the data.

Ridsdill-Smith observed that dung of cattle dosed subcutaneously with abamectin at 0.2 mg/kg and collected at various time points for up to 11 weeks after treatment did not affect survival of adult *O. binodis*, fed on the dung for 16 days.[96] *O. binodis* is not found in the continental United States. Dung collected at one and two weeks, but not four weeks or longer, post-dose did reduce brood ball production (compared to dung containing levamisole, the control). No immature (larval) beetles survived in brood balls made from feces collected one week post-dose. Survival was approximately 55%, 65% and 100% (corrected for controls) in brood balls from feces excreted two, four and eight weeks post-dose, respectively.

The presence of abamectin residues in the dung of cattle 3-5 days after a subcutaneous dose of 0.2 mg/kg delayed, but did not abrogate, egg laying by newly emerged female *O. binodis*.[97] Importantly, the effect of abamectin residues in dung on egg laying by *O. binodis* dung beetles was reversed when the beetles switched from feeding on

residue-containing dung to dung from untreated cattle. Adult mortalities for the newly emerged female beetles, which fed on the maximum residue-containing dung for 8 weeks (an unreasonably long period of exposure, given the pattern of use of abamectin and normal beetle behavior), or for 2 weeks followed by 6 weeks on control dung, were about 20% greater than those for the control group.

The effect to dung beetles reported most frequently in studies with abamectin or ivermectin residues in cattle dung is that upon larval development (adult emergence). Further, as larvae are the life-stage of dung beetles most sensitive to abamectin and ivermectin residues in dung, this is a parameter which allows comparison of the toxicity of abamectin, ivermectin and eprinomectin, and the sensitivity of various species of dung beetles, to these residues. Relevant data are presented in Figures 6 and 7 and Table 9. Little or no impact is found for ivermectin and abamectin residues in cattle dung excreted 10-21 and 28 days post dose, respectively, except with *O. binodis* (a species not found in the continental U.S.) on dung from abamectin-treated cattle. Eprinomectin should not affect emergence of progency of *O. gazella* or *E. intermedius* from dung excreted 7-10 days after dosing, based on the NOEC of 64.7 ppb for both species and the excretion pattern of the drug in the feces as illustrated in Figure 2.

Probit analysis of data on the emergence of adults of a number of dung beetle species from dung from ivermectin-dosed cattle has been carried out. These data were compiled from a number of studies. The probit analysis indicates that 10, 50 and 90% emergence can be expected with dung excreted 4, 9 and 18 days post dose, respectively (see Figure 7). As dung from abamectin-treated cattle inhibits emergence of *O. gazella* for about one week longer post dose than does dung from ivermectin-treated cattle, at least 50% emergence with abamectin can be expected by 3 weeks post dose, and 90% or greater by 4 weeks, with most species of dung beetles.

FIGURE 6

Percent of Emergence of Dung Beetles with Respect to Time Post Dose of Dung Excretion

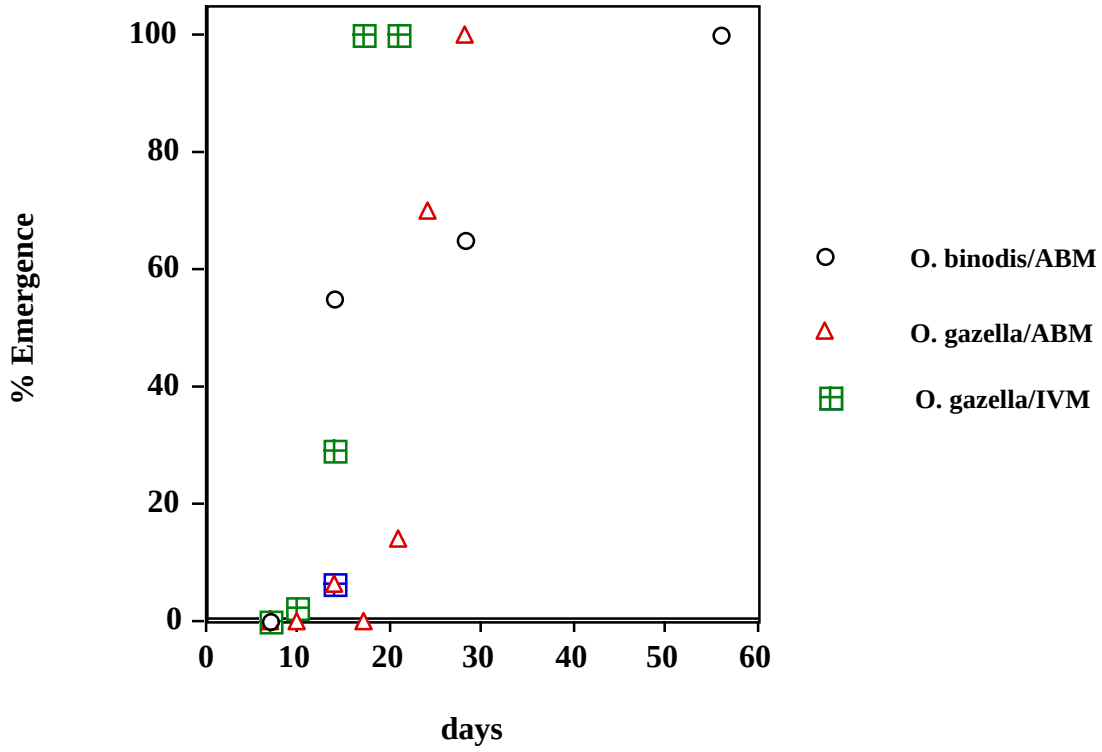


FIGURE 7

Percent of Emergence of Dung Beetles and Probit Analysis of Emergence with Respect to Time of Dung Excretion Post Dosing of Cattle with Ivermectin

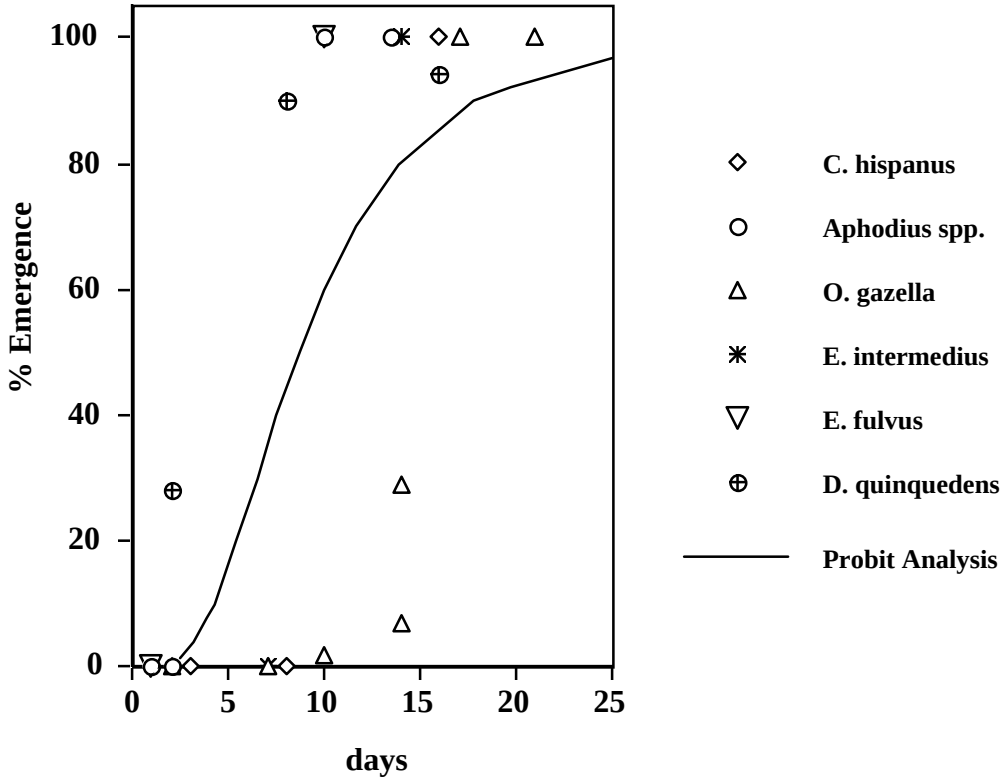


TABLE 9

Sensitivity of Dung Beetle Larval Development/Adult Emergence to Ivermectin, Abamectin and Eprinomectin

Residues in Dung of Cattle Dosed Subcutaneously at 0.2 mg/kg with Ivermectin or Abamectin or Topically at 0.5 mg/kg with Eprinomectin

Species	Days Post-Dose of Dung With No Effect on Larval Development/Adult Emergence		
	IVERMECTIN	ABAMECTIN	EPRINOMECTIN
O. gazella [a]	17 (Sommer and Nielsen[86]) 21 (Fincher[85])	28 (Roncalli[76]) [b]	7-10[f]
Aphodius spp.[a]	10 (Madsen *et al.*[87]) 13-14 (Sommer *et al.*[88])	----------	
E. intermedius [a]	14 (Fincher [85])	----------	7-10[f]
O. binodis [c]	------	56 (Ridsdill-Smith[96])[d]	
C. hispanus [c]	16 (Wardhaugh and Rodriguez-Menendez [92])	----------	
D. quinquedens [c]	16 (Sommer *et al.* [89]) [e]	----------	
E. fulvus [c]	10 (Lumaret *et al.* [90]) [g]	----------	
Aphodius spp. [a]	Concentration of 125 ppb not toxic (Strong and Wall[84])	----------	

a Found in the U.S.
b Adult emergence was about 14% and 70% with dung collected 21 and 24 days post-dose, respectively.
c Not found in the U.S.
d Adult emergence (survival) was about 55% and 65% (corrected for control) with dung collected 2 and 4 weeks post-dose, respectively.
e 94% emergence
f Based on results of studies ASR-14487 (APPENDIX C-5) and ASR-14602 (APPENDIX D-14)
g Relative to controls, a slight delay in development, but no inhibition or increased mortality, was observed.

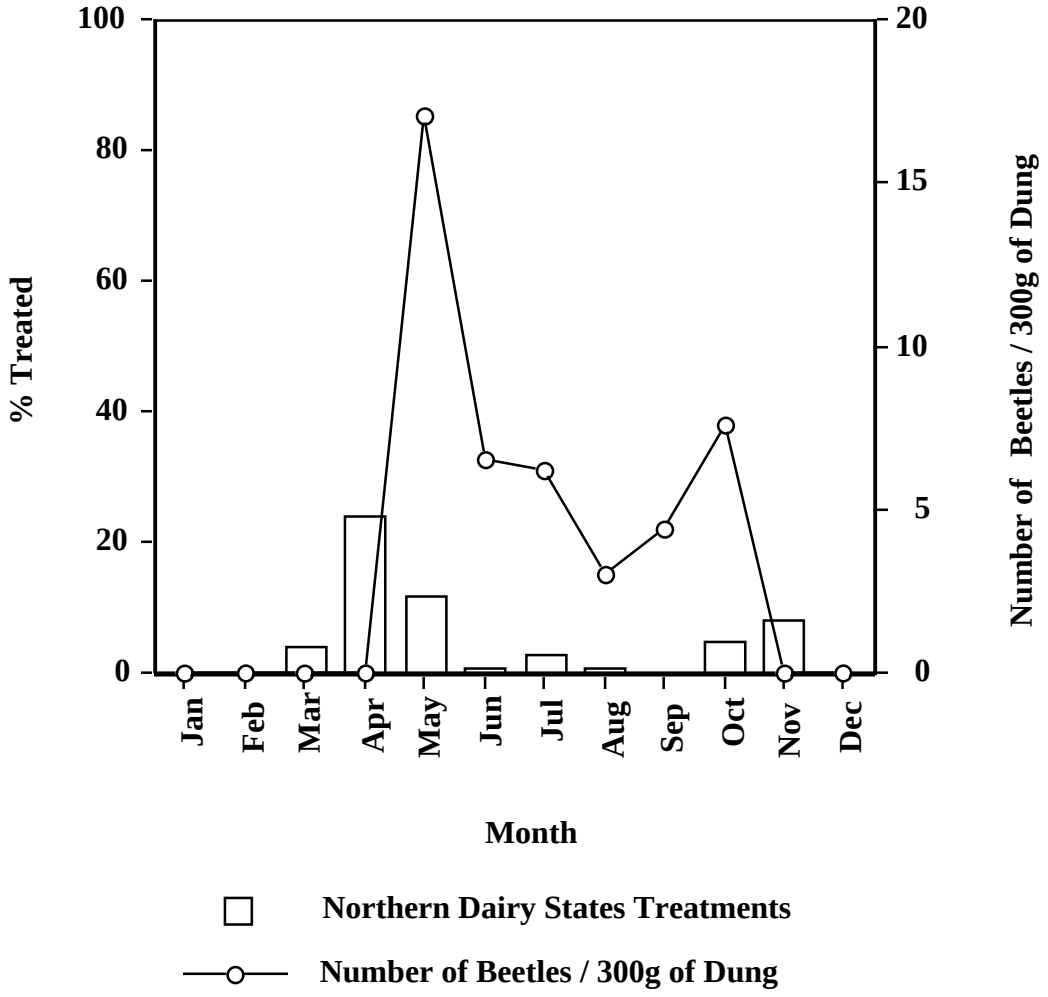
(g) Use and exposure scenarios

The overall assessment of any impact of eprinomectin residues in cattle dung upon dung beetle populations is based on the estimated actual usage of anthelmintics and ectoparasiticides. The use patterns are then compared with the activity patterns for dung beetles in those regions for which such activity data are available.

It is clear from the data presented in Figure 8 that there are two major peaks of dung beetle activity (mainly *Aphodius* spp.) for Minnesota, a representative northern dairy state.[49,98] Christensen and Dobson reported the presence, in March, of viable *Aph. fimetarius* eggs in overwintered cattle dung pats in Indiana (also considered a northern dairy state).[99] Larvae and pupae may also overwinter in pats. Thus, the early peak of dung beetle activity (May) in Minnesota likely arises from newly emerged, as well as some overwintering, adults (Moon, R.D., personal communication). As neither dung beetle activity peak in Minnesota coincides with the main usage months for anthelmintic in this region, any impact of anthelmintic residues upon reproduction will be low. As noted in Tables 2 and 3, except for April, the monthly percentages of cattle treated are all well below 20%. For May and October, the two months of peak dung beetle activity, anthelmintic usage is estimated to be only 12 and 5%, respectively, hence only a small proportion of fresh dung pats will contain anthelmintic or ectoparasiticide residues. The month of greatest anticipated anthelmintic usage, April, occurs one month prior to the major peak of dung beetle activity (May). As dung beetles are only attracted to fresh dung, and as only dung in those pats excreted for up to 7-10 days post-dose by eprinomectin-treated cattle will inhibit dung beetle emergence, only a small percentage to none of the pats excreted in May by the cattle treated in April will affect dung beetle emergence. Further, as only a small fraction of cattle will be treated in May, the non-treated animals will provide large numbers of residue-free pats. Thus, the presence of ample amounts of residue-free dung for use by dung beetles is assured, and there will be no adverse impact on dung beetle populations from use of IVOMEC EPRINEX Pour-On.

FIGURE 8

Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics versus Numbers of Dung Beetles by Month in Minnesota



In east central Texas there is a broad period of dung beetle (mainly *Onthophagus* and *Canthon* spp.) activity, including reproductive activity, from April to September; see Figure 9.[72] *O. gazella*, for example, are commonly found throughout these regions and are active from spring through the summer and into the autumn. This period of activity does not coincide with any major time of usage of anthelmintics or ectoparasiticides. Indeed, the month of greatest anticipated anthelmintic usage in the South Central region (October; see Tables 2 and 3) occurs well beyond the time of major dung beetle activity and involves anthelmintic treatment of only about 21% of the pastured cattle. This pattern of beetle activity should be representative of the South Central and Lower Southeast regions (Blume, R.R., personal communication). Hence there will be no long-term impact on dung beetle populations.

The month of major anthelmintic usage in the Upper Southeast region is April, for which estimated use in pastured cattle is 21% (Table 2). This is two months prior to major beetle activity observed in Missouri in June.[79] There is little anthelmintic usage during June through September, when beetle activity is high. Thus, the exposure of dung beetles (mainly *Aphodius* spp.) in Missouri to cattle dung containing eprinomectin residues will be low (see Figure 10). These results should be applicable to the Upper Southeast Region. A large majority of dung pats will not contain anthelmintic residues during any part of this month. Thus, in both scenarios there will be no impact on dung beetle populations from use of IVOMEC EPRINEX Pour-On.

In the Pacific Eastern range, the month of highest estimated anthelmintic usage in beef cattle is April (Table 2). This is at least one month prior to the major peak of dung beetle activity in north central California; the activity represents emergence of mainly *Aphodius* spp. especially *Aph. fimetarius*. These dung beetles in this region (data for the western foothills of the Sierra Nevada Mountains) reproduce during the autumn and winter (second peak of activity) Anderson, J. R., personal communication), a time

of little anthelmintic usage.[67,78] The data presented in Figure 11 clearly demonstrate there is very little coincidence of anthelmintic or ectoparasiticide usage and dung beetle activity. This assessment should be applicable to the inland Mediterranean climatic areas of the other states of the Pacific region as well. With respect to the Coastal pasture region of the Pacific Coast states, seasonal activity of cattle dung beetles in Marin County, CA (a representative area) appears to be close to that for the north central California inland area, with *Aph. fimetarius* females ovipositing from October through February-March, and maximum oviposition in November and December (Anderson, J. R., personal communication). No usage of anthelmintics occurs in October, November or February in the Coastal pasture region; maximal usage of anthelmintic in beef cattle is in December and January (estimated values of 37 and 9% in the Pacific Coastal region for these two months, Table 2). Thus, in one of the two months of maximal oviposition activity, all dung pats will be free of anthelmintic residues and hence non-toxic to larval beetles. Even in December, the other peak month for oviposition, less than 40% of the cattle will be treated with anthelmintics (only a fraction of those with eprinomectin), and thus the large majority of pats will be free of residue. The large majority of larvae will, therefore, not be exposed to eprinomectin residues at inhibitory levels.

Treatments of dairy cows with anthelmintics and ectoparasiticides and the anthelmintic treatments for beef cattle in the Pacific region are summed in Table 3. The overall treatments per month were divided by the total cattle in both regions in Table 3, unlike the subdivision of the region into Pacific Eastern and Pacific Coastal in Table 2. This was done because the dairy management practices do not follow the same geographic subdivision seen for the beef cattle management practices. Nevertheless, inclusion of dairy cow treatments and numbers of dairy cows in the estimated percentages of treated cattle on pasture (Table 3), does not alter the assessment.

FIGURE 9

Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics in South Central and Lower Southeast Regions versus Numbers of Dung Beetles by Month in Texas

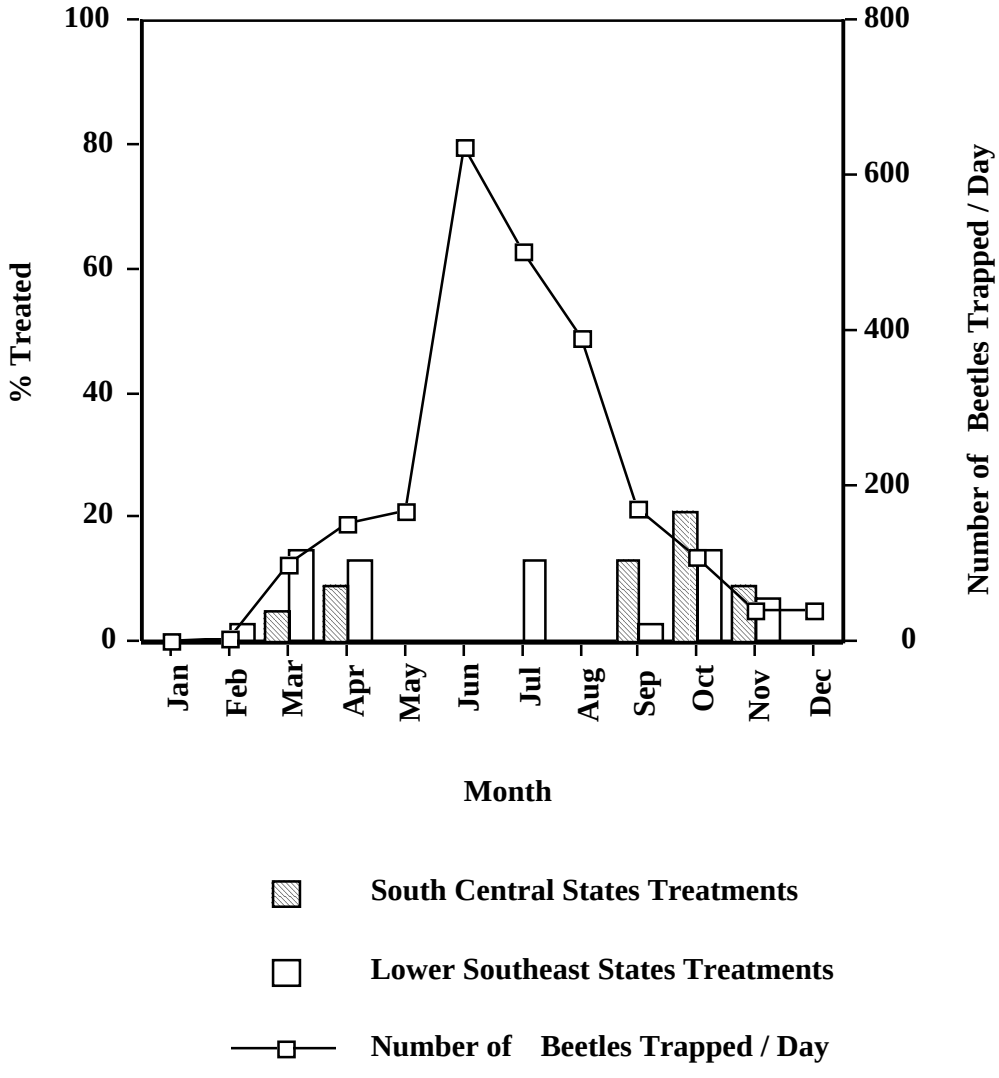


FIGURE 10

Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics versus Numbers of Dung Beetles by Month in Missouri

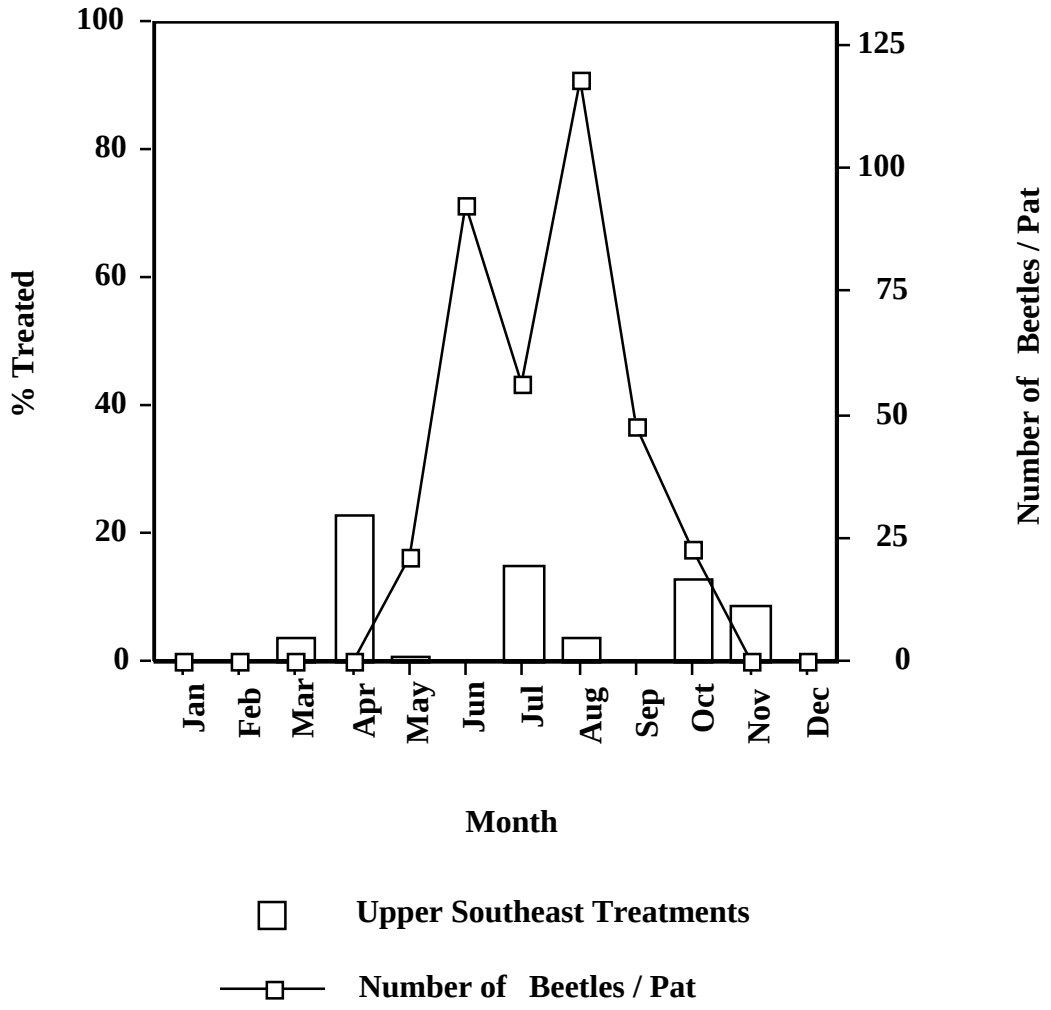
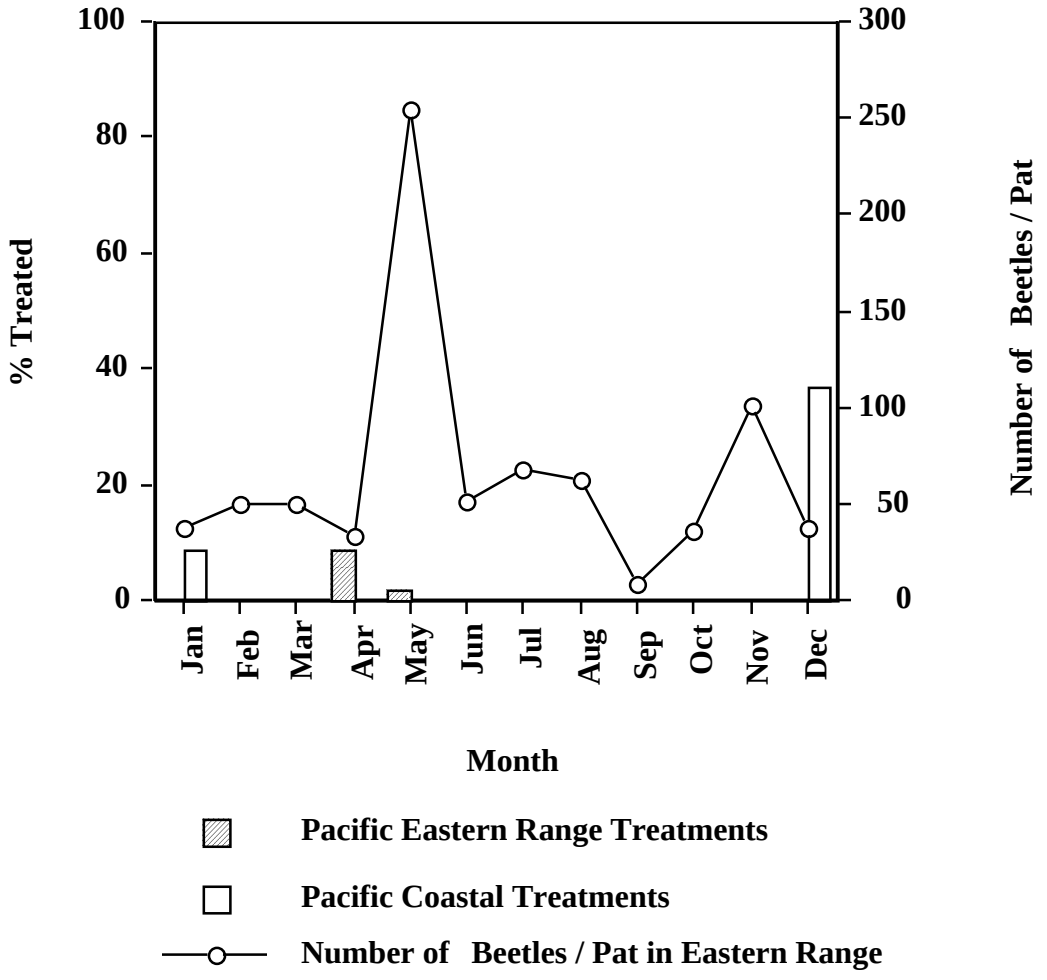


FIGURE 11

Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics versus Numbers of Dung Beetles by Month in California



The estimated actual use of anthelmintics in beef cattle for the New England region reaches 33% in October and 41% in November (Table 2), but in this and other regions such as the Big Sky (maximum of 17% in October) and Plains (maximum of 18% in October) in which winters are severe, anthelmintics are administered just prior to removal of cattle from pasture. Further, in regions with cold winters there will be little or no dung beetle activity in the late fall or winter months. Inclusion of the anthelmintic and ectoparasiticide treatments for dairy cows on pasture in the New England region (Table 3) does not appreciably alter the percentages. Thus, few cattle in these regions would be treated with anthelmintics or ectoparasiticides while on pasture.

The estimated actual percentages of pastured cattle treated with anthelmintics in the Southwest region are low throughout the year. The percentages of treated pastured cattle are 13% (Table 2) or less in any month.

In Hawaii, the greatest estimated percentage of pastured cattle treated with anthelmintics is 16% (Table 2). Most of the anthelmintic treatments in the spring are given to stockers which are treated before they are shipped off of the islands for growing and finishing, and hence treatment of these cattle would not contribute to anthelmintic residues on pastures.

Based on the estimated actual scenario, much less than 40% of the dung beetle larvae populations would be exposed to feces from cattle treated with anthelmintics or ectoparasiticides. Even if as much as 40% of the larval dung beetles were exposed to dung containing inhibitory levels of eprinomectin during the peak month of reproduction/larval development, this would not result in a long-term impact on dung beetle populations because of the operation of various compensatory mechanisms.

These compensatory mechanisms are based on two behavior characteristics of dung beetles which facilitate recolonization and compensate for any temporarily reduced populations of adult dung beetles which might result because of reduced emergence of a new generation. One of the characteristics is the mobility of adult dung beetles

which allows them to move readily between locales and recolonize an area which may have for any reason a low population density. Studies by Eschle *et al* and Hanski demonstrated that dung beetles will be attracted to dung from at least one mile away, and migration of dung beetles over long distances has been well documented.[46,47] In-flying dung beetles from other areas and refugia will reproduce using the readily available non-toxic dung pats being excreted by cattle treated weeks previously. The second characteristic that will aid in maintaining the dung beetle population is density-dependent reproduction (Anderson, J. R., personal communication) (see Section 8. A. xiii. a. 3).[69,70] Lowered densities of dung beetles in pats can lead to increased egg laying and brood ball production per female, thus in part compensating for lower numbers of egg-laying females.[69,70] In addition, even if there are fewer eggs per pat, an enhanced success rate for larval development occurs because of reduced competition among the larvae for food and habitat space (Anderson, J. R., personal communication). Both of these behavior patterns will serve to maintain the population of dung beetles in a locale where use of IVOMEC EPRINEX Pour-On might cause a decrease in the number of adults in a succeeding generation.

Even if there were a locale in which all of the cattle were treated during a month of major dung beetle reproductive activity, the compensatory factors would be expected to attenuate any effects upon populations of dung beetles. Thus, there will not be a long-term impact upon these populations.

(3) Effects on dung pat degradation/ decomposition

Eprinomectin administered topically to cattle ultimately enters the environment via the dung, either as residual drug or metabolites, or by sloughing off with the hair or hide. Dung pats undergo degradation, returning nutrients to the soil, and offer certain insects sites and food necessary for their successful reproduction. Degradation of dung pats is a complicated series of events, involving a wide variety of animate and inanimate forces. The rate of decomposition of dung pats is extremely variable, and depends upon many factors [e.g., climate, season, soil type, faunal inhabitants and microclimate.[73,87,100] A general discussion of dung decomposition

and degradation is presented in this section, as an introduction to the assessment of the impact of avermectins on dung degradation and on certain dung fauna and flora. Dung pat degradation is important not only because it results in recycling of nutrients to the soil, but also because a low rate of degradation can have an adverse economic impact arising from the smothering of new vegetation and inhibiting its growth. Loss of useful forage may result from the phenomenon known as "grazing avoidance", i.e., cattle not eating grass growing in the immediate vicinity of fecal pats.

(a) Effects of biological components

Worms, fungi, bacteria and insects (both adult and larval forms) are members of the bovine dung community, all playing roles in the removal and decomposition of dung. During the wet season in the tropics, dung-collecting and dung-burying beetles may degrade an entire dung pat from a large herbivore within 24 hours of deposition; however, in temperate ecosystems, dung beetles do not play a major role in dung pat removal.[73] Rather, decomposition of dung is primarily a microbiological decomposition process, with the bacteria and fungi of decay serving as major contributors.[101] Earthworms also play a key role in the dung degradation process.[73,102] Dung-breeding insects, including flies, are present in temperate areas and are also included among animals associated with decaying dung. They colonize dung directly, laying their eggs in the dung, upon which the developing larvae feed. Insect larvae and microorganisms, colonizing a dung pat, provide a route for the molecular removal (via metabolism) of organic material from the pat. Tunneling by insects (larval and adult forms) increases aeration of the pat and facilitates deeper penetration of aerobic bacteria and the entrance of fungi into the pat.

(b) Effects of physical/mechanical components

Just as there is a biological component to the decomposition and degradation of dung pats, physical and/or mechanical factors also play a key role in pat degradation. Weathering (rain, frost and snow, freezing, thawing, dehydration) and resultant pat cracking is very important in the breakdown of dung pats.[73,102-104] Heavy

and frequent rains disrupt dung pats, and Dickinson and colleagues reported that irrigation of a pasture, to simulate continuously wet weather, promoted the disappearance of cattle dung.[105] In contrast, hot, dry, sunny weather retards pat degradation, as the dung quickly develops a hard outer crust retarding entrance of insects; further, activity of earthworms, bacteria and fungi proceed more slowly under dry conditions and in the winter.[101,105] Growth of new vegetation through cracks in dung pats contributes to further pat degradation. Trampling and scattering of pats by cattle lead to the breakdown of pats (especially on pastures with high stocking rates), as does disturbance by birds (e.g., the Western meadowlark, *Sturnetta neglecta*) scratching and pecking in dung piles in their search for insects and undigested seeds.[101,104]

Intense pasture management geared to maximum forage production for high stocking rates with high value cattle usually involves mechanical activities (mowing, harrowing, dragging of chains and chain-link fencing) which contribute to enhanced dung pat degradation. Irrigation of pastures will facilitate biologically based routes of dung decomposition.

(c) Effects of insecticide (lindane) upon pat degradation

Merritt and Anderson and Anderson *et al.*, studied the relationship between cattle feces devoid of insects (created by adding lindane to control feces at the high rate of 282 ppm) and increased dung fouling of pastures.[68,104] These authors concluded that pat degradation rates are determined more by the season of the year when pats are deposited, and the type of pasture on which they are dropped, than by insect activity. Fastest degradation occurred on cleared but irrigated pasture, and the slowest was observed for non-irrigated pastures with no shade. Increase in time required for degradation was greatest for lindane-containing pats (compared to insecticide-free pats) put outdoors in May and early June (a time of high insect activity); the impact of the lindane upon degradation was least with pats placed on irrigated pastures. Further, little difference in degradation rates was noted during other times of the year between insecticide-treated pats and control pats.

(d) Pat weights and surface areas

Anderson *et al.*, reported that comparative losses in weight between treated and control pats "had little biological or practical meaning" in rangeland pasture.[104] The two important criteria were the pat surface area smothering new growth, and the length of time a pat remains in the pasture. How much less a pat weighed as it aged was not an important criterion. Most cattle dung pats initially contain 75 to 90% water, and significant differences in weight loss can occur among pats with no important effect on the area of ground covered.[104] Weight loss of pats can approach 70 to 80% during a hot, dry spell of a month's duration, resulting entirely from evaporative loss of water.[68]

(e) Effects of avermectins on dung pat degradation

Several studies have investigated the effects of ivermectin on dung pat degradation. Methodologies used in these studies were not consistent; natural and artificially formed pats were used and methods for assessing degradation included measurements of wet weight, dry weight, organic matter content, pat diameter or pat area. For a recent review on the importance of methodology in the interpretation of the factors affecting the degradation of dung and for suggestions on standardizing conditions, see Barth.[106]

In a report by Schmidt, there was no apparent impact upon the disintegration on pasture of artificially formed (1.5 kg or less in weight) dung pats produced by cattle which had received ivermectin (0.2 mg/kg via intramuscular injection) compared to the disintegration of pats from control cattle.[35]

Wall and Strong also investigated the impact of excreted ivermectin upon fecal pat degradation.[83] Ivermectin was given continuously to 200-kg calves at 0.04 mg/kg/day via ruminal bolus. They concluded that degradation in cattle-free pasture of 2000-g pats, prepared from feces containing ivermectin residues, was prolonged compared to that of pats prepared from control feces. These artificially formed pats were several times the weight of those typically

deposited on pastures in trials with cattle. These authors used differences in wet weight of control and experimental (i.e., ivermectin residue-containing) pats with time for a quantitative estimate of the difference in rates of pat decomposition, and speculated that ivermectin treatment could lead to an increase in the amount of pasture land fouled by dung. Results from field studies demonstrate that this speculation is not born out in reality. Since the control pats were "largely degraded within 100 days," the practical significance of a relative difference between small numbers is not clear. Additionally, any differences in moisture content (another important factor for pat area and degradation according to Barth) between the control and experimental pats could have lead to the observations.[106] When the data were presented using a more-conventional plotting method, it was apparent that the originally reported data did largely reflect the moisture content of the pats, not decomposition.[91] The importance of diminution of wet weight by pats, with respect to their degradation and environmental impact, has been discounted by other researchers.[74,104]

Schaper and Liebisch reported that, compared to dung pats from control cattle, dung pats from cattle that received ivermectin subcutaneously at 0.2 mg/kg did not exhibit delayed degradation.[107] Twenty-one cattle were treated at 3 and 8 weeks after the start of the grazing season in northern Germany. Fresh dung was collected two days after the first treatment and then weekly thereafter. Standardized 1.5-kg artificial pats were deposited in a fenced-in area of pasture along with pats from untreated cattle. The moisture content of pats from both groups were equalized before deposition. Six control cattle grazed on the pasture but outside of the fenced-in area. Pat areas were determined by serial photography at regular intervals over 21 weeks. Schaper and Liebisch also found no differences in numbers of adult or larval dung beetles between treatment groups; however, numbers of diptera and nematodes were reduced in the pats from cattle treated with ivermectin.[107]

McKeand *et al.* also found no delay in the degradation of natural pats of cattle treated with the pour-on formulation of ivermectin.[108] Cattle were treated at 3, 8 and 13 weeks

after spring turnout in western Scotland. Jacobs *et al.* also examined the degradation of natural dung pats from cattle treated with the pour-on formulation at 3, 8 and 13 weeks after turnout onto pastures in the UK.[109] They found no feces remaining just before the next grazing season on pastures grazed by treated or control cattle. Rates of degradation of pats were not determined and lungworm infections necessitated treating all control cattle at least once during the trial with parenteral ivermectin.

Madsen *et al.* prepared artificial 0.1-kg dung pats from feces from a single heifer treated subcutaneously with ivermectin 24 hours previously and placed the pats into clay pots containing composted garden soil.[110] Similar pots were prepared from feces from heifers treated with other anthelmintics or from non-treated heifers. To each pot was added a mixture of earthworms. The pots were covered although holes allowed access for insects. The pots were placed outdoors in the early summer in Denmark under an open shelter where they were watered frequently. Within a period of 42 to 55 days, all pats, except those from the ivermectin-treated heifer, had disappeared completely. Complete disappearance of the pats containing ivermectin residues was observed by day 98. Thus, in the absence of normal weathering mechanisms and when interactions with some biotic species are prevented, effects of ivermectin on dung-living dipterian larvae might affect dung degradation.

Madsen *et al.* also compared the organic matter content of formed pats of 1-kg weight from cattle given ivermectin subcutaneously at 0.2 mg/kg b.w. with that from control animals.[87] As the pats aged in the pasture, the percentage of initial organic matter decreased more slowly in pats excreted by treated animals one or twenty days post-dosing than for comparable pats from control animals. Organic matter of pats deposited by treated animals 30 days post-dosing decreased at a rate comparable to that of controls. In Denmark, dung degradation was also measured by percentage of initial pat organic matter using formed 1-kg pats which were placed on nylon mesh screening and under chicken wire to prevent breakup of the pats by birds.[88] Pat degradation was diminished for dung collected 1 - 2 days post-treatment (0.2 mg/kg subcutaneous injection) or up to 13 - 14 days post-treatment (0.5 mg/kg topical application) compared to ivermectin-free dung.

Sommer *et al.* found no differences, related to treatment of cattle with ivermectin, in the amount of cattle dung buried in fields by afrotropical dung beetles in Zimbabwe.[66] Artificial, 1-kg pats were prepared from dung from control cattle and from cattle treated on 2, 8 or 16 days prior with ivermectin subcutaneously at 0.2 mg/kg body weight. After five days of exposure, most of the residual dung was inextricably mixed with soil; however, the total amounts of non-buried dung organic matter were determined from loss of weight on ignition data.

No significant effects upon feces degradation were observed with respect to use of ivermectin in horses. Ewert *et al.* and DiPietro *et al.* reported that multiple dewormings with ivermectin did not result in prolonged dung degradation leading to increased pasture fouling as determined by aerial survey mapping.[111,112] However, Herd *et al.* reported that delayed degradation occurred with dung pats from horses treated with ivermectin.[113]

Three studies were conducted by Merck to determine whether ivermectin in dung from calves treated with an IVOMEC SR Bolus affected dung pat degradation, grazing avoidance or fauna populations.[81,82,114] There were no treatment-related effects for dung pat degradation or grazing avoidance. There were, however, treatment-related effects on dung fauna, especially upon insect pests.

Wallace *et. al.* noted extensive weight loss of pats deposited by both bolus-treated and control calves.[82] There were no treatment-related effects upon pat weight loss or upon reduction of dung pat areas over time. Similar results were found in a trial conducted in Lauterbach, West Germany.[81] The surface areas of fecal pats deposited on days 21/22, 70 and 119 post-treatment from control calves and those given an IVOMEC SR Bolus were followed for over eight months. Degradation of the pats from the IVOMEC SR Bolus-treated calves appeared to be somewhat reduced compared to that for pats from control calves beginning one and one-half to two months post-initiation of treatment. However, statistical analysis of these data revealed no difference ($p>0.10$) between treatments in respect to average surface area or change in area over time for dung pats deposited on Days 21/22 or 70.

After adjusting for initial differences, control pats deposited on day 119 were slightly larger than ivermectin pats 7 to 49 days after deposition and slightly smaller 63 to 147 days after deposition; the difference was less than 1 cm^2 at 175 days. By 8-9 months both sets of pats were essentially degraded. Further, the decrease in organic matter content of control and ivermectin residue-containing pats was treatment-independent. Madsen *et al.* suggested that decrease in organic matter of dung pats is an indication of rate of dung pat disappearance.[87] Based upon these results, ivermectin treatment would not be expected to increase pasture fouling and loss of new growth because of smothering.

To determine the effect of anthelmintic drugs upon the production and disappearance of cattle dung on pastures, a two-year study was conducted by scientists from the Agrichemical Evaluation Unit, University of Southampton, at the Merck farm in Hoddesdon, Hertfordshire, UK.[114] Treatments include controls, ivermectin bolus (8 mg/day for approximately 90 days), ivermectin injection (0.2 mg/kg at 3, 8 and 13 weeks) and oxfendazole bolus (750 mg at five intervals of approximately 21 days each). The functionality of the ivermectin bolus was supported by fecal EPG counts. There were no treatment-related differences between groups in the rate of dung deposition (weight of dung collected at monthly intervals) and accumulation of dung on the pastures, i.e., no significant difference ($P>0.05$) in the dry weights of cumulative standing dung.

The rate of decomposition/degradation under natural conditions of dung pats from calves was investigated by locating 40 fresh pats in each paddock in July. At this time, the ivermectin bolus had been operational for two months, hence there was drug residue in the dung. Ten of these natural pats were collected in each paddock immediately following deposition, as were ten each at monthly intervals for three months. The dry weight of each collected pat was determined and a mean value calculated for each paddock at each time point. The collection procedure was repeated with pats deposited in September, at which time the IVOMEC SR Bolus was no longer delivering ivermectin. The results from this

experiment (both July and September depositions, ivermectin-containing and ivermectin-free pats, respectively) show that weights of the pats decreased with time, and rate of decrease was not effected by treatment ($P > 0.05$). With respect to the second part of the study (initiated in the Spring of 1989), there were no significant ($P > 0.05$) differences among treatments for dung deposition rates, weight of dung collected at monthly intervals, or rate of decomposition/degradation of natural dung pats.

Another key component of the U.K. trial involved taking transects of fields, monitoring the development of grazing avoidance patches, and ascertaining whether the areas of the patches differed among treatment groups.[114] No significant differences ($P > 0.05$) were found among treatments for either year.

In summary, with pats deposited by cattle on pasture and allowed to degrade naturally under field conditions, the presence of ivermectin residues, even in feces from cattle which received an IVOMEC® SR Bolus, has no significant effect upon pat degradation. Delays in degradation of artificially formed pats from ivermectin-treated cattle have been reported. It appears that the methodology utilized in the study, in addition to abiotic and biotic factors, can influence the results of dung degradation studies. Based on the results of dung pat degradation/decomposition studies with ivermectin, where no effects were seen, eprinomectin will not affect dung pat degradation/ decomposition.

(4) Summary of hazard assessment for insects and dung pat degradation/decomposition

The above assessments demonstrate that there will be negligible risk to the terrestrial environment resulting from the use of eprinomectin as a topically applied endectocide on cattle. Eprinomectin will neither persist nor accumulate in terrestrial ecosystems. Although eprinomectin hydrolyzes very slowly in the dark, it should photodegrade rapidly on surfaces exposed to sunlight and it binds tightly to soil, where it degrades aerobically. Concentrations of eprinomectin in feces

will not affect adult dung beetles and will have a similar or shorter period of effect than other available avermectins on the emergence of dung beetle larvae. Hence, no effects on dung beetle populations are expected from the estimated use of IVOMEC EPRINEX Pour-On on cattle in a region because:

- Anthelmintic use is highly variable within a region and throughout the year.
- Not all eligible cattle would be treated with IVOMEC EPRINEX Pour-On.
- High usage rates would be expected to be scattered throughout a region; used by some, but not all, cattle managers.
- Most dung beetle species which are found on open pastures in the United States are not bovine dung specialists. Native, forest-dwelling species, which are adapted to use deer dung, do not generally feed on cattle dung and would therefore not be routinely exposed to eprinomectin residues.
- Usage of anthelmintics in pastured cattle in most regions does not coincide with peak periods of dung beetle reproduction.
- Although eprinomectin residues in dung may inhibit larval development, a high percentage of emergence can be expected from dung excreted by cattle at approximately one week post-dose.
- In regions where treatment and reproduction may be coincident, the percentage of animals treated is low and sufficient dung would be available for reproduction.
- Repopulation of areas with reduced populations is expected to occur because of density-dependent reproduction within the area and migration of highly mobile dung beetles into the area.

No dung-dependent insects are known to be listed or considered by government authorities as endangered or threatened. Blume listed 450 species of insects associated with bovine droppings on pasture. None is listed as endangered or threatened.

Dung-breeding and dung-feeding insects comprise only one of the factors involved in the decomposition and degradation of dung pats. It is very unlikely that any effects on these species will have a major impact upon dung pat degradation or dung dispersal. Dung beetles play, at most, only a minor role in the U.S. in degradation of cattle dung or its removal from pastures. Removal of dung from pastures in the U.S. is not an efficient process even during periods of high dung beetle activity. Bacteria, fungi, earthworms, weathering, trampling, action of birds and foraging animals and pasture management techniques all play very important roles in dung pat disappearance. The highest expected concentrations of eprinomectin-related residue in feces from cattle are well below those that would be expected to have an effect upon bacteria, fungi or earthworms. Since eprinomectin is not expected to affect the role of dung beetles or other biotic species including earthworms in dung dispersal or its removal from pastures in temperate climates and based upon the results of the field study, treatment of cattle with IVOMEC EPRINEX Pour-On would not be expected to inhibit dung pat degradation and thus not increase pasture fouling or cause loss of new growth because of smothering. Hence, no impact upon pastures would be caused by use of IVOMEC EPRINEX Pour-On.

(5) Hazard assessment for other terrestrial organisms

Given the low concentrations of eprinomectin expected in soil as a result of the use of IVOMEC EPRINEX Pour-On on cattle, the lack of buildup of drug residues in soil, the low toxicity of the compound relative to its expected concentrations in soil and excreta and the rapid decrease post-dose of residue levels in feces (dung pats), no deleterious effects are expected towards terrestrial organisms. For example, eprinomectin has no significant antimicrobial effects at concentrations as high as 1000 ppm, a value of almost 1.5 million-times greater than the initial concentration, 0.69 ppb, of eprinomectin expected in soil fertilized with residue-containing manure. Hence, the risk to microbes in the terrestrial ecosystem is remote. Likewise, the maximum concentration of eprinomectin residues in a field right after the spring application of manure in the second year, 1.1 ppb, is 268,000-times lower than the no-mortality level to earthworms, 295 ppm in dry soil, and 85,000-times lower than the lowest level tested, 90.8 ppm, where sublethal weight losses occurred. Even in dry dung pats, where maximum

concentrations of residues would be ~1200 - 4800 ppb (see Section 6.F.ii.), eprinomectin residues would be 61- to 246-fold below the no-mortality level to earthworms and 19- to 76-fold below the level where sublethal weight losses were observed. The initial concentration, 0.69 ppb, of eprinomectin expected in soil fertilized with residue-containing manure is over 680-fold lower than the lowest NOEC level for phytotoxicity towards terrestrial plants grown in sand, 0.47 ppm. Therefore, phytoxicity will not occur from the use of eprinomectin.

C. Hazard Assessment Summary

It is highly improbable that use of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle will have a detrimental effect on the environment.

- Eprinomectin is unlikely to move through the environment (low water solubility, tight binding to organic matter and especially soil).
- Eprinomectin degrades readily in the environment (photodegradation, aerobic breakdown by soil microorganisms).
- Eprinomectin is present at a very low concentration (maxima of 0.69 or 1.1 ppb for annual or semiannual application, respectively, with no accumulation or persistence) in soil fertilized with manure from treated cattle.
- At concentrations that will be present in soil fertilized with residue-containing manure, eprinomectin is not phytotoxic or toxic to aquatic ecosystems, plants, earthworms, fungi, bacteria or avians.
- Eprinomectin use is not expected to adversely affect populations of dung beetles or their dispersal of dung.
- Under study conditions when foraging-related mechanisms were prevented, residues of some macrocyclic lactones in dung pats slightly reduced the rate of dung degradation. However, grazing avoidance has not been reported in any field trials with cattle treated with macrocyclic lactones. Under conditions of use, eprinomectin is not expected to affect dung degradation or grazing avoidance.

9. Use of resources and energy consumption

The use of raw materials utilized to manufacture eprinomectin and IVOMEC EPRINEX Pour-On for Beef and Dairy cattle are in ample commercial supply.

No effects upon endangered or threatened species or upon historic property are anticipated.

10. Mitigation measures

The measures taken to avoid potential adverse environmental impacts associated with the manufacture of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle include proper disposal of Liquid and Solid Waste as described in Section 6 of this Environmental Assessment.

A statement similar to that following appears on the label to minimize the potential adverse impacts associated with the use and disposal of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle.

ENVIRONMENTAL SAFETY: Studies indicate that when eprinomectin comes in contact with the soil, it readily and tightly binds to the soil and becomes inactive. Free eprinomectin may adversely affect fish and certain aquatic organisms. Do not contaminate water by direct application or by the improper disposal of drug containers. Dispose of containers in an approved landfill or by incineration.

11. Alternatives to the proposed action

At this time there are no alternatives to chemotherapeutic agents for treatment and control of the important endo- and ectoparasites of cattle. IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle has two important attributes. It has a very broad spectrum and therefore obviates the need for multiple treatments with different agents; and it results in the release into the environment of negligible amounts of active ingredient and metabolites. From an environmental standpoint, IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle poses an environmental risk which is minimal compared to the alternatives.

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The undersigned officials certify that the information presented is true, accurate and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

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15. Appendices

APPENDIX A

Calculation of Eprinomectin-Related Residue Concentrations in Soil Resulting From Use of Cattle Manure as Fertilizer
(See Sec. 6.G.iv.)

Manure from Beef Cattle:

$$\frac{47 \text{ mcg residue}^a}{\text{kg manure}} \times \frac{1 \text{ kg}}{2.2 \text{ lb}} \times \frac{2000 \text{ lb}}{1 \text{ ton (U.S.)}} \times \frac{1 \text{ mg}}{1000 \text{ mcg}} = \frac{42.7 \text{ mg residue}}{\text{ton (U.S.) manure}}$$

$$\frac{42.7 \text{ mg residue}}{\text{ton manure}} \times \frac{15 \text{ tons manure}}{\text{acre}} \times \frac{1 \text{ acre}}{43{,}560 \text{ ft}^2} \times \frac{1000 \text{ mcg}}{\text{mg}} = \frac{14.7 \text{ mcg residue}}{\text{ft}^2}$$

$$1 \text{ ft}^2 \times 6\text{" deep} \times \frac{144 \text{ in}^2}{\text{ft}^2} \times \frac{16.4 \text{ cm}^3}{\text{in}^3} \times \frac{1.5 \text{ g}}{\text{cm}^3 \text{ soil}} = 21{,}254 \text{ g soil in 1 ft}^2 \times 6\text{" deep volume}$$

$$\frac{14.7 \text{ mcg residue}}{21{,}254 \text{ g soil}} \times \frac{1000 \text{ ng}}{\text{mcg}} = 0.69 \text{ ng/g (ppb) residue}$$

a "Worst-case" concentration based on one application per 130 days and 22 kg of manure (feces and urine) excreted per day from an animal weighing 270 kg upon entering a feedlot.

Manure from Dairy Cows:

$$\frac{27 \text{ mcg residue}}{\text{kg manure}}^{a} \times \frac{1 \text{ kg}}{2.2 \text{ lb}} \times \frac{2000 \text{ lb}}{1 \text{ ton (U.S.)}} \times \frac{1 \text{ mg}}{1000 \text{ mcg}} = \frac{24.5 \text{ mg residue}}{\text{ton (U.S.) manure}}$$

$$\frac{24.5 \text{ mg residue}}{\text{ton manure}} \times \frac{15 \text{ tons manure}}{\text{acre}} \times \frac{1 \text{ acre}}{43{,}560 \text{ ft}^2} \times \frac{1000 \text{ mcg}}{\text{mg}} = \frac{8.44 \text{ mcg residue}}{\text{ft}^2}$$

$$1 \text{ ft}^2 \text{ x } 6\text{" deep} \times \frac{144 \text{ in}^2}{\text{ft}^2} \times \frac{16.4 \text{ cm}^3}{\text{in}^3} \times \frac{1.5 \text{ g}}{\text{cm}^3 \text{ soil}} = 21{,}254 \text{ g soil in } 1 \text{ ft}^2 \text{ x } 6\text{" deep volume}$$

$$\frac{8.44 \text{ mcg residue}}{21{,}254 \text{ g soil}} \times \frac{1000 \text{ ng}}{\text{mcg}} = 0.40 \text{ ng/g (ppb) residue}$$

a "Worst-case" concentration based on two applications per year and 50 kg of manure (feces and urine) excreted per day from a 500-kg dairy cow.

APPENDIX B

Table of Contents

APPENDIX B-1

Material Safety Data Sheet for Eprinomectin Bulk Drug Substance

MATERIAL SAFETY DATA SHEET

PRODUCT NAME: EPRINOMECTIN **PAGE: 1 OF 9**
PLANT MSDS CODE: CH-132 **Date: 11/96**

1. Chemical Product and Company Identification

Manufacturer----------	**MERCK & CO. INC.** **One Merck Drive** **P.O. Box 100** **Whitehouse Station, NJ 08889-100** **1-908-423-1000**
Emergency Telephone Number------	**1-908-594-5555**
Label Name----------	Eprinomectin
Chemical Name----------	Component B_{1a}: (4"R)-4"-(acetylamino)-5-0-demethyl-4"-deoxyavermectin A_{1a}; Component B_{1b}: (4"R)-4"-(acetylamino)-5-0-demethyl-25-de(1-methylpropyl)-4"-deoxy-25-(1-methylethyl)avermectin A_{1a}
Synonyms----------	MK-0397; L-653,648 Component B_{1a}: 4"-epiacetamido-4"-deoxyavermectin B1a Component B_{1b}: 4"-epiacetylamino-4"-deoxyavermectin B_{1b} (4"-epiacetamido-4"-deoxyavermectin B_{1b})
Material Statistical Number-----	2-32395
Material Product Number---------	22814
Intended Use----------	Antiparasitic

2. Composition/Information on Ingredients

Component	Molecular Formula	Molecular Weight	CAS Number	Percent (%)
Eprinomectin (B_{1a})	$C_{50}H_{75}O_{14}N$	914	133305-88-1	90% or greater
(B_{1b})	$C_{49}H_{73}O_{14}N$	900	133305-89-2	10% or less

EC Label---------- Xn, R25, R48, N, R50

3. Hazards Identification

Appearance---------- White crystalline powder

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Emergency Overview----------	WARNING! Toxic if swallowed. Danger of serious damage to health by prolonged exposure. Very toxic to aquatic organisms.
Potential Health Effects--------	Toxic by ingestion. May cause nerve damage based on animal data. Overexposure may cause dilation of pupils, muscle tremor and incoordination.

4. First-Aid Measures

Eye Contact----------	Flush eyes with plenty of water for 15 minutes. Get medical attention if irritation develops.
Skin Contact----------	Wash with soap and water. Get medical attention if irritation develops.
Inhalation----------	If inhaled, remove to fresh air. Get medical attention if symptoms appear. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
Ingestion----------	If ingested, call a physician or Poison Control Center immediately. Drink one or two glasses of water and induce vomiting by gently touching the back of the throat with finger. Repeat until vomit fluid is clear. Do not induce vomiting or give anything by mouth to an unconscious person.
Note to Physicians----------	Toxicity following accidental ingestion can be minimized by inducing vomiting within one half hour of exposure. Since eprinomectin is believed to produce effects that mimic enhancement of GABA activity in animals, it is probably wise to avoid drugs that enhance GABA activity (barbiturates, benzodiazepines, valproic acid) in patients with potentially toxic eprinomectin exposure.

5. Fire-Fighting Measures

Flash Point (^{o}C/^{o}F)----------	Not applicable
Flash Point Test Method---------	Not applicable

*** Continued on next page: ***

Flammable Limits-LEL (%)----------	Not applicable
-UEL (%)----------	Not applicable
Autoignition Temperature (^{o}C/^{o}F)-------	Not available
Oxidizing Properties- ----------	Not available
Combustibility Information----------	Not available
Dust Explosivity Information----------	Not available
Shock Sensitivity----------	Not available
Fire/Explosion Hazards----------	Can form an explosive mixture with air in dusty conditions.
Extinguishing Media----------	Use carbon dioxide or dry chemical fire extinguishers.
Special Fire Fighting Procedures-------	Avermectins are very toxic to certain aquatic organisms. Contain all runoff water. See spill procedures section. All exposed personnel and equipment should be decontaminated at the site. Use full protective clothing and self-contained breathing apparatus.

Hazardous Decomposition Products Resulting From a Fire-- If involved in a fire, toxic gases (including CO, CO_2), toxic dust and smoke may be generated.

6. Accidental Release Measures

Personal Precautions----------	Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment. (Section 8) Follow all fire fighting procedures (Section 5).
Environmental Precautions-------	Eprinomectin is very toxic to certain aquatic species. Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills and fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer.
Methods for Cleaning Up---------	If emergency personnel are unavailable, vacuum or carefully scoop up spilled material and place in an appropriate container for disposal by incineration.

*** Continued on next page: ***

Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills or fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer. Residual surface material should be removed with towels moistened with methanol.

For additional assistance in the U.S., CHEMTREC provides a toll-free Hotline for chemical emergencies regarding spills, leaks, exposure or accidents: 1-800-424-9300.

7. Handling and Storage

Handling---------- Open handling must be limited to only very small quantities.

Storage---------- Store at less than 8°C (46.4°F) protected from light.

Other---------- Controlled access to the work area is strongly recommended. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Signs shall be posted indicating the compound in question and its associated hazards.

8. Exposure Controls/Personal Protection

Exposure Guidelines

Component	OSHA Permissible Exposure Limit	ACGIH Threshold Limit Value	Merck Exposure Control Limit
Eprinomectin	Not established	Not established	25 mcg/m3 (8hr-TWA)

Engineering Controls

Ventilation---------- Open handling must be limited. Local exhaust ventilation or use of a ventilated enclosure (e.g., fume hood) is required.

Personal Protective Equipment

Eye/Face Protection---------- Safety glasses are required as a minimum.

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Hand/Arm Protection----------	Latex gloves of equal or greater protection are required.
Respiratory Protection----------	Appropriate respiratory protection is recommended if there is the potential for overexposure to dust or aerosols.
Additional Protective Equipment-	Laboratory coat or work uniform is required. Disposable outer garments are required if there is the potential for contact with dust.

9. Physical and Chemical Properties

Appearance----------	White crystalline powder
Odor/Threshold Level (ppm)------	Not available
pH----------	7.3
Boiling Point/Range (^{o}C/^{o}F)-----	Not applicable
Melting Point/Range (^{o}C/^{o}F)-----	Not available
Solubility in water----------	Water solubility = 3.5 mg/L. Acetonitrile solubility est. greater than 300 mg/ml.
Partition Coefficient (Kow)-----	2.5 x 10E5
Specific Gravity (Water=1)------	1.23 g/cm^{3}
Vapor Density (Air=1)----------	4x10E-6 at 22.5^{o}C (72.5^{o}F)
Vapor Pressure (mmHG @ ^{o}C/^{o}F)---	Not available
Volatile Components (% w/w)-----	Not available

10. Stability and Reactivity

Stability----------	When stored under normal conditions (see Section 7) this product is expected to be stable for greater than 12 months. Any deterioration poses no safety concern.
Conditions to Avoid----------	Avoid temperatures greater than 8^{o}C (46.4^{o}F) and protect from light.
Incompatibilities----------	Can be hydrolyzed by strong caustic solution.
Hazardous Polymerizations-------	Not available
Hazardous Decomposition Products-	Not available

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11. Toxicological Information

Potential Route(s) of Entry------ Ingestion No
Inhalation Yes
Skin No

Toxicity Data

TEST	SPECIES	ROUTE	RESULTS
ALD50	Rat	Oral	55 mg/kg
ALD50	Mouse	Oral	70 mg/kg
Irritation	Rabbit	Ocular	Practically non-irritating
Skin Sensitization (Maximization)	Guinea Pig	Intradermal/ Dermal	Not a skin sensitizer

Effects of Acute Exposure

Eye Contact---------- Practically non-irritating in a primary ocular irritation study in rabbits.

Skin Contact---------- No irritation data available. Eprinomectin was negative in a skin sensitization study in guinea pigs.

Inhalation---------- No data available.

Ingestion---------- Toxic by ingestion. Overexposure may cause dilation of pupils, muscle tremor and incoordination.

Effects of Chronic Exposure----- Eprinomectin is an anti-parasitic compound for use in cattle. It inhibits transmission of nerve impulses in susceptible parasites, thereby causing paralysis and death.

In 3-month oral toxicity studies, eprinomectin produced neurotoxic effects including tremors (rats), ataxia, death, and mydriasis (dogs) and sciatic nerve degeneration (rats and dogs). Decreased body weight gain, organ weight changes and arrest of ovarian follicular maturation were also observed in rats. The no-effect levels for rats and dogs were 5 mg/kg/day and 0.8 mg/kg/day, respectively.

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In a 1-year oral toxicity study in dogs, degenerative changes in the brain were observed at 2 mg/kg/day (no-observed-effect level = 1 mg/kg/day).

In oral developmental toxicity studies in rabbits and rats, eprinomectin produced no evidence of developmental toxicity at doses up through 8 and 12 mg/kg/day, respectively. Treatment-related effects were noted in the maternal animals including slowed pupillary reflex and mydriasis (dilated pupils) unresponsive to light in rabbits (no-effect levels = 2 and 1.5 mg/kg/day in rabbits and rats respectively).

In an oral multigeneration reproduction study in rats, mortality, tremors, decreased body weight gain and decreased fertility were observed. The no-observed-effect level in the multigeneration study is 1.5 mg/kg/day for all of the above changes.

In a Secretion in Rat Mothers' Milk study, tremors were noted in pups whose mothers were given doses in excess of 0.9-1.5 mg/kg/day of eprinomectin. The overall milk/plasma ratio was approximately 3:1.

Eprinomectin was negative in a battery of genotoxicity assays.

Carcinogen Designation---------- Not listed as a carcinogen by OSHA, NTP, or IARC.

Medical Conditions Aggravated by Exposure-- Not available

12. Ecological Information

Environmental Fate---------- Eprinomectin is practically insoluble in water (3.5 mg/L) and highly hydrophobic based upon its octanol/water coefficient (Log Kow = 5.4). Avermectins are not biologically lipophilic. Bioaccumulation studies indicate that the avermectins have bioconcentration factors less than 100. It degrades rapidly in sunlight (t1/2=0.29 days in summer and 1.1 days in winter). The soil binding constant (Koc) is greater than or equal to 3000. Based upon the

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lowest K_d (adsorption distribution coefficient) derived experimentally, equal or greater than 98% of eprinomectin is expected to partition to soil in a 1:1 soil to water mixture. Therefore, it is not likely to be readily available to aquatic organisms. Approximately fifty percent of soil-bound eprinomectin is degraded aerobically in 64 days at 22°C. Due to its low vapor pressure and strong affinity for soil, eprinomectin is not expected to partition to air.

Environmental Effects---------- Eprinomectin is very toxic to certain aquatic organisms and toxic to other species.

LC50 Daphnia Magna, 48 hrs.	0.45 ppb (0.00045 mg/L)
EC50 Rainbow Trout, 96 hrs.	1.2 ppm (1.2 mg/L)
EC50 Bluegill Sunfish, 96 hrs	0.37 ppm (0.37 mg/L)

13. Disposal Considerations

Waste Disposal Information------ Eprinomectin is very toxic to certain aquatic species. Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills and fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer. Residual surface material should be removed with towels moistened with methanol.

Incinerate all spill materials and residues at temperatures greater than 600°C.

14. Transport Information

Shipping Description

U.S. DOT---------- Toxic solid, organic, N.O.S. (Eprinomectin), 6.1, UN2811, PG II

IATA/ICAO---------- Toxic solid, organic, N.O.S. (Eprinomectin), 6.1, UN2811, II

IMO---------- Toxic solid, organic, N.O.S. (Eprinomectin), Class 6.1, UN2811, P.G. II

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15. Regulatory Information

U.S. Federal Regulations-------	Not available
International Regulations-------	Not available
State Regulations----------	Not available

16. Other Information

Date Prepared----------	June 1996
Last Revision Date----------	Not applicable
MSDS Coordinator----------	1-908-423-7926

Disclaimer: While this information and recommendations set forth are believed to be accurate as of the date hereof, MERCK & CO, INC. makes no warranty with respect hereto and disclaims all liability from reliance thereon.

APPENDIX B-2

B-2 Material Safety Data Sheet for IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle (Haarlem, Holland Facility)

MATERIAL SAFETY DATA SHEET

PRODUCT NAME:	**IVOMEC® EPRINEX™ POUR-ON FOR BEEF AND DAIRY CATTLE**	**PAGE: 1 OF 10**
PLANT MSDS CODE:	**AGHO-037**	**Date: 11/96**

1. Chemical Product and Company Identification

Manufacturer---------- **MSD AGVET**
DIVISION OF MERCK SHARP & DOHME B.V.
WAARDERWEG 39
2031 BN HAARLEM
THE NETHERLANDS

Emergency Telephone Number **8:30 A.M. to 4:30 P.M. - 023 5 153 153**

National Poison Information Center:
24 hrs/day 7 days/wk - 030-2 748 888

Label Name---------- Ivomec® Eprinex™ Pour-On for Beef and Dairy Cattle

Chemical Name---------- Active ingredient:
Component B_{1a} (90% or greater): (4"R)-4"-(acetylamino)-5-0-demethyl-4"-deoxyavermectin A_{1a};
Component B_{1b} (10% or less): (4"R)-4"-(acetylamino)-5-0-demethyl-25-de(1-methylpropyl)-4"-deoxy-25-(1-methylethyl)avermectin A_{1a}

Synonyms (Common)---------- Eprinomectin Pour-On for Beef and Dairy Cattle

(Chemical)---------- Active ingredient:
Component B_{1a}: 4"-epiacetamido-4"-deoxyavermectin B1a
Component B_{1b}: 4"-epiacetylamino-4"-deoxyavermectin B_{1b} (4"-epiacetamido-4"-deoxyavermectin B_{1b})

Material Statistical Number----- Not applicable

Material Product Number-------- 30250 - 250 mL
30251 - 1 Litre
Squeeze-measure-pour bottles from high density polyethylene with polypropylene tamper-evident caps.

30252 - 2.5 L
30253 - 5.0 L
HDPE collapsible backpacks have tamper-evident high-density polyethylene caps.

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Intended Use---------- Topically applied (back-line) antiparasitic solution for the treatment and control of internal and external parasites of cattle. Applied from closed measuring systems: either squeeze/measure/ pour bottle or application gun drawing from a collapsible reservoir.

2. Composition/Information on Ingredients

Component	Molecular Formula	Molecular Weight	CAS Number	Percent (%)
Eprinomectin (B_{1a})	$C_{50}H_{75}O_{14}N$	914	133305-88-1)	0.5
(B_{1b})	$C_{49}H_{73}O_{14}N$	900	133305-89-2)	
Non-hazardous ingredients	Not avail.	Not avail.	Not available	99.5

EC Label---------- N, R50

3. Hazards Identification

Appearance---------- Clear, slightly yellow-colored solution

Emergency Overview---------- CAUTION!
Veterinary antiparasitic drug.
May be harmful if ingested.
Very toxic to aquatic organisms.
Avoid contact of spilled materials or runoff with soil or surface waterways.

Potential Health Effects-------- Accidental ingestion may cause tremors, dilated pupils and incoordination. Repeated or prolonged exposure may cause nerve damage based upon animal studies.

4. First-Aid Measures

Eye Contact---------- In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if irritation develops and persists.

Skin Contact---------- Wash with soap and water. Get medical attention if irritation develops.

Inhalation---------- If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if symptoms appear.

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Ingestion----------	If ingested, call a physician or Poison Control Center immediately. Drink one or two glasses of water and induce vomiting by gently touching the back of the throat with finger. Repeat until vomit fluid is clear. Do not induce vomiting or give anything by mouth to an unconscious person.
Note to Physicians----------	Toxicity following accidental ingestion can be minimized by inducing vomiting within one half hour of exposure. Since eprinomectin is believed to produce effects that mimic enhancement of GABA activity in animals, it is probably wise to avoid drugs that enhance GABA activity (barbiturates, benzodiazepines, valproic acid) in patients with potentially toxic eprinomectin exposure.

5. Fire-Fighting Measures

Flash Point (^{O}C/^{O}F)----------	220^{O}C (428^{O}F)
Flash Point Test Method---------	Not available
Flammable Limits-LEL (%)--------	Not available
-UEL (%)--------	Not available
Autoignition Temperature (^{O}C/^{O}F)-	Not available
Oxidizing Properties----------	Not available
Combustibility Information------	Not available
Dust Explosivity Information----	Not applicable
Shock Sensitivity----------	Not available
Fire/Explosion Hazards----------	Not available
Extinguishing Media----------	Use Carbon dioxide, foam or dry chemical.
Special Fire Fighting Procedures-	Avermectins are very toxic to certain aquatic organisms. Contain all runoff water. See spill procedures section. All exposed personnel and equipment should be decontaminated at the site. Use full protective clothing and self-contained breathing apparatus.

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Hazardous Decomposition Products Resulting From a Fire-- Carbon monoxide, carbon dioxide and oxides of nitrogen and sulfur may be released in a fire.

6. Accidental Release Measures

Personal Precautions---------- Emergency personnel involved in spill cleanup should wear full protective clothing (cap, waterproof coveralls and jacket, and rubber boots). Wear goggles and impervious rubber gloves (neoprene/ nitrile/polyvinyl chloride) when handling spilled material.

Environmental Precautions------- Eprinomectin is very toxic to certain aquatic species. Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills and fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer.

Methods for Cleaning Up--------- If emergency personnel are unavailable, absorb small spills on vermiculite or other suitable absorbing material and place in a sealed container for disposal. Dike large spills and transfer to an appropriate container for disposal. Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills or fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer. Residual surface material should be removed with towels moistened with methanol.

Incinerate all spill materials and residues at temperatures greater than 600^{o}C.

For additional assistance in the U.S., CHEMTREC provides a toll-free Hotline for chemical emergencies regarding spills, leaks, exposure or accidents: 1-800-424-9300.

7. Handling and Storage

Handling---------- Avoid direct contact with eyes and skin.

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Storing---------- Store bottle in carton to protect from light and avoid prolonged storage above 40°C (104°F).

Other---------- Keep this and all chemicals out of the reach of children.

8. Exposure Controls/Personal Protection

Exposure Guidelines

Component	OSHA Permissible Exposure Limit (PEL)	ACGIH Threshold Limit Value (TLV)	Merck Exposure Control Limit (ECL)
Eprinomectin	Not established	Not established	25 ug/m3 (8hr-TWA)

Engineering Controls

Ventilation---------- Not necessary for normal use. For manufacturing, local exhaust ventilation is recommended if aerosols are present.

Personal Protective Equipment

Eye/Face---------- Normal Use: None required.
Manufacturing: Safety glasses are recommended if there is a potential for direct eye contact.

Hand/Arm Protection---------- Normal Use: None required.
Manufacturing: Latex gloves or gloves of equal or greater protection are recommended.

Respiratory Protection---------- Normal Use: None required.
Manufacturing: Respiratory protection is recommended if the potential for exposure to aerosols exists.

Additional Protective Equipment- Appropriate clothing should be worn to avoid direct contact.

9. Physical and Chemical Properties

Appearance---------- Clear, slightly yellow-colored solution

Odor/Threshold Level (ppm)------ Practically odorless

pH---------- Not available

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Boiling Point/Range (^{O}C/^{O}F)-----	Not available
Melting Point/Range (^{O}C/^{O}F)-----	Not applicable
Solubility in water----------	Insoluble in water. Soluble in 90% alcohol.
Partition Coefficient (Kow)-----	Not available
Specific Gravity (Water=1)------	0.91-0.92
Vapor Density (Air=1)----------	Not available
Vapor Pressure (mmHG @ ^{O}C/^{O}F)---	Not available
Volatile Components (% w/w)-----	Not available

10. Stability and Reactivity

Stability----------	When stored under normal conditions this product is expected to be stable for 24 months. Any deterioration poses no safety concern.
Conditions to Avoid----------	Avoid prolonged exposure to excessive heat (above 40^{O}C) and direct sunlight. Store away from oxidizing materials.
Incompatibilities----------	Plastic packing materials such as polystyrene, low density polyethylene (high pressure)(LDPE), and PVC should not be used.
Hazardous Polymerizations-------	Will not occur.
Hazardous Decomposition Products-	If involved in a fire carbon monoxide, carbon dioxide and oxides of nitrogen and sulfur may be released.

11. Toxicological Information

Primary Route(s) of Entry-------	Inhalation: Unlikely with normal use Ingestion: Unlikely with normal use Skin Contact: Unlikely with normal use

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Toxicity Data

For formulation

TEST	SPECIES	ROUTE	RESULTS
LD50	Mouse	Oral	Greater than 5,000 mg/kg
Irritation	Rabbit	Ocular	Practically non-irritating
Skin Sensitization (Buehler)	Guinea Pig	Dermal/Dermal	Not a skin sensitizer
1-Month	Mini-swine	Dermal	Mildly irritating due to vehicle

For Eprinomectin

ALD50	Rat	Oral	55 mg/kg
ALD50	Mouse	Oral	70 mg/kg
Irritation	Rabbit	Ocular	Practically non-irritating
Skin Sensitization (Maximization)	Guinea Pig	Intradermal/ Dermal	Not a skin sensitizer

Effects of Acute Exposure

Eye Contact---------- The formulation was practically non-irritating to the eyes of rabbits without ocular wash and non-irritating when followed by ocular water wash.

Skin Contact---------- Both the vehicle and formulation were mildly irritating in a 1-month dermal study in miniswine. The formulation and active ingredient were negative in guinea pig skin sensitization assays.

Inhalation---------- No data available for the formulation or the active ingredient.

Ingestion---------- The formulation was practically non-toxic orally in mice (LD50 is greater than 5 g/kg). Eprinomectin was toxic by ingestion to mice and rats (LD50 is 55-70 mg/kg). Signs of toxicity included ataxia (incoordination), tremors and death.

Effects of Chronic Exposure--- No data available for the formulation. Eprinomectin is a second generation avermectin used as an anti-parasitic agent in cattle. It inhibits transmission of nerve impulses in susceptible parasites, thereby causing paralysis and death.

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In a 1-month dermal study in miniswine, both the vehicle and formulation were mildly irritating to the application site. No signs or histologic evidence of neurotoxicity were observed.

In 3-month oral toxicity studies, eprinomectin produced neurotoxic effects including tremors (rats), ataxia, death, mydriasis (dogs) and sciatic nerve degeneration (rats and dogs). Decreased body weight gain, organ weight changes and arrest of ovarian follicular maturation were also observed in rats. The no-effect levels for rats and dogs were 5 mg/kg/day and 0.8 mg/kg/day, respectively.

In a 1-year oral toxicity study in dogs, bile thickening and degenerative changes in the brain were observed at 2 mg/kg/day (no-effect level = 1 mg/kg/day).

In oral developmental toxicity studies in rabbits and rats, eprinomectin produced no evidence of developmental toxicity at doses up through 8 and 12 mg/kg/day, respectively. Treatment-related effects were noted in the maternal animals including slowed pupillary reflex and mydriasis (dilated pupils) unresponsive to light (no-effect levels = 2 and 1.5 mg/kg/day in rabbits and rats respectively).

In an oral multigeneration reproduction study in rats, mortality, tremors, decreased body weight gain and decreased fertility were observed. The no-observed-effect level in the multigeneration study is 1.5 mg/kg/day for all of the above changes.

In a Secretion in Rat Mothers Milk study, tremors were noted in pups whose mothers were given doses in excess of 0.9-1.5 mg/kg/day of eprinomectin. The overall milk/plasma ratio was approximately 3:1.

Eprinomectin was negative in a battery of genotoxicity assays.

*** Continued on next page ***

Carcinogen Designation---------- Not listed as a carcinogen by OSHA, NTP, or IARC. Carcinogenicity studies have not been conducted with eprinomectin.

Medical Conditions Aggravated by Exposure-- Not available

12. Ecological Information

Environmental Fate---------- Avermectins are not biologically lipophilic. Bioaccumulation studies indicate that the avermectins have bioconcentration factors less than 100. Eprinomectin is practically insoluble in water (3.5 mg/L) and highly hydrophobic based upon its octanol/water coefficient (Log Kow = 5.4). It degrades rapidly in sunlight (t1/2=0.29 days in summer and 1.1 days in winter). The soil binding constant (Koc) is greater than or equal to 3000. Based upon the lowest K_d (adsorption distribution coefficient) derived experimentally, equal or greater than 98% of eprinomectin is expected to partition to soil in a 1:1 soil to water mixture. Therefore, it is not likely to be readily available to aquatic organisms. Approximately fifty percent of soil-bound eprinomectin is degraded aerobically in 64 days at 22^{o}C. Due to its low vapor pressure and strong affinity for soil, eprinomectin is not expected to partition to air.

Environmental Effects---------- Eprinomectin is very toxic to certain aquatic organisms and toxic to other species.

For Pure Eprinomectin

LC50 Daphnia Magna, 48 hrs.	0.45 ppb	(0.00045 mg/L)
EC50 Rainbow Trout, 96 hrs.	1.2 ppm	(1.2 mg/L)
EC50 Bluegill Sunfish, 96 hrs.	0.37 ppm	(0.37 mg/L)

13. Disposal Considerations

Waste Disposal Information------ Eprinomectin is very toxic to certain aquatic species. Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills and fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer.

*** Continued on next page ***

Residual surface material should be removed with towels moistened with methanol.

Incinerate all spill materials and residues at temperatures greater than 600^{o}C.

14. Transport Information

Shipping Description	
U.S. DOT----------	Not regulated
IATA/ICAO----------	Not regulated
IMO----------	Not regulated
ADR-RID----------	Not available

15. Regulatory Information

U.S. Federal Regulations--------	Not available
International Regulations-------	Not available
State Regulations----------	Not available

16. Other Information

Date Prepared----------	June 1996
Last Revision Date----------	November 1996
MSDS Coordinator----------	1-908-423-7926 Merck & Co, Inc. One Merck Drive P.O. Box 100, WS2F-48 Whitehouse Station, NJ 08889-0100 U.S.A.

Disclaimer: While this information and recommendations set forth are believed to be accurate as of the date hereof, MERCK & CO, INC. makes no warranty with respect hereto and disclaims all liability from reliance thereon.

APPENIDX B-3

B-3 Material Safety Data Sheet for IVOMEC® EPRINEX™ Pour-On for Beef and Dairy Cattle (Barceloneta, Puerto Rico Facility)

MATERIAL SAFETY DATA SHEET

PRODUCT NAME: **IVOMEC® EPRINEX™ POUR-ON FOR BEEF AND DAIRY CATTLE** **PAGE: 1 OF 10**
PLANT MSDS CODE: **PR-068** **Date: 11/96**

1. Chemical Product and Company Identification

Manufacturer---------- **MERCK SHARP & DOHME**
QUIMICA DE PUERTO RICO, INC.
P.O. BOX 601
BARCELONETA, PUERTO RICO; 00617

Emergency Telephone Number----- **(809) 846-3620 (P.R.)**
1-908-594-5555 (U.S.)

Label Name---------- Ivomec® Eprinex™ Pour-On for Beef and Dairy Cattle

Chemical Name---------- Active ingredient:
Component B_{1a} (90% or greater): (4"R)-4"-(acetylamino)-5-0-demethyl-4"-deoxyavermectin A_{1a};
Component B_{1b} (10% or less): (4"R)-4"-(acetylamino)-5-0-demethyl-25-de(1-methylpropyl)-4"-deoxy-25-(1-methylethyl)avermectin A_{1a}

Synonyms (Common)---------- Eprinomectin 0.5% Pour-On for Cattle
(Chemical)---------- Active ingredient:
Component B_{1a}: 4"-epiacetamido-4"-deoxyavermectin B1a
Component B_{1b}: 4"-epiacetylamino-4"-deoxyavermectin B_{1b} (4"-epiacetamido-4"-deoxyavermectin B_{1b})

Material Statistical Number----- Not applicable

Material Product Number--------- 30250 - 250 mL Squeeze-measure-pour
30251 - 1 Litre bottles from high density polyethylene with polypropylene tamper-evident caps.

30252 - 2.5 L HDPE collapsible
30253 - 5.0 L backpacks have tamper-evident high-density polyethylene caps.

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Intended Use---------- Topically applied (back-line) antiparasitic solution for the treatment and control of internal and external parasites of cattle. Applied from closed measuring systems: either squeeze/measure/ pour bottle or application gun drawing from a collapsible reservoir.

2. Composition/Information on Ingredients

Component	Molecular Formula	Molecular Weight	CAS Number	Percent (%)
Eprinomectin (B_{1a})	$C_{50}H_{75}O_{14}N$	914	133305-88-1)	0.5
(B_{1b})	$C_{49}H_{73}O_{14}N$	900	133305-89-2)	
Non-hazardous ingredients	Not avail.	Not avail.	Not available	99.5

EC Label---------- N, R50

3. Hazards Identification

Appearance---------- Clear, slightly yellow-colored solution

Emergency Overview---------- CAUTION!
Veterinary antiparasitic drug.
May be harmful if ingested.
Very toxic to aquatic organisms.
Avoid contact of spilled materials or runoff with soil or surface waterways.

Potential Health Effects-------- Accidental ingestion may cause tremors, dilated pupils and incoordination. Repeated or prolonged exposure may cause nerve damage based upon animal studies.

4. First-Aid Measures

Eye Contact---------- In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if irritation develops and persists.

Skin Contact---------- Wash with soap and water. Get medical attention if irritation develops.

Inhalation---------- If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if symptoms appear.

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Ingestion----------	If ingested, call a physician or Poison Control Center immediately. Drink one or two glasses of water and induce vomiting by gently touching the back of the throat with finger. Repeat until vomit fluid is clear. Do not induce vomiting or give anything by mouth to an unconscious person.
Note to Physicians----------	Toxicity following accidental ingestion can be minimized by inducing vomiting within one half hour of exposure. Since eprinomectin is believed to produce effects that mimic enhancement of GABA activity in animals, it is probably wise to avoid drugs that enhance GABA activity (barbiturates, benzodiazepines, valproic acid) in patients with potentially toxic eprinomectin exposure.

5. Fire-Fighting Measures

Flash Point (^{O}C/^{O}F)----------	220^{O}C (428^{O}F)
Flash Point Test Method---------	Not available
Flammable Limits-LEL (%)--------	Not available
-UEL (%)--------	Not available
Autoignition Temperature (^{O}C/^{O}F)-	Not available
Oxidizing Properties----------	Not available
Combustibility Information------	Not available
Dust Explosivity Information----	Not applicable
Shock Sensitivity----------	Not available
Fire/Explosion Hazards----------	Not available
Extinguishing Media----------	Use Carbon dioxide, foam or dry chemical.
Special Fire Fighting Procedures-	Avermectins are very toxic to certain aquatic organisms. Contain all runoff water. See spill procedures section. All exposed personnel and equipment should be decontaminated at the site. Use full protective clothing and self-contained breathing apparatus.

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Hazardous Decomposition Products Resulting From a Fire-- Carbon monoxide, carbon dioxide and oxides of nitrogen and sulfur may be released in a fire.

6. Accidental Release Measures

Personal Precautions---------- Emergency personnel involved in spill cleanup should wear full protective clothing (cap, waterproof coveralls and jacket, and rubber boots). Wear goggles and impervious rubber gloves (neoprene/ nitrile/polyvinyl chloride) when handling spilled material.

Environmental Precautions------- Eprinomectin is very toxic to certain aquatic species. Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills and fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer.

Methods for Cleaning Up--------- If emergency personnel are unavailable, absorb small spills on vermiculite or other suitable absorbing material and place in a sealed container for disposal. Dike large spills and transfer to an appropriate container for disposal. Avoid contact of spilled material with soil. Do not allow any water potentially contaminated with eprinomectin including storm water, runoff from spills or fire fighting activities and contaminated wastewater to enter any waterway, drain or sewer. Residual surface material should be removed with towels moistened with methanol.

Incinerate all spill materials and residues at temperatures greater than 600^{o}C.

For additional assistance in the U.S., CHEMTREC provides a toll-free Hotline for chemical emergencies regarding spills, leaks, exposure or accidents: 1-800-424-9300.

7. Handling and Storage

Handling---------- Avoid direct contact with eyes and skin.

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Storing---------- Store bottle in carton to protect from light and avoid prolonged storage above 40°C (104°F).

Other---------- Keep this and all chemicals out of the reach of children.

8. Exposure Controls/Personal Protection

Exposure Guidelines

Component	OSHA Permissible Exposure Limit (PEL)	ACGIH Threshold Limit Value (TLV)	Merck Exposure Control Limit (ECL)
Eprinomectin	Not established	Not established	25 ug/m3 (8hr-TWA)

Engineering Controls

Ventilation---------- Not necessary for normal use. For manufacturing, local exhaust ventilation is recommended if aerosols are present.

Personal Protective Equipment

Eye/Face---------- Normal Use: None required.
Manufacturing: Safety glasses are recommended if there is a potential for direct eye contact.

Hand/Arm Protection---------- Normal Use: None required.
Manufacturing: Latex gloves or gloves of equal or greater protection are recommended.

Respiratory Protection---------- Normal Use: None required.
Manufacturing: Respiratory protection is recommended if the potential for exposure to aerosols exists.

Additional Protective Equipment- Appropriate clothing should be worn to avoid direct contact.

9. Physical and Chemical Properties

Appearance---------- Clear, slightly yellow-colored solution

Odor/Threshold Level (ppm)------ Practically odorless

pH---------- Not available

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Boiling Point/Range (^{O}C/^{O}F)-----	Not available
Melting Point/Range (^{O}C/^{O}F)-----	Not applicable
Solubility in water----------	Insoluble in water. Soluble in 90% alcohol.
Partition Coefficient (Kow)-----	Not available
Specific Gravity (Water=1)------	0.91-0.92
Vapor Density (Air=1)----------	Not available
Vapor Pressure (mmHG @ ^{O}C/^{O}F)---	Not available
Volatile Components (% w/w)-----	Not available

10. Stability and Reactivity

Stability----------	When stored under normal conditions this product is expected to be stable for 24 months. Any deterioration poses no safety concern.
Conditions to Avoid----------	Avoid prolonged exposure to excessive heat (above 40^{O}C) and direct sunlight. Store away from oxidizing materials.
Incompatibilities----------	Plastic packing materials such as polystyrene, low density polyethylene (high pressure)(LDPE), and PVC should not be used.
Hazardous Polymerizations-------	Will not occur.
Hazardous Decomposition Products-	If involved in a fire carbon monoxide, carbon dioxide and oxides of nitrogen and sulfur may be released.

11. Toxicological Information

Primary Route(s) of Entry-------	Inhalation:	Unlikely with normal use
	Ingestion:	Unlikely with normal use
	Skin Contact:	Unlikely with normal use

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Toxicity Data

For formulation

TEST	SPECIES	ROUTE	RESULTS
LD50	Mouse	Oral	Greater than 5,000 mg/kg
Irritation	Rabbit	Ocular	Practically non-irritating
Skin Sensitization (Buehler)	Guinea Pig	Dermal/Dermal	Not a skin sensitizer
1-Month	Mini-swine	Dermal	Mildly irritating due to vehicle

For Eprinomectin

ALD50	Rat	Oral	55 mg/kg
ALD50	Mouse	Oral	70 mg/kg
Irritation	Rabbit	Ocular	Practically non-irritating
Skin Sensitization (Maximization)	Guinea Pig	Intradermal/ Dermal	Not a skin sensitizer

Effects of Acute Exposure

Eye Contact---------- The formulation was practically non-irritating to the eyes of rabbits without ocular wash and non-irritating when followed by ocular water wash.

Skin Contact---------- Both the vehicle and formulation were mildly irritating in a 1-month dermal study in miniswine. The formulation and active ingredient were negative in guinea pig skin sensitization assays.

Inhalation---------- No data available for the formulation or the active ingredient.

Ingestion---------- The formulation was practically non-toxic orally in mice (LD50 is greater than 5 g/kg). Eprinomectin was toxic by ingestion to mice and rats (LD50 is 55-70 mg/kg). Signs of toxicity included ataxia (incoordination), tremors and death.

Effects of Chronic Exposure------ No data available for the formulation. Eprinomectin is a second generation avermectin used as an anti-parasitic agent in cattle. It inhibits transmission of nerve impulses in susceptible parasites, thereby causing paralysis and death.

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In a 1-month dermal study in miniswine, both the vehicle and formulation were mildly irritating to the application site. No signs or histologic evidence of neurotoxicity were observed.

In 3-month oral toxicity studies, eprinomectin produced neurotoxic effects including tremors (rats), ataxia, death, mydriasis (dogs) and sciatic nerve degeneration (rats and dogs). Decreased body weight gain, organ weight changes and arrest of ovarian follicular maturation were also observed in rats. The no-effect levels for rats and dogs were 5 mg/kg/day and 0.8 mg/kg/day, respectively.

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Medical Conditions Aggravated by Exposure-- Not available

12. Ecological Information

Environmental Fate---------- Avermectins are not biologically lipophilic. Bioaccumulation studies indicate that the avermectins have bioconcentration factors less than 100. Eprinomectin is practically insoluble in water (3.5 mg/L) and highly hydrophobic based upon its octanol/water coefficient (Log Kow = 5.4). It degrades rapidly in sunlight (t1/2=0.29 days in summer and 1.1 days in winter). The soil binding constant (Koc) is greater than or equal to 3000. Based upon the lowest K_d (adsorption distribution coefficient) derived experimentally, equal or greater than 98% of eprinomectin is expected to partition to soil in a 1:1 soil to water mixture. Therefore, it is not likely to be readily available to aquatic organisms. Approximately fifty percent of soil-bound eprinomectin is degraded aerobically in 64 days at 22^{o}C. Due to its low vapor pressure and strong affinity for soil, eprinomectin is not expected to partition to air.

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13. Disposal Considerations

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any waterway, drain or sewer. Residual surface material should be removed with towels moistened with methanol.

Incinerate all spill materials and residues at temperatures greater than 600^{o}C.

14. Transport Information

Shipping Description	
U.S. DOT----------	Not regulated
IATA/ICAO----------	Not regulated
IMO----------	Not regulated
ADR-RID----------	Not available

15. Regulatory Information

U.S. Federal Regulations--------	Not available
International Regulations-------	Not available
State Regulations----------	Not available

16. Other Information

Date Prepared----------	June 1996
Last Revision Date----------	November 1996
MSDS Coordinator----------	1-908-423-7926 Merck & Co, Inc. One Merck Drive P.O. Box 100, WS2F-48 Whitehouse Station, NJ 08889-0100 U.S.A.

Disclaimer: While this information and recommendations set forth are believed to be accurate as of the date hereof, MERCK & CO, INC. makes no warranty with respect hereto and disclaims all liability from reliance thereon.

APPENDIX C

Summaries of Fate Studies

C-1 McCauley, J. A., 1993. Determination of Physical Properties of L-653,648-000X.

C-2 Venkataraman, K. and N. I. Narasimhan, 1993. The Sorption and Desorption of 4"-Epiacetylamino-4"-Deoxyavermectin B_1 (L-653,648) with Soils (AEDM-81).

C-3 Green-Erwin, M., K. Venkataraman and N. I. Narasimhan, 1994. Depletion of Radioresidues in Tissues of Cattle Dosed Topically With a Single Dose of Radiolabeled MK-0397 (Trial CA-368).

C-4 Venkataraman, K. and N. I. Narasimhan, 1995. Metabolism of [^{3}H]-MK-0397 in Cattle Following a Topical Application (ADMES-3).

C-5 Batty, A. F. and D. Barth, 1995. MK-0397/Topical/Cattle/Safety/ Environmental Safety/Residue/Dung Residue Depletion/Dung Residue Disappearance.

C-6 Venkataraman, K. and N. I. Narasimhan, 1993. Photodegradation of 4"-Epiacetylamino-4"-Deoxyavermectin B_{1a} (L-653,648) in Aqueous Solution Under Sunlight (AEDM-75).

C-7 Yan, Z., 1995. Aerobic Biodegradation in Soil with ^{14}C-MK-0397 (^{14}C-L-653,648).

C-8 Venkataraman, K and N. I. Narasimhan, 1995. The Hydrolytic Stability of 4"-Epiacetylamino-4"-Deoxyavermectin B_1 (MK-0397) (AEDM80).

APPENDIX C-1

McCauley, J. A., 1993. Determination of Physical Properties of L-653,648-000X.

The objective was to determine the density, octanol/water partition coefficient, water solubility, melting point, sublimation (vapor) pressure, dissociation constant (pKa) and ultraviolet absorbance of eprinomectin (L-653,648). The lot of eprinomectin used for these studies was L-653,648-000X016. The experimental methodologies were based on generally accepted scientific principles with particular references to the FDA Environmental Assessment Technical Assistance Handbook (1987) and the Official Journal of the European Communities (Vol. 27, 1984).

The density, 1.23 ± 0.04 g/cm3, was determined in triplicate by the solvent displacement method. Water was used to calibrate the volume of the pynchnometers and hexane was used as the displacement solvent. Room temperature averaged 22.0 ± 0.2°C during the experiment.

The partition (distribution) coefficient between octanol and pH 6.8 phosphate buffer was determined by the shake-flask method. The average Log P was 5.4 ± 0.3 based on nine determinations. The octanol and buffer were preequilibrated with each other. Aliquots from a stock solution of eprinomectin in octanol were added to various volumes of octanol and buffer and stirred by magnetic stirrers or on a rotating stirrer for approximately two hours. The samples were then centrifuged and the layers separated. The concentration of eprinomectin was determined in each layer by HPLC analysis utilizing a standard HPLC-UV area versus concentration curve.

The water solubility in unbuffered water at pH 7.26 ± 0.09 was determined to be 0.0035 ± 0.0002 mg/mL (3.5 ± 0.2 ppm) at 25.0°C. Three quantities of eprinomectin were weighed and added to glass ampoules or tubes. A measured volume of filtered water was added to each tube and the tubes were sealed. One tube was placed into a 30.2° water bath for 5 hours. The three tubes were then placed into a 25.0°C water bath. On successive days, a single tube was removed from the bath, the solution was filtered through a 0.22 micron filter and the filtrate was analyzed for eprinomectin by UV absorption. The tube preconditioned at 30.2° was analyzed on day 2.

The melting point of 163-166°C (2°C/min N_2) was determined by differential scanning calorimetry (DSC). The DSC experiments disclosed a complex thermal behavior which was dependent on heating rate and atmosphere. Under nitrogen, the melting endotherm showed a dependence upon heating rate indicative of some thermal decomposition accompanying the melting. The DSC instrument was calibrated using Millipore water, indium and tin at three heating rates.

The sublimation (vapor) pressure was determined to be 4±1 x 10^{-6} torr at 22.5 ± 0.9°C by the gas saturation method. Nitrogen gas, dried over molecular sieves, was passed over the test compound at a measured constant flow rate. The carrier gas then flowed through a C8 adsorbent for a measured time. The adsorbent was then extracted with methanol and the quantity of desorbed eprinomectin was determined by HPLC. The adsorption efficiency of the adsorbent and the subsequent recovery efficiency were also determined. The sublimation (vapor) pressure was calculated from an equation which includes the weight of compound adsorbed, the gas flow rate, the time of flow, the temperature, a gas constant and the molecular weight of the compound. The results from three experiments were averaged.

The dissociation constant was determined by potentiometric titration of eprinomectin in 50% aqueous methanol with standardized solutions of sodium hydroxide and hydrochloric acid. No dissociation constant (pKa) was found between 3 and 10, consistent with the molecular structure of eprinomectin.

The ultraviolet absorption spectrum was determined in 50% acetonitrile in water and is characterized by a maximum absorbance at 244 nm and an A1% 1cm of 343. Duplicate determinations were performed using a calibrated spectrophotometer.

APPENDIX C-2

Venkataraman, K. and N. I. Narasimhan, 1993. The Sorption and Desorption of 4"-Epiacetylamino-4"-Deoxyavermectin B_1 (L-653,648) with Soils (AEDM-81).

The objective was to measure soil sorption/desorption of eprinomectin. Three soils from the U.S.A. were used: Iowa (IA) loam, California (CA) loam/sandy loam and Missouri (MO) clay loam. Methods in the Environmental Assessment Technical Assistance Handbook, Food and Drug Administration, Washington, D. C., March 1987, Technical Assistance Document 3.08 Sorption and Desorption were followed. Wherever appropriate, the methods were modified to accommodate OECD guidelines for testing of chemicals, 106 Adsorption/Desorption, dated 05/12/81.

[^{3}H]eprinomectin solutions were prepared in ethanol from non-radiolabeled L-653,648-000X016 and the tritium-labeled major component (B_{1a}) of eprinomectin. Samples containing 1.0 g soil, 5.0 g 0.01 M $CaCl_2$ solution, and a 50 mcL aliquot of [^{3}H]eprinomectin were protected from light, mixed for 22 hours, then centrifuged. Concentrations of in test solutions were 2.03, 0.40, 0.08 and 0.02 mcg/mL. Eprinomectin in solution after equilibration was quantitated by scintillation counting of the aqueous layer, and bound eprinomectin was quantitated by determining the difference between added and aqueous radioactivity. Soil samples were desorbed three times by replacing the equilibrated solution with fresh $CaCl_2$ solution. Mass balance was determined after the third desorption step at the 2.03 mcg/mL level by extracting all three soils with methanol and summing radioactivity in aqueous and methanol extracts. Recovery of radioactivity was quantitative for all three soils, indicating eprinomectin did not significantly bind to glass in the presence of soil. The methanolic extracts were also analyzed chromatographically; eprinomectin did not undergo any degradation during the soil binding study.

Equilibration was achieved in all soils within two hours of mixing. Total mass balance data showed eprinomectin does not bind significantly to glass in the presence of soil. The binding of eprinomectin to soil is quite strong and somewhat irreversible. Approximately 86.8% to 94.2% of the eprinomectin was bound in the sorption step and only 6.7% to 16.8% was desorbed in subsequent desorptions. The distribution coefficients (K_d) for sorption and desorption between bound and solubilized eprinomectin were determined to be 88.2, 53.1 and 133.5 for the IA, CA and MO soils, respectively. The coefficients for binding to organic carbon (K_{OC}) determined from sorption and desorption data ranged from 3231 to 9208. The FDA Technical Assistance Document 3.08, "Sorption/Desorption" states that "compounds having a K_{OC} value of around 1,000 are quite tightly bound to organic matter in soil and are

considered to be immobile." Eprinomectin would therefore be classified as immobile in these soils and highly unlikely to leach out of soil. Although K_d values and percent soil organic carbon did not correlate well, a good correlation existed between the K_d values and the percent clay, percent silt and percent sand in the soils.

The adsorption-desorption data were fitted to the Freundlich equation. For all the soils the value of n was very close to 1.0 and the Freundlich constant K was approximately equal to the equilibrium constant K_d.

Eprinomectin will be strongly bound to soils and will not leach out into surface or ground waters.

APPENDIX C-3

Green-Erwin, M., K. Venkataraman and N. I. Narasimhan, 1994. Depletion of Radioresidues in Tissues of Cattle Dosed Topically With a Single Dose of Radiolabeled MK-0397 (Trial CA-368).

Twelve commercial breed (Angus and Hereford) beef cattle of less than one year of age (body weight range 274 - 336 kg), six steers and six heifers, were dosed topically with [5-^{3}H]-eprinomectin (MK-0397) in Miglyol 840 / 0.01% BHT formulation. Both components of eprinomectin, the major component (AAB_{1a}) and the minor component (AAB_{1b}), were present in the formulation and both were radiolabeled. The dose was applied topically at the rate of 500 mcg/kg (1.0 mL of a 0.5% formulation for every 10 kg weight of the animal), which is 1.0 x the proposed use rate. Three cattle were sacrificed at each time point: 7, 14, 21 and 28 days post dose. Two control animals, a steer and a heifer, were sacrificed 7 days after the other animals were dosed. Total radioactive residue levels and the concentration of the AAB_{1a} component of the parent drug in plasma were determined by scintillation counting and reverse-phase HPLC, respectively. Peak radioresidue levels in plasma were in the range of 4.35 - 21.10 ppb. The peak concentrations of AAB_{1a} in plasma were in the range of 7.33 - 19.74 ng/mL. Urine and feces were collected from the two steers assigned to the 28 day sacrifice group. Only a small percent of the applied dose, 0.35%, was found in the urine through 28 days. A larger percent of the applied radiolabeled dose, 14.3%, was excreted in the feces in the same time period. Analysis of the hide samples from the three Day-28 sacrifice group cattle revealed that 54.0% of the initially applied radiolabeled dose remained on the hide. RP-HPLC of methanolic extracts of hide samples indicated that eprinomectin was not significantly metabolized or degraded in hide and it represented 89% of the total extractable radioactivity. Several degradates were observed in the hide extracts and all the degradates collectively accounted for the remaining 11% of the extractable radioactivity.

The total radioresidue levels and the marker residue levels in tissues were determined by scintillation spectrometry and reverse-phase HPLC, respectively. The major component of eprinomectin, AAB_{1a}, was identified as the marker residue since AAB1a depletes at the same rate as that of the total radioresidues and is the major residue in all the tissues. The radioresidue levels and the marker residue levels in tissues followed the order: liver > kidney > fat > dose site muscle > muscle. The average radioresidue levels in liver, kidney, fat, dose site muscle and muscle were 977, 181, 34, 24 and 8 ppb (or ng/g), respectively on day 7. In the same tissues, the average marker residue levels were 807, 161, 30, 17 and 6 ppb, respectively. Both the radioresidue and the marker residue levels in liver were approximately two orders of magnitude higher than the corresponding levels in muscle.

Radioresidue levels in tissues were highest in the day-7 treatment group animals and continued to decrease with time in the other groups. The total residue levels and the marker residue levels in muscle were less than 10 ppb and 8 ppb, respectively in all cattle from day 7 onwards.

Feces samples were collected daily until day 14 and then on day 21 and day 28. The peak radioresidue levels in feces were 215.3 ppb (day 9) for steer #6239 and 137.3 ppb (Day 12) for steer #6251. Overall 19.35% and 15.60% of the administered dose was excreted in feces of steers #6239 and #6251, respectively. Excreta was collected as a mixture of feces and urine from heifer #6244, and 17.97% of the dose was eliminated in the excreta. The radioresidue levels in urine were less than 4.4 ppb for steer #6239 and less than 8.7 ppb for steer #6251 throughout the study. Overall in both steers, only 0.45% of the administered dose was excreted in urine.

In all tissues except dose site muscle, the depletion rate constants for the total radioresidues were approximately 0.086 $days^{-1}$ indicating that the residues in these tissues were depleting in parallel. Indeed, the half-life for the depletion of radioresidues were 8.6, 8.1, 7.9 and 7.8 days, respectively in liver, kidney, fat and muscle. Due to large inter-animal variation, the dose site muscle had a longer depletion half-life of 36.1 days. The lack of good fit in the depletion data of the dose site muscle makes this value of 36.1 days suspect. The half-lives for the depletion of AAB_{1a} in liver, kidney, fat and muscle were 9.6, 7.5, 8.1 and 8.5 days, respectively. In dose-site-muscle, the half-life for the depletion of marker residue was 29.4 days but the lack of fit in the dose-site-muscle data shows that 29.4 days may not be a true measure of the half-life in this tissue. The depletion rates and the half-lives for the marker residue were very nearly the same as that of the radioresidues, suggesting that the marker residue depletes in parallel with the total residues in all the tissues from day 7 through day 28. Since the radioresidues in liver were the highest among tissues and they deplete nearly at the same rate in other tissues, liver is proposed as the target tissue. The rates of depletion of AAB_{1a} levels in the tissues are very nearly the same as that of the radioresidues in the corresponding tissues and AAB_{1a} is being proposed as the marker residue in all the tissues.

APPENDIX C-4

Venkataraman, K. and N. I. Narasimhan, 1995. Metabolism of [^{3}H]-MK-0397 in Cattle Following a Topical Application (ADMES-3).

Twelve cross-bred beef cattle of less than one year of age (body weight range 274 - 336 kg), six steers and six heifers, were dosed topically with [5-^{3}H]-eprinomectin (MK-0397) in Miglyol 840 / 0.01% BHT formulation in Study CA-368 and provided biological samples for this study. Both components of eprinomectin, the major component (AAB_{1a}) and the minor component (AAB_{1b}), were present in the formulation and both were radiolabeled. The dose was applied topically at the rate of 500 mcg/kg (1.0 mL of a 0.5% formulation for every 10 kg weight of the animal), which is 1.0 x the proposed use rate. Three cattle were sacrificed at each time point: 7, 14, 21 and 28 days post dose. Two control animals, a steer and a heifer, were sacrificed 7 days after the other animals were dosed. Blood was collected prior to dosing and every twelve hours post-dosing till day 14 and daily thereafter from all available animals. Liver, kidneys, fat, muscle and dose-site muscle were collected from all the animals upon sacrifice. Urine and feces were collected daily from the two steers assigned to the day-28 withdrawal group.

Urine, plasma, and aqueous homogenates of liver, kidney and feces samples from the two steers in the day-28 group were lyophilized and the radioactivity in the aqueous sublimates (tritiated water) was determined by scintillation spectrometry. The tritiated water levels were less than 1 ppb in all tissues and the maximum amount of loss of tritium label was estimated to be less than 0.5% of the initially applied topical dose. The tritium label at the 5-position of eprinomectin was demonstrated to be stable and suitable for use in radioresidue studies.

Day-3 a.m. and day-7 a.m. plasma samples from all the cattle were pooled by sex and the composite plasma samples were analyzed to obtain metabolite profiles. The metabolite profiles in plasma indicate that there were no differences in the metabolism due to the time of blood collection or due to gender. In plasma, eprinomectin amounted to 94.8% of the total residues. AAB_{1a} is the most abundant residue in plasma and accounted for 87.4% of the total radioresidues in the plasma.

The liver, kidney, fat, muscle and dose-site muscle tissues were solvent extracted and the extracts were processed using aminopropyl solid phase extraction cartridges. The drug and related residues were quantitatively eluted by organic solvents and the eluates were evaporated to dryness. The residues were reconstituted in methanol/water and analyzed by reversed phase HPLC. The metabolite profile in any given tissue was independent of

the gender of theanimal and of the day of collection of the sample. The metabolite profiles were qualitatively and quantitatively similar in liver, kidney, fat, muscle and dose-site muscle. In most of the tissues there were five to seven metabolites; most of them very minor and contributed in the range of 1 - 2% of the total radioresidues. The only exception was in muscle in which metabolite M5 was 3.9% of the total radioresidues.

AAB_{1a} was the most abundant radioresidue and, on average in the samples analyzed, accounted for 86.4%, 86.2%, 86.7%, 82.0%, 83.3%, 87.4% and 78.3% of the total radioresidues in liver, kidney, fat, muscle, dose-site muscle, plasma and feces, respectively. Eprinomectin was metabolized only to a very small extent and accounted for 94.8%, 94.5%, 93.9%, 89.9%, 91.2%, 94.8% and 85.9% of the total radioresidues in liver, kidney, fat, muscle, dose-site muscle, plasma and feces, respectively.

Composite feces samples (days 1 through 14 post dose) from the day-28 group steers and other selected feces samples from all three day-28 group cattle were solvent extracted and the extracts were analyzed by reversed phase HPLC. Eprinomectin is excreted mostly through feces, and very little *via* urine; so the metabolite profiles in feces are indicative of the fate of eprinomectin in beef cattle. The amounts of radioactivity excreted in feces and urine through day-28 post dose were 14.3% and 0.35% of the dose, respectively. In feces, metabolite M1 accounted for 7.4% of the total radioactivity and was the only major metabolite of eprinomectin. Eprinomectin was excreted in cattle feces without being metabolized to a large degree and accounted for 85.9% of the total radioresidues. AAB_{1a} was also the most abundant residue in cattle feces also, and represented 78.3% of the total residues. The metabolite profiles of the composite feces do not differ significantly from those of the samples collected daily. Also the metabolite profiles in steer feces were nearly identical to those from the heifer feces.

Eprinomectin is not extensively metabolized in cattle following topical application. In all biological matrices, AAB_{1a} is the single most abundant residue. The contribution of AAB_{1a} to the total radioresidue levels remained relatively constant from day 7 through day 28 - for example, between 84% and 90% in liver, the proposed target tissue; therefore AAB_{1a} is an appropriate marker residue.

APPENDIX C-5

Batty, A. F. and D. Barth, 1995. MK-0397/Topical/Cattle/Safety/ Environmental Safety/ Residue/Dung Residue Depletion/Dung Residue Disappearance.

The purpose of this study (ASR 14487) was to determine the concentration of MK-0397 in fresh dung of cattle following a single treatment at the recommended dose with MK-0397 in the proposed market formulation and to follow the disappearance of MK-0397 residues in dung exposed to environmental conditions on pasture. Nine 10-month-old Friesian male castrate cattle weighing 259-324 kg were treated with MK-0397 0.5% w/v solution at 1 mL/10 kg bodyweight once topically. Three additional 10-month-old Friesian male castrate cattle served as untreated controls. To determine the concentration of MK-0397 in fresh dung, fecal samples were collected from each of the treated and control cattle prior to treatment on Day 0, again at three and seven days after treatment and thereafter at weekly intervals to Day 70. The disappearance of MK-0397 residues in aged dung pats was determined by collecting and mixing dung from each animal on the third and fourth days after treatment to provide a series of ~750-g artificial pats, which were placed onto pasture which was fenced off within the paddock grazed by the trial cattle. Pats were placed on mats of nylon mesh and were protected from interference from birds by placing netting over them at a height of ~ 1 m. At 7, 21, 35, 49, 63, 77, 105 and 126 days after placement, one randomly selected pat from each animal was collected and, after blending, subsampled for percentage dry matter and organic matter estimation and for MK-0397 assay. At 21, 49 and 63 days after placement, an additional randomly selected pat from each animal was collected and separated into crust and inner layers, then each layer was processed and subsampled as for whole pats.

Residues of MK-0397 B_{1a} less than 2 ng/g were non-detectable (reported as n.d.). Residues equal to or greater than 2 ng/g, but less than 4 ng/g, were identified but not quantified (reported as n.q.). Residues above or equal to 4 ng/g were identified and quantified. The water content of the fresh fecal samples assayed ranged from 84.91% to 91.41%.

The assays of fresh feces were discontinued for the treated animals when at least two consecutive fecal samples had non-detectable (n.d.) levels of MK-0397 B_{1a}. MK-0397 B_{1a} mean levels (standard deviations) in fresh feces on a wet-weight basis were 292 (118), 295 (126), 42 (26) and 8.1 (4.7) on trial days 3, 3/4, 7 and 14, respectively. Mean residue levels were not quantified on trial day 21 and not detectable on days 28, 35 and 42. The ranges of individual residue levels in fresh feces were 112-462, 122-458, 13.3-97.1, and n.d. - 17.6 ppb on 3, 3/4, 7 and 14 days post dose, respectively. MK-0397 B_{1a} mean levels (standard deviations) in fresh feces on a dry-weight basis were 2353 (961), 2372 (996), 385 (275), 58 (41) and 2.9 (1.1) ppb on trial days 3, 3/4, 7 and 14, respectively.

Whole pat material (pats formed from bulk collections of Day 3/4 and deposited on Day 4) from all nine treated animals was assayed for up to 126 days after deposition. Based on the MK-0397 B_{1a} content per gram of dry matter, no depletion of residues was seen within the trial period. However, the weight of the pats decreased over time and the mean total amount of MK-0397 B_{1a} per pat decreased from 246 mcg at deposition to 185 mcg at 105 days after deposition and to 137 mcg at 126 days after deposition.

The water content in inner and crust layers largely corresponded to what was found for the complete pats. The range was from 14.98% to 54.82% and from 14.08% to 23.69% in inner and crust layers, respectively.

Inner and crust layers of pats formed from bulk collections of Day 3/4 and deposited on Day 4 from all nine treated animals were assayed for up to 63 days after deposition. Based on the MK-0397 B_{1a} content per gram of dry matter, no marked differences between MK-0397 B_{1a} residues of inner and crust layers were seen.

Pats from treated and control animals degraded at the same rate, based on a comparison of the pat dry weights on days 105 and 126.

APPENDIX C-6

Venkataraman, K. and N. I. Narasimhan, 1993. Photodegradation of 4"-Epiacetylamino-4"-Deoxyavermectin B_{1a} (L-653,648) in Aqueous Solution Under Sunlight (AEDM-75).

The photodegradation of eprinomectin (4"-epiacetylamino-4"-deoxyavermectin) B_{1a} (L-653,648) was carried out in aqueous solution to determine the half-life for the disappearance of the test chemical. The major component of eprinomectin is designated as eprinomectin B_{1a} (>90% by weight of eprinomectin) and it differs from the minor component by one methylene unit. Eprinomectin B_{1a} tritium labeled at the 5-position ([5-^{3}H]eprinomectin B_{1a}) was exposed to sunlight in 13 x 100 mm screw-capped glass tubes in air-saturated water containing 1% acetonitrile as co-solvent. The exposure took place from noon on July 9, 1991 to noon on July 15, 1991. p-Nitroacetophenone (PNAP), dissolved in air-saturated water containing 0.049M pyridine, was also exposed to sunlight under identical conditions during the same period and served as the actinometer. Foil-wrapped sample tubes containing the test chemical or PNAP were also exposed to sunlight and served as controls. The test chemical concentration was 3.133 nM and that of PNAP was 9.712×10^{-6} M. A total of six tubes (three [5-^{3}H]eprinomectin B_{1a} tubes and three PNAP tubes) were removed at each predetermined time point. Control tubes were removed only at noon on all exposure days. All samples were analyzed by reverse-phase HPLC. The test chemical and PNAP concentrations at each time point were averaged based on analyses of triplicate samples. The concentrations of the test chemical and PNAP in all control tubes were very nearly equal to their respective zero-time values and hence no corrections were applied to exposed sample concentrations.

Since the concentration of the test chemical decreased to a very low value of 1.4% of the initial level, the data for time points 2 days and beyond were not included in further calculations. The photodegradation data for the test chemical and PNAP were fitted to a single-exponential curve. From the equation of the best-fit curve, the rate constant and half-life for the photodegradation of [5-^{3}H]eprinomectin B_{1a} were determined to be -2.56 days^{-1} and 0.27 days, respectively. The corresponding values for PNAP were -0.45 days^{-1} and 1.53 days, respectively. The rate constants and the half-lives were also calculated from the best-fit line obtained by linear regression of Ln {C(0)/C(t)} versus time data. The rate constants and half-lives for the photo-degradation of [5-^{3}H]eprinomectin B_{1a} and PNAP determined by this second method were identical to the results obtained by the first method. The ratio of the rate constants was determined by a third method recommended by the EPA and FDA. The ln{C(0)/C(t)} data (0 - 1.4 days) for PNAP was plotted against that of [5-^{3}H]L-653,648 B_{1a}. The ratio (k_p^c / k_p^a) of the photolysis rate constants (slope of the best-fit line) was calculated to be 5.49.

From the absorbance data for [5-^{3}H]eprinomectin B_{1a}, the summer, fall and winter solar irradiance values for 40° N, the quantum yield for PNAP in the experimental actinometer, and the rate constant ratio, the photodegradation rates for [5-^{3}H]eprinomectin B_{1a} were computed. The maximum photodegradation rates for the test chemical under clear skies at the surface of flat bodies of water were calculated to be 2.42 day^{-1} for summer, 1.11 day^{-1} for fall, and 0.63 day^{-1} for winter. The corresponding minimum half-lives for the photodegradation of [5-^{3}H]eprinomectin B_{1a} would be 0.29 day for summer, 0.62 day for fall, and 1.10 day for winter. If 95% confidence limits are imposed, the seasonal minimum half-lives (in days) would be in the range 0.21 - 0.33 for summer, 0.46 - 0.73 for fall and 0.82 - 1.29 for winter. Because of very short half-life, [5-^{3}H]eprinomectin B_{1a}, and therefore eprinomectin, will undergo rapid photodegradation. The photodegradation of eprinomectin under environmental conditions will be extremely rapid. If eprinomectin were to enter surface water, it would be rapidly degraded and therefore would not be of environmental concern.

APPENDIX C-7

Yan, Z., 1995. Aerobic Biodegradation in Soil with ^{14}C-MK-0397 (^{14}C-L-653,648).

The purpose of this study was to determine the fate of eprinomectin in soil under aerobic conditions. The study was conducted in compliance with GLP regulations at ABC Laboratories, Inc., Columbia, MO. This 64-day aerobic soil biodegradation study with [^{14}C]eprinomectin was conducted following the FDA Technical Assistance Document 3.12, "Aerobic Biodegradation in Soil."

Three test soils were employed: sandy loam (soil 1), loam (soil 2), and silt loam (soil 3). All three soils were collected in Grand Forks County, ND, and sieved through a 2-mm mesh screen. [^{14}C]eprinomectin possessed a radiochemical purity of >99% by TLC and ~98% by HPLC analyses. A reference chemical, [^{14}C]glucose, was tested concurrently to monitor the viability of the microbial population.

The test apparatus consisted of 125-mL flasks containing 50 g of soil (dry weight). The flasks were each connected to a series of glass scintillation vials which served as backflow, volatile and CO_2 traps. Nine test systems were prepared for each of three soil types. Three replicates contained the test chemical, [^{14}C]eprinomectin at 10 mg carbon/50 g soil; three replicates contained the reference chemical, [^{14}C]glucose, at 10 mg carbon/50 g soil; and three replicates served as blank soil controls and contained neither test nor reference chemical. Purified distilled water was added to each sample to give a moisture content of 70% of field capacity. The test was conducted in the dark at 22±3°C. All test systems were aerated every few days to maintain aerobic incubation conditions. All test flasks were weighed on days 0, 42 and 64 to determine if the soil had dried out during the test. The trapping solutions were removed on days 1, 2, 3, 4, 5, 6, 7, 12, 14, 21, 28, 35, 42, 49, 56, and 64 for analysis of $^{14}CO_2$ production. The percent biodegradability was calculated as a function of the $^{14}CO_2$ production in the test systems as compared to the amount applied. After the 64-day aerobic incubation, about 59.55, 59.13, and 65.30% of the applied reference compound was biodegraded to $^{14}CO_2$ in soils 1, 2, and 3, respectively, which verified the microbial inoculum in these three soil types was viable and active. For the test compound, an average of 3.62, 2.87, and 2.91% of applied ^{14}C-activity was recovered as $^{14}CO_2$ following the same incubation period in soils 1, 2, and 3, respectively. This indicates that [^{14}C]eprinomectin was slowly mineralized in all three soil types tested. In all cases, the production of ^{14}C-volatiles (other than $^{14}CO_2$) was negligible (0.01-0.02% of applied ^{14}C-activity). On day 64, test soil samples were extracted with acetone followed by methanol. Extracted soils were combusted to quantitate non-extractable residues. The overall ^{14}C-mass balance is taken as the summation of total volatile ^{14}C-residues, total extractable ^{14}C-residues, and total ^{14}C-nonextractable residues.

The ^{14}C-mass balance for test compound [^{14}C]eprinomectin of soils 1, 2, and 3 was 99.81, 97.84, and 95.03%, respectively. The total extractable ^{14}C-residues accounted for 77.24, 75.94, and 74.54% of applied [^{14}C]eprinomectin activity in soils 1, 2, and 3, respectively. The total nonextractable ^{14}C-residues accounted for 18.94, 19.01, and 17.57% of applied [^{14}C]eprinomectin activity in soils 1, 2, and 3, respectively. Total volatile ^{14}C-residues (including $^{14}CO_2$) accounted for 3.63, 2.89, and 2.92% of applied [^{14}C]eprinomectin activity in soils 1, 2, and 3, respectively.

To verify the presence of parent compound remaining in the soil at the end of the study, solvent extracts of the samples were analyzed by both high performance liquid chromatography and thin-layer chromatography. The results showed that the test compound, [^{14}C]eprinomectin, underwent some degradation (about 25% of the extracted ^{14}C-activity was determined as several transformation products of unknown structures, and 60-73% of the extracted ^{14}C-activity, and 47-55% of the applied ^{14}C-activity, remained as parent compound). The [^{14}C]eprinomectin on day 64 as a percent of ^{14}C-activity applied to soils 1, 2, and 3 was 46.59, 47.11 and 49.58%, respectively, by HPLC and 51.34, 51.89 and 54.56%, respectively, by TLC.

Thus, eprinomectin degrades in soil with a half-life of approximately 64 days in three soils at 22 ±3°C. The extent of mineralization (as determined by $^{14}CO_2$ evolution) was low (2.9-3.6%).

APPENDIX C-8

Venkataraman, K and N. I. Narasimhan, 1995. The Hydrolytic Stability of 4"-Epiacetylamino-4"-Deoxyavermectin B_1 (MK-0397) (AEDM80).

The objective of this study was to determine the rate of hydrolysis and half-life of eprinomectin (MK-0397; 4"-epiacetylamino-4"-deoxyavermectin B_1) in aqueous buffers at different pH values so that the environmental fate of the chemical can be predicted. Methods in the Environmental Assessment Technical Assistance Handbook, Food and Drug Administration, Washington, D.C., March 1987, Technical Assistance Document 3.09 Hydrolysis were followed. Wherever appropriate, the methods were modified to accommodate OECD guidelines as described in the Official Journal of European Communities, C. 10. Degradation - Abiotic Degradation: Hydrolysis as a Function of pH, dated 19/9/84.

Eprinomectin is a mixture of two homologous compounds. The major component is designated as AAB_{1a} (> 90% by weight of eprinomectin) which differs from the minor component, designated as AAB_{1b}, by a single methylene group. In this study, only the major component AAB_{1a} was subjected to hydrolysis. The rate of hydrolysis of the minor component is expected to be very similar to that of the major component since the methylene group is not expected to influence the rate of hydrolysis.

A preliminary test was conducted with ^3H- AAB_{1a} at an initial concentration of 25.5×10^{-9} M in aqueous buffers at pH 4.0, 5.0, 7.0, and 9.0. The hydrolysis was performed at 50°C for 5 days in screw-capped vials which were protected from light to avoid photolysis. The concentrations of AAB_{1a} remaining at the end of 5 days were determined by reverse phase HPLC and an on-line, flow-through radioactivity detector. Slightly greater than 10% of the AAB_{1a} was hydrolyzed in the preliminary test. Therefore a definitive hydrolysis rate test was performed as required by US FDA TAD and OECD guidelines. The study design was very similar to that of preliminary test except the study was carried out at 25°C and samples were withdrawn at 0, 3, 7, 14, 21 and 28 days. The results indicate that at 25°C, there was less than 5% hydrolysis of AAB_{1a} on day 28 at all pH values. At each pH value, a plot of the logarithm of AAB_{1a} concentration versus time was obtained. By linear regression analysis, the equation of the best-fit line was obtained. From the slope of the best-fit line, the hydrolysis rate constant (k) and half-life ($t_{1/2}$) were calculated. The half-lives for the hydrolysis of AAB_{1a} at pH 4, 5, 7, and 9 were estimated to be 622, 614, 2026, 414 days respectively. A chemical with a half-life of greater than 1 year at 25°C is considered to be hydrolytically stable. Because of the long half-life (> 1 year) of AAB_{1a}, it is hydrolytically stable and the same will be true for the minor component.

APPENDIX D
Effects Study Reports

D-1 Graves, W. C. and J. P. Swigert, 1994. L-653,648: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*).

D-2 Graves, W. C. and J. P. Swigert, 1995. L-653,648 (MK-397): A 96-Hour Flow Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*).

D-3 Graves, W. C. and J. P. Swigert, 1995. L-653,648 (MK-397): A 96-Hour Flow Through Acute Toxicity Test with the Bluegill Sunfish (*Lepomis macrochirus*).

D-4 Campbell, S. M. and J. B. Beavers, 1994. L-653,648 (MK-397) An Acute Oral Toxicity Study with the Bobwhite.

D-5 Campbell, S. M. and J. B. Beavers, 1994. L-653,648: An Acute Oral Toxicity Study with the Mallard.

D-6 Campbell, S. M. and J. B. Beavers, 1995. L-653,648 (MK-397) A Dietary LC_{50} with the Northern Bobwhite.

D-7 Campbell, S.M. and J. B. Beavers, 1995. L-653,648 (MK-397) A Dietary LC_{50} with the Mallard.

D-8 Salvatore, M.J., 1991. Antimicrobial Spectrum Profile for L-653,648.

D-9 Palmer, S.J. and J.B. Beavers, 1995. L-653,648 (MK-0397): A Subacute Toxicity Study With the Earthworm (*Lumbricus Terrestris).*

D-10 Thompson, S. G. and J. P. Swigert. 1994. L-653,648: A 14-Day Toxicity Test with the Fresh Water Alga (*Selenastrum capricornutum*).

D-11 Zhao, P. L., 1995. Minimum Inhibitory Concentration and No Observed Adverse Effect Concentration for the 14-Day Toxicity Test of MK-397 to the Freshwater Alga

D-12 Feutz, E. and L. Stuerman, 1995. Determining the Effects of MK-397 on Seed Germination and Root Elongation.

D-13 Feutz, E. and L. Stuerman, 1995. Determination of the Effects of MK-397 on Seedling Growth

D-14 Faidley, T., T. Murphy, S. Nicolich, P. Kochbarski and W. Langholff, 1995. MK-0397/Safety/Environmental Safety/Dung Fauna/Dung Beetle

APPENDIX D-1

Graves, W. C. and J. P. Swigert, 1994. L-653,648 : A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (Daphnia magna).

The purpose of this study was to determine the acute toxicity of eprinomectin (L-653,648; MK-397) to the cladoceran, Daphnia magna, under flow-through test conditions. This test was conducted at the Wildlife International Ltd. aquatic toxicology facility in Easton, MD. Procedures followed "Daphnia Acute Toxicity" (U.S. FDA Environmental Assessment Technical Assistance Document 4.08, 1987) and "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (ASTM Standard E 729-88, 1988). The L-653,648-000X021 used in this study was 95.4% pure. Daphnids were from laboratory stocks cultured at Wildlife International Ltd. The toxicity test was conducted in 8-L Teflon®-lined chambers, each of which contained 6.5 L of test solution under flow-through conditions (average of 14 media exchanges per 24 hours in each test vessel). Test water (not chlorinated) was groundwater collected from a well and filtered before testing. During the test this water had a total hardness and alkalinity as $CaCO_3$ of 140-144 mg/L and 188-190 mg/L, respectively, and a conductivity of 320-330 mcmhos/cm. The pH was between 8.2 and 8.4, the temperature between 20.0 and 20.1°C, and the dissolved oxygen concentration between 8.1 and 8.8 mg/L. Nominal concentrations of eprinomectin tested included: 0, 0.45, 0.76, 1.3, 2.1, and 3.5 mcg a.i./L. The corresponding mean measured concentrations were <0.10 mcg a.i./L for the dilution water and solvent control (0.1 ml acetone per L), 0.37, 0.64, 1.2, 1.8 and 3.3 mcg a.i./L, respectively.

Twenty daphnids were equally distributed between 2 replicates of each treatment. After 48 hours of exposure, daphnids in the negative control had 100% survival with no visually observed sublethal effects (i.e., lethargy; floating on surface, resubmerged, appeared normal; or floating on surface, resubmerged, appeared lethargic). Daphnids in the solvent control and in several treatment solutions were observed floating during the test; no diluent control daphnids were found floating. A dose-response pattern of immobilization and death existed at the 24- and 48-hour observation periods in spite of the floating. Based on the mortality/immobility data for 24 and 48 hours of exposure of daphnids to eprinomectin, the 24- and 48-hour EC_{50} values (95% confidence limits) were 1.6 (1.4-1.8) and 0.45 (0.37-0.64) mcg a.i./L (ppb), respectively. The 48-h no mortality concentration was less than 0.37 mcg a.i./L, the lowest concentration tested.

APPENDIX D-2

Graves, W. C. and J. P. Swigert, 1995. L-653,648 (MK-397): A 96-Hour Flow Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*).

The purpose of this study was to estimate the acute toxicity of eprinomectin (L 653,648; MK-397) to rainbow trout (*Oncorhynchus mykiss*) during a 96-hour exposure period under flow-through test conditions. This test was conducted at the Wildlife International Ltd. facility in Easton, Maryland. The study was conducted according to procedures in the Food and Drug Administration Environmental Assessment Technical Assistance Document 4.11 and ASTM Standard E 729-88. The batch L-653,648-000X021 was 95.4% pure. Rainbow trout used in the test were obtained as eyed eggs from Troutlodge, Inc., McMillin, WA, and hatched from cultures maintained by Wildlife International. The fish were held approximately 69 days prior to the test and the juvenile fish were acclimated to test conditions for approximately 50 hours prior to test initiation.

Test chambers were Teflon® lined, 25-L polyethylene aquaria filled with 15 L of test water. No aeration was provided to the test chambers during the test. A continuous flow diluter was used to deliver each concentration of test substance, a solvent (acetone) control, and a well-water control. Syringe pumps were used to deliver the five test substance stock solutions and solvent control into mixing chambers assigned to each treatment and control group. Two replicate test chambers were maintained in each treatment and control group with 10 rainbow trout in each test chamber. The average length and weight of control fish measured at the end of the test was 42 mm (±2.2 mm) with a range of 38 to 45 mm, and 1.2 g (±0.23 g) with a range of 0.84 to 1.5 g. Loading was determined to be 0.13 g fish/L. Nominal concentrations of eprinomectin tested included, 0, 0.19, 0.32, 0.54, 0.90 and 1.5 mg a.i./L. Mean measured concentrations were determined from samples of test water collected at the beginning of the test and at 24, 48, 72 and 96 hours. Mortality and behavioral/physical abnormalities (i.e., loss of equilibrium, lethargy, erratic swimming) were determined at various times during the 96-hour exposure.

The mean measured concentrations of eprinomectin for the study were 0, 0.21, 0.37, 0.63, 1.1 and 1.8 mg a.i./L. Mean measured concentrations were used in calculation of LC_{50} and NOEC values. Water temperatures were within the limits of the range established for this test and averaged 12.1°C over the 96-hour period. Dissolved oxygen concentrations exceeded 60% of saturation throughout the test. The pH ranged from 8.0 to 8.3.

The 96-hour LC_{50} value for rainbow trout exposed to eprinomectin was 1.2 mg a.i./L. The 95% confidence limits were 0.99 and 1.4 mg a.i./L and the slope of the concentration response curve was 6.3. The 96-hour no observed effect concentration, determined by a visual examination of the mortality and observations data, was 0.37 mg a.i./L. Observations of loss of equilibrium and lethargy were noted for several fish in the 0.63 mg a.i./L treatment group after 48-hours. By 72 hours, fish in this treatment group exhibited erratic swimming and were observed lying on the bottom of the test chambers and exhibiting little movement. By test termination, 10% of the fish in this treatment group had died. Five percent of the rainbow trout exposed at the 1.1 mg a.i./L treatment level died by 48 hours and by test termination, there was 30% mortality. Mortality in the 1.8 mg a.i./L treatment group, the highest concentration tested, was 35% within 48 hours of test initiation, and 95% by 96 hours.

APPENDIX D-3

Graves, W. C. and J. P. Swigert, 1995. L-653,648 (MK-397): A 96-Hour Flow-Through Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*).

The purpose of this study was to estimate the acute toxicity of eprinomectin (L 653,648; MK-397) to bluegill sunfish (*Lepomis macrochirus*) during a 96-hour exposure period under flow-through test conditions. This test was conducted at the Wildlife International Ltd. facility in Easton, Maryland. The study was conducted according to procedures in the Food and Drug Administration Environmental Assessment Technical Assistance Document 4.11 and ASTM Standard E 729-88. The batch L-653,648-000X021 was 95.4% pure. Bluegill sunfish used in the test were obtained as juveniles from Northeastern Biologists, Inc., Rhinebeck, NY. The fish were held approximately 14 days prior to the test and were acclimated to test conditions for approximately 49 hours prior to test initiation.

Test chambers were Teflon® lined, 25-L polyethylene aquaria filled with 15 L of test water. No aeration was provided to the test chambers during the test. A continuous flow diluter was used to deliver each concentration of test substance, a solvent (acetone) control, and a well-water control. Syringe pumps were used to deliver the five test substance stock solutions and solvent control into mixing chambers assigned to each treatment and control group. Two replicate test chambers were maintained in each treatment and control group with 10 bluegill sunfish in each test chamber. The average length and weight of 10 control fish measured at the end of the test was 26 mm (±2.4 mm) with a range of 23 to 30 mm, and 0.41 g (±0.16 g) with a range of 0.24 to 0.72 g. Loading was determined to be 0.045 g fish/L. Nominal concentrations of eprinomectin tested included 0, 0.13, 0.22, 0.36, 0.60 and 1.0 mg a.i./L. Mean measured concentrations were determined from samples of test water collected at the beginning of the test and at 24, 48, 72 and 96 hours. Mortality and behavioral/physical abnormalities (i.e., appearance of darker color, lethargy) were determined at 3, 6, 24, 48, 72, and 96 hours.

The mean measured concentrations of eprinomectin for the study were 0, 0.14, 0.25, 0.41, 0.69 and 1.2 mg a.i./L. Mean measured concentrations were used in calculation of LC_{50} and NOEC values. Water temperatures were within the limits of the range established for this test and averaged 22^{o} C over the 96-hour period. Dissolved oxygen concentrations exceeded 60% of saturation throughout the test. The pH ranged from 8.2 to 8.4.

The 96-hour LC_{50} value for bluegill sunfish exposed to eprinomectin was 0.37 mg a.i./L. The 95% confidence limits were 0.33 and 0.42 mg a.i./L and the slope of the concentration response curve was 9.9. The 96-hour no observed effect concentration, determined by a visual examination of the mortality and observations data, was 0.14 mg a.i./L. Observations of lethargy were noted for all remaining fish in the 0.41 mg a.i./L treatment group after 48-hours. By 96 hours, there was 65% mortality in this treatment group. Observations of lethargy, discoloration, and lying on the bottom and exhibiting little movement were seen for several fish in the 0.25 mg a.i./L treatment group after 96 hours. By test termination there was 5% mortality in this treatment group.

APPENDIX D-4

Campbell, S. M. and J. B. Beavers, 1994. L-653,648 (MK-397): An Acute Oral Toxicity Study with the Bobwhite.

The purpose of this study was to determine the acute toxicity of eprinomectin (MK-0397; L-653,648-000X021) in the northern bobwhite (*Colinus virginianus*) when administered as a single oral dose. This test was conducted by Wildlife International Ltd, Easton, Maryland. Bobwhite quail were from Top Flight Quail Farm, Belvidere, NJ. Seventy 26-week old quail were randomly assigned within sex to one control and six treatment groups (five males and five females per group). Feed was withheld from all birds for 15 hours prior to treatment. Temperature averaged 20.7°C ±1.8°C (S.D.) and relative humidity 53% ± 15% (S.D.). Photoperiod was 8 hr of light daily. Technical MK-0397 [95.4% pure active ingredient (a.i.)], was given as a single oral dose by gelatin capsule. Nominal doses (adjusted to be 100% a.i.) of eprinomectin were: 0, 62.5, 125, 250, 500, 1000 and 2000 mg a.i./kg body weight. Symptoms of toxicity, mortality and abnormal behavior were recorded daily for two weeks after treatment. Body weights and feed consumption were measured at several points during the 2 week post-dosing period.

Severity of toxicity was related to dose. At 62.5 mg a.i./kg, signs of toxicity were first noticed about 4 hours after dosing and persisted through the afternoon of Day 1. All birds appeared normal on day two. Symptoms were more severe at higher doses. Signs of toxicity typical of intoxication were reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness, gaping, ruffled appearance, wing beating, lethargy, loss of righting reflex, prostrate posture, convulsions, depression and coma. There was 50% mortality at the 250 mg a.i./kg dosage, 90% at 500 mg a.i./kg, 100% at 1000 mg a.i./kg and 100% at 2000 mg a.i./kg eprinomectin. The acute oral LD_{50} of eprinomectin in bobwhite quail is determined to be 272 mg a.i./kg. The no-effect level is estimated to be lower than 62.5 mg a.i./kg, the lowest level tested, based on signs of toxicity noted at 62.5 mg a.i./kg. The slope of the dose response curve was 5.7. The no-mortality level was 125 mg a.i./kg.

APPENDIX D-5

Campbell, S. M. and J. B. Beavers, 1994. L-653,648: An Acute Oral Toxicity Study with the Mallard.

The purpose of this study was to determine the acute toxicity of eprinomectin (MK-0397; L-653,648-00X021) in the mallard (*Anas platyrhynchos*) when administered as a single oral dose. This test was conducted by Wildlife International Ltd, Easton, Maryland. Mallard ducks were from Whistling Wings, Hanover, Illinois. Eighty, 19-week old ducks were randomly assigned within sex to one control and seven treatment groups (five males and five females per group). Feed was withheld from all ducks for 15 hours prior to treatment. Eprinomectin was given as a single oral dose in a gelatin capsule. Birds were housed indoors at 21.0°C ± 2.1°C (S.D.), 64% ± 12% (S.D.) relative humidity and 8-hr of light daily. Nominal doses of eprinomectin (adjusted to be 100% a.i.) were: 0, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 mg a.i./kg body weight. Technical eprinomectin (L-653,648-00X021) was 96.3% pure. Symptoms of toxicity and mortality were recorded daily for two weeks.

At the 7.8 mg a.i./kg dosage, signs of toxicity were displayed on days 1 and 2 only. In addition, birds given 15.6 mg a.i./kg showed signs of toxicity on day 1 and through the afternoon of day 3 (2 birds were found dead on day 1). At 31.3 and 62.5 mg a.i./kg, signs of toxicity were noted within 2 hours of dosing. Signs of toxicity typical of intoxication were reduced reaction to external stimuli (sound and movement), wing droop, prostrate posture, lower limb weakness, loss of coordination, loss of righting reflex, the use of wings for stabilization, depression, lethargy, minor muscle fasciculations, inability to stand and coma. Mortality in all ten birds was seen by day 2 in the 62.5 mg a.i./kg group. There was 100% mortality on day 1 at 125, 250 and 500 mg a.i./kg of MK-0397. The acute oral LD_{50} (95% confidence limits), corrected for 96.3 percent purity, was 24 (18-32) mg a.i./kg. The slope of the dose response curve was 5. The no observed effect dosage was determined to be lower than 7.8 mg a.i./kg, the lowest dose tested, based on the signs of toxicity noted at that dosage. The no-mortality level was 7.8 mg a.i./kg.

APPENDIX D-6

Campbell, S. M. and J. B. Beavers, 1995. L-653,648 (MK-397) A Dietary LC_{50} with the Northern Bobwhite.

The purpose of this study was to determine the eight day dietary LC_{50} of eprinomectin (MK-397) in the northern bobwhite (*Colinus virginianus*). The test was conducted by Wildlife International Ltd., Easton, Maryland. Quail eggs were obtained from Wildlife International's production flock. The birds were from the same hatch and phenotypically indistinguishable from wild birds. Ten-day old quail were assigned to each of control and 6 treatment groups by indiscriminate draw. All birds were acclimated to the caging from the day of hatch until initiation of the test. During the test the average ambient room temperature was 25.7^{o}C +/- 1.7 ^{o}C (SD) with an average relative humidity of 46% +/- 10%(SD). The photoperiod was sixteen hours of light per day.

All birds were observed at least once daily. A record was maintained of mortality, signs of toxicity and abnormal behavior. The test diets were prepared by mixing the test substance (95.4% a.i.) into the diet with corn oil and acetone at a concentration of approximately 2% each. The dietary concentrations were adjusted to 100% active ingredient. The nominal dietary test concentrations were 316, 562, 1000, 1780, 3160 and 5620 ppm a.i. Samples of test diets were taken to verify homogeneity, stability and test concentrations.

During the exposure period, the control group received an amount of carrier vehicle in their diet, equivalent to the greatest amount used in the eprinomectin-treated diets. Following the five-day exposure period, all groups were given untreated feed for three days.

There were no mortalities in the control group and the 316, 562, or 1000 ppm a.i. treatment groups. There was 60% mortality at the 1780 ppm, 90% at the 3160 ppm and 100% at the 5620 ppm test concentrations. At the 316 ppm a.i. test concentration, signs of toxicity were first noted on the morning of Day 2, and continued through Day 5. Signs of toxicity were reduced reaction to external stimuli (sound and movement), lethargy, depression, wing-droop, a ruffled appearance, shallow and rapid respiration and lower limb weakness. Exposure to higher concentrations of eprinomectin resulted in more prolonged, and similar, signs of toxicity.

There was a slight reduction in body-weight gain among birds in the 316 and 562 ppm a.i. test concentrations during the exposure period. A reduction in food consumption was noted among birds at all concentrations during Days 0 - 2, and among birds at the 316, 562, 1000, 1780, and 3160 ppm a.i. test concentrations during Days 3-5.

Analyses of homogeneity samples indicated that the test substance was uniformly distributed in the diet with a maximum coefficient of variation of 7.83%. Concentrations of test sustance in the verification samples ranged from 98 - 104% of nominal. Concentrations of the Day 5 freezer stability samples ranged from 88 - 106% of the Day 0 values, documenting stability of eprinomectin in the diet.

The LC_{50} for northern bobwhite exposed to eprinomectin was determined to be 1813 ppm a.i., with a 95% confidence interval of 1420 to 2312 ppm a.i. The slope of the dose response curve was 7. The no-mortality level was 1000 ppm a.i., and the no-observed effect concentration was lower than 316 ppm a.i. based on signs of toxicity and effect upon body weight at that concentration.

APPENDIX D-7

Campbell, S. M. and J. B. Beavers, 1995. L-653,648 (MK-397): A Dietary LC_{50} with the Mallard.

The purpose of this study was to determine the eight day dietary LC_{50} of eprinomectin (MK-397) in the mallard (*Anas platyrhynchos*). The test was conducted by Wildlife International Ltd., Easton, Maryland. Ducklings were obtained from Whistling Wings, Hanover, Illinois. The birds were from the same hatch, and phenotypically indistinguishable from wild birds. Ten-day old ducklings were assigned to each of four control and 6 treatment groups by indiscriminate draw. All birds were acclimated to the caging from the day of receipt until initiation of the test, During the test the average ambient room temperature was 24.0^{o}C +/- 1.4 ^{o}C (SD) with an average relative humidity of 56% +/- 14%(SD). The photoperiod was sixteen hours of light per day.

All birds were observed at least once daily. A record was maintained of mortality, signs of toxicity and abnormal behavior.

The test diets were prepared by mixing the test substance (95.4% a.i.) into the diet with corn oil and acetone at a concentration of approximately 2% each. The dietary concentrations were adjusted to 100% active ingredient. The nominal dietary test concentrations were 100, 178, 316, 562, 1000, and 1780 ppm a.i. Samples of test diets were taken to verify homogeneity, stability and test concentrations.

During the exposure period, the control groups received an amount of carrier vehicle in their diet, equivalent to the greatest amount used in the eprinomectin-treated diets. Following the five-day exposure period, all groups were given untreated feed for three days.

There were no mortalities in the control group and the 100 or 178 ppm a.i. treatment groups. There was 10% mortality at the 316 ppm, 80% at the 562 ppm, and 100% at both the 1000 and 1780 ppm test concentrations. At the 100 ppm a.i. test concentration, signs of toxicity were first noted on the morning of Day 1, and continued through Day 5. Signs of toxicity were reduced reaction to external stimuli (sound and movement), lethargy, loss of co-ordination and lower limb weakness. Exposure to higher concentrations of eprinomectin resulted in more prolonged, and severe, signs of toxicity.

There was a slight reduction in body-weight gain among birds in the 100 and 178 ppm a.i. test concentrations during the exposure period. A reduction in food consumption was noted among birds at all concentrations during Days 0 - 5. A continued slight reduction in food consumption was noted in birds at the 178 and 316 ppm a.i. concentration during the post-exposure observation period.

Analyses of homogeneity samples indicated that the test substance was uniformly distributed in the diet with a maximum coefficient of variation of 2.63%. Concentrations of test substance in the verification samples ranged from 97 - 103% of nominal. Concentrations of the Day 5 freezer stability samples ranged from 97 - 105% of the Day 0 values, documenting stability of eprinomectin in the diet.

The LC_{50} for mallard duck exposed to eprinomectin was determined to be 447 ppm a.i., with a 95% confidence interval of 357 to 558 ppm a.i. The slope of the dose response curve was 9. The no-mortality level was 178 ppm a.i., and the no-observed effect concentration was lower than 100 ppm a.i. based on signs of toxicity and effect upon body weight at that concentration.

APPENDIX D-8

Salvatore, M. J., 1991. Antimicrobial Spectrum Profile.

The purpose of this study was to determine the toxicity of eprinomectin to a variety of microorganisms in a standard antimicrobial, disc-plate susceptibility test (Zimmerman, *et al.* 1970). The study was conducted at Merck Research Laboratories, Rahway, NJ, USA. Eprinomectin (L-653,648-00X16) at 1 mg/mL in methanol was used to saturate 6.3 mm (0.25-inch) diameter filter paper discs, which were then air dried and applied to the surface of seeded agar plates. The plates were examined for zones of inhibition after 16 to 24 hours of incubation at either 25° or 37° C. The species tested were *Bacillus* Sp., *Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Bacillus subtilis, Sarcina lutea, Salmonella gallinarum, Vibrio percolans, Xanthomonas vesicatoria, Escherichia coli, Pseudomonas stutzeri, Klebsiella pneumoniae, Aerobacter aerogenes, Erwinia atroseptica, Corynebacterium pseudodiphtheriticum, Streptococcus faecium, Streptococcus agalactiae, Proleus mirabilis, Micrococcus flavus, Streptomyces* Sp., *Saccharomyces cerevisiae, Candida albicans, Aspergillus niger, Bordetella bronchiseptica and Penicillium* Sp. In all, 52 test were performed; some species were incubated at both 25° and 37° C, some species were incubated in the presence and absence of lactamases, and both normal and antibiotic-resistant strains of some species were included in the screen. For all organisms, no significant (10 mm or greater) zones of inhibition were seen, indicating that eprinomectin has no antimicrobial activity even at a concentration of 1000 ppm.

APPENDIX D-9

Palmer, S.J. and J.B. Beavers, 1995. L-653,648 (MK-0397): A Subacute Toxicity Study With the Earthworm (*Lumbricus terrestris).*

The purpose of the study was to determine the toxicity (LC_{50}) of eprinomectin (L-653,648; MK-0397) to the earthworm, *Lumbricus terrestris.* The study was conducted at Wildlife International Ltd., Easton, Maryland. The worms were supplied by Shore Sportsman, Trappe, Maryland. All worms used in the test were mature with clitellum. The worms were acclimated in artificial soil for 14 days prior to the initiation of the test. The test material was 94.7% pure and test concentrations were adjusted to 100% active ingredient.

The artificial soil used for the test was prepared by mixing manure from non-medicated rabbits and de-ionized water into an artificial soil substrate consisting of 70% quartz sand, 20% kaolin clay, and 10% sphagnum peat. The pH of the soil was adjusted to pH 6.03 using calcium carbonate. Water was added to achieve a final moisture content of approximately 25%. Approximately 2000 g of prepared soil was placed in each of four replicate test chambers for each treatment and control group.

The test was conducted at nominal test concentrations of 100, 178, 316, 562 and 1000 mg a.i./kg dry soil, based on results from screening tests. A control group was maintained concurrently. Four replicate test chambers were maintained in each treatment and control group with 10 worms in each chamber. The worms were observed for burrowing behavior approximately 1/2 hour after test initiation. The worms were observed for mortality and signs of toxicity (behavioral or pathological abnormalities) on Days 7, 14, 21, and 28 of the test. The total weight of the worms in each test chamber was measured at test initiation and termination. Cumulative mortality percentages in the treatment groups were used to determine the LC_{50} value. The no mortality and no observed effect concentrations were determined by visual examination of the mortality, body weight, and clinical observation data (thin, soft, reduced reaction to mechanical stimuli). Temperature and relative humidity were measured twice daily throughout the test period. During the test, the temperature and relative humidity averaged 13.5°C +/- 0.2°C (SD) and 63% +/-8% (SD), respectively. Light intensity readings taken directly over each test chamber during the test averaged 597 +/-109 Lux. The soil pH ranged from 6.8 to 8.0, and moisture content ranged from 24.1% to 27.0%. The soil temperature ranged from 18°C to 22°C at soil preparation on Day 0, and then examined relatively constant at 13°-14°C from Day 7 to Day 28. Results of analyses to measure test concentrations were averaged from samples collected 0, 7, 14, 21, and 28 days of the test. The mean measured concentrations were 90.8, 165, 295, 531, and 951 mg a.i./kg dry soil and were 91 to 95% of nominal values.

By 1/2 hour after test initiation all worms were either burrowing into the soil or were completely under the soil surface, except for 1 worm in the 951 mg ai/kg treatment group that was still on the surface.

There were no mortalities in the control group by test termination. There were no mortalities among worms in the 90.8 and 165 mg a.i./kg treatment groups. One worm in the 90.8 mg a.i./kg dose group appeared thin on Day 7, and one worm each in dose groups 90.8 and 165 mg a.i./kg appeared thin and soft on Day 28. Although there were no mortalities among worms in the 295 mg a.i./kg, all 40 worms appeared thin and soft from Day 7 through Day 28 of the test. In the 531 and 951 mg a.i./kg treatment groups, the rate of mortality was 10% and 30%, respectively. Beginning on Day 7 of this test, the worms in these treatment groups were noted as thin and soft, with some worms exhibiting a reduced reaction to external stimuli.

The LC_{50} value for earthworms exposed to eprinomectin in an artificial soil was determined to be greater than 951 mg a.i./kg dry soil, the highest concentration tested. The no mortality concentration was 295 mg a.i./kg dry soil. The no observed effect concentration was less than 90.8 mg a.i./kg dry soil, the lowest concentration tested, based on a treatment-related loss in body weight among worms in this treatment group.

APPENDIX D-10

Thompson, S. G. and J. P. Swigert, 1994. L-653,648: A 14-Day Toxicity Test with the Fresh Water Alga (*Selenastrum capricornutum*).

A phytotoxicity test was conducted to determine the effect of eprinomectin (L 653,648; MK-397) on the fresh water unicellular green alga *Selenastrum capricornutum*. This test was conducted at the Wildlife International Ltd. aquatic toxicology facility in Easton, MD. Procedures were adapted from "Algal Assay" (U.S. FDA Environmental Assessment Technical Assistance Document 4.01, 1987), "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (EPA 600/4-85/014) and "Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae" (ASTM Standard Guide 1218-90E, 1990). The L653,648-000X021 used in this study was 95.8% pure. The *Selenastrum capricornutum* culture was from laboratory stocks cultured at Wildlife International Ltd. Cultures (1×10^5 cells/ml) were incubated at 24±2°C, 4040-4570 lux illumination in sterile 250-ml Erlenmeyer flasks containing 100 ml test medium for 14 days. Nominal concentrations of eprinomectin tested included: 3.8, 7.5, 15, 30, 60 and 120 mg active ingredient (a.i.)/L (ppm), plus negative and solvent control groups. Mean measured concentrations of the Day 0 and 14 analyses were 3.8, 7.0, 15, 29, 58 and 119 mg a.i./L. Triplicate flasks were tested at each dose level. Samples were collected at approximately 48-hr intervals during the 14-day exposure for the determination of cell densities.

Mean cell density in the solvent control was inhibited by 34% compared to mean cell density in the negative control. The difference between the negative and solvent controls was statistically significant ($p<0.05$). Therefore, all statistical evaluations were made by comparing the treatment groups to the solvent control replicates.

There were no statistically significant ($p<0.05$) differences in mean cell density between the solvent control and the 3.8, 7.0, and 15 mg a.i./L treatment groups. Mean cell density in the 15 mg a.i./L treatment group was reduced by 31% compared to the solvent control. Although the reduction in cell density observed in that treatment group was not statistically significant ($p<0.05$), the effect upon algal growth followed the dose-response curve and was thereby considered to be treatment related. Mean cell densities in the 29, 58 and 119 mg a.i./L treatment groups were inhibited by 64, 60 and 60%, respectively. The effect upon algal growth in those treatments was statistically significant ($p<0.05$) compared to the solvent control group and was considered treatment related.

The minimum inhibitory concentration (MIC) for *Selenastrum capricornutum* exposed to eprinomectin for 14 days was determined to be 29 mg a.i./L. The 14-day no observed adverse effect concentration was 7.0 mg a.i./L.

APPENDIX D-11

Zhao, P. L., 1995. Minimum Inhibitory Concentration and No Observed Adverse Effect Concentration for the 14-Day Toxicity Test of MK-397 to the Freshwater Alga.

Analyses of data (see APPENDIX D-10) included the t-test and a dose-response trend test (Tukey *et al*., 1985).

Mean cell density in the solvent control was inhibited by 34% compared to the negative control. The difference between the negative and solvent controls was statistically significant ($p<0.05$). Therefore, all statistical evaluations were made by comparing the treatment groups to the solvent control replicates.

There were no differences ($p\geq0.59$) in mean log cell density on day 14 between the solvent control and the 3.8 and 7.0 mg a.i./L treatment groups. Mean cell densities in the 15, 29, 58 and 119 mg a.i./L treatment groups were inhibited by 31, 64, 60 and 60%, respectively. The effect upon algal growth in those treatments was significant ($p<0.05$) compared to the solvent control group and was considered treatment related. The trend test indicated no treatment-related decreasing trend in cell density across the solvent control, 3.8 and 7.0 mg a.i./L levels ($p=0.46$), however, there was a treatment-related decreasing trend in cell density across the solvent control, 3.8, 7.0 and 15 mg a.i./L levels ($p<0.01$). Therefore, the no observed adverse effect concentration (NOAEC) based on cell density is 7.0 mg a.i./L and the minimum inhibitory concentration (MIC) is 15 mg a.i./L.

Specific growth rates for each replicate at each time interval were also determined. The maximum mean specific growth rates (mu-max) were obtained during days 2 to 4 for the controls and the 3.8, 7.0 and 15 mg a.i./L treatment groups and during days 4 to 6 for the 29, 58 and 119 mg a.i./L treatment groups. There were no differences ($p\geq0.084$) between the maximum mean specific growth rates between the solvent control and the 3.8, 7.0 and 15 mg a.i./L treatment groups. The trend test indicated no concentration-related relationship on mu-max across the solvent control, 3.8, 7.0 and 15 mg a.i./L levels ($p=0.532$). Inclusion of the 29 mg a.i./L data in the trend test resulted in a nearly significant ($p=0.056$) dose response, while inclusion of doses above 29 mg a.i./L produced significant ($p<0.01$) dose responses. Therefore, the no observed adverse effect concentration (NOAEC) based on the maximum mean specific growth rates (mu-max) is 15 mg a.i./L and the minimum inhibitory concentration (MIC) is 29 mg a.i./L.

APPENDIX D-12

Feutz, E. and L. Stuerman, 1995. Determining the Effects of MK-397 on Seed Germination and Root Elongation.

A phytotoxicity test was conducted with six representative species of terrestrial plants [cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), soybean (*Glycine max*), perennial ryegrass (*Lolium perenne*), tomato (*Lycopersicon esculentum*), and wheat (*Triticum aestivum*)] to determine the effects of MK-397 on seed germination and root elongation. This test was conducted by Analytical Bio-Chemistry (ABC) Laboratories, Inc., Columbia, MO according to the U.S. Food and Drug Administration (FDA) Technical Assistance Document 4.06. The MK-397 purity was 94.7%. Nominal concentrations were 1.0, 10, 100 and 1000 ppm. Mean measured concentrations of MK-397 in water containing 2% acetone were 0.81, 8.5, 95 and 1300 ppm using high performance liquid chromatography. Vehicle (2% acetone in water) and control (water) blanks were also tested. MK-397 did not completely dissolve into 2% aqueous acetone at the nominal concentrations of 100 and 1000 ppm. Thus, these test concentrations were suspensions. At the 10 and 1 ppm nominal concentrations, MK-397 was dissolved. Each treatment had six replicates, with 50 seeds in each germination dish per replicate. None of the seeds had been previously treated with any seed protectants. Temperatures were maintained at 25^{o} C (+/- 2^{o} C), with relative humidity greater than 95%. Seeds were observed periodically. The observation times were dependent upon the species tested. Germination was defined as the emergence of the primary root 3 mm outside the seed coat. When the average radicle length in the control was greater than 20 mm, testing was concluded. Root elongation data were collected from 10 randomly-chosen germinated seeds from each replicate. Measurements were made to the nearest millimeter using standard rulers incremented in 1 mm segments. All percent germination and radicle length measurements were transformed into ASCII files to be used in an ANOVA statistical program to analyze all data for significant differences compared to the vehicle control treatment.

Percent germination data indicated that MK-397 at 1300 ppm, the highest concentration tested, did not cause results different from the vehicle control for any of the six species tested. Thus, the no observed effect concentration (NOEC) for seed germination is 1300 ppm.

A significant difference in radicle lengths between the vehicle control and those from the 1300 ppm treatment (but not the 95 ppm treatment) was observed for cucumber and soybean. A significant difference in radicle lengths was noted between the 1300 and 95 ppm treatment (but not the 8.5 ppm treatment), and the vehicle control for ryegrass, lettuce, tomato and wheat. Thus, the NOEC values for root elongation were 95 ppm for cucumber and soybean, and 8.5 for ryegrass, lettuce, tomato and wheat.

APPENDIX D-13

Feutz, E. and L. Stuerman, 1995. Determination of the Effects of MK-397 on Seedling Growth.

A phytotoxicity test was conducted with six representative species of terrestrial plants [cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), soybean (*Glycine max*), perennial ryegrass (*Lolium perenne*), tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*)] to determine the effects of eprinomectin, MK-397, on seedling growth. This test was conducted by Analytical Bio-Chemistry (ABC) Laboratories, Inc., Columbia, MO according to the U.S. Food and Drug Administration (FDA) Technical Assistance Document 4.07. The MK-397 purity was 94.7%. Nominal concentrations were 1.0, 10, 100 and 1000 ppm. Mean measured concentrations of MK-397 in sand were 0, 0.47, 6.5, 68 and 710 ppm using high performance liquid chromatography. Vehicle (acetone) and control (water) blanks were also tested. Each treatment had five replicates, with 5 plants per replicate. Temperatures were maintained at 25^{o} C (+/- 4^{o} C), with relative humidity greater than 55 %. Seedlings were transplanted into pots containing the control or MK -397 treated sand. The seedlings were cultured in an environmentally controlled room for 21 days. Seedlings were subirrigated daily with one-half strength Hoagland's nutrient solution. Shoot length measurements were made on all seedlings on study days 0, 7, 14, and 21. Shoot and root weight measurements of the individual plants were made after drying for a minimum of 3 days at 40 -60^{o}C. All length and weight measurements were transformed into ASCII files for statistical analysis using the SAS statistical program. A one-way analysis of variance (ANOVA) was first run to determine the suitability of pooling the control and vehicle blank treatments. Dunnett's one-tailed method at alpha = 0.05 was used for comparison of the MK-397 treatments to the control(s). The NOEC values determined for percent inhibition of shoot length and shoot and root weights were based on the highest concentration which was not significantly different from the control treatment. The values of the NOEC were based on the mean measured concentrations from the day 0 and day 21 analysis. Results from the analysis of shoot length data indicated statistically significant inhibition of length occurred with all species following exposure to MK-397. Visual observations of the seedlings made weekly during the study indicated stunted growth as the most prevalent treatment effect. The effects in the 100 and 1000 ppm nominal treatments were suspected to be partly associated with the physical transformation associated with coating the sand with the test chemical. This change in the physical properties of the sand were noted by the decreased ability of the sand to wick-up the nutrient solution via sub-irrigation as readily as the controls and 1 and 10 ppm nominal treatments.

Based on the mean measured concentration of MK-397 during the study, the NOEC for shoot length was 0.47 ppm for cucumber, perennial ryegrass, tomato, and wheat, and 6.5 ppm for lettuce and soybean.

The results from the analysis of shoot weight mimicked the shoot length data for the six species. The NOEC for cucumber, perennial ryegrass, tomato, and wheat shoot weight was 0.47 ppm. The NOEC for lettuce and soybean shoot weight was 6.5 ppm. The root weight data also indicated growth inhibition with all of the species. Similarly, the NOEC was 0.47 ppm for cucumber, perennial ryegrass, tomato, and wheat. The NOEC for lettuce and soybean was 6.5 ppm.

APPENDIX D-14

Faidley, T, T. Murphy, S. Nicolich, P. Kochbarski and W. Langholff, 1995. MK-0397/Safety/Environmental Safety/Dung Fauna/Dung Beetle .

The purpose of this study (ASR 14602) was to determine the effect of MK-0397 in feces on adult and larval dung beetles in two species, *Onthophagus gazella* (Fabricius) and *Euoniticellus intermedius* (Reiche). The dung beetles were acquired from colonies maintained by Merck & Co., Inc. at the Branchburg Farm facility. Feces were collected from one female and 3 male castrate Holstein cattle, 2 years of age. Animals had not been treated with an avermectin or any insecticide within 100 days prior to fecal collection. Fecal material was homogenized, divided into seven, 5-kg aliquots and MK-0397 in 5 mL of dimethylformamide was added at 0 (vehicle-treated control), 6.7, 20, 60, 180 and 540 ppb to six of the aliquots. The remaining 5-kg aliquot served as a non-treated control. All samples were homogenized to ensure uniform distribution of the drug/vehicle. An aliquot of approximately 30 g was taken from each sample for MK-0397 analysis. The remaining feces from each aliquot was divided into 12 approximately 400-g sub-samples. The sub-samples were coded, blinding personnel conducting the dung beetle bioassay.

Fecal samples spiked with 0, 6.7, 20, 60, 180 and 540 ppb of MK-0397 were found on assay to contain an average of 0.0, 7.0, 24, 64.7, 166 and 590 ppb, respectively. Fecal pats were placed on top of soil in plastic pails and three male-female pairs of *O. gazella* or *E. intermedius* beetles were placed in each pail. There were 6 pails per treatment for each species. *E. intermedius* pails were maintained at 28°C and 45 to 52% relative humidity. *O. gazella* pails were maintained at 26.5°C to 28.5°C and 41 to 69% relative humidity. Adult capture was begun on the 7th day after adding the adults to the pails. The last live adult beetle of both species was captured on the 9th day. Live adults of both sexes were recovered from all pails. No treatment-related differences were noted in the number of live, breeding adults captured.

E. intermedius progeny were first captured on the 24th day and *O. gazella* progeny on the 27th day. On the 43rd day for the *E. intermedius* beetles and the 41st day for the *O. gazella* beetles, pails were emptied and the number of brood balls, dead adults, live and dead progeny and live and dead larvae were counted. No live progeny were recovered from any of the 166 or 590 ppb pails.

For all calculations the actual levels of MK-0397 found in the feces were used. The NOEL was calculated for each species separately using a trend test. A linear regression of the number of live progeny on the natural logarithm of (dose +1) was done, including all doses. If the slope was significantly ($p<0.10$) different from zero, the highest dose was dropped from the model and the analysis was repeated. This procedure continued until the slope was not significantly different from zero ($p>0.10$). The highest dose in the last regression analysis was then declared the NOEL. Prior to the analysis the data from the two control groups (untreated and solvent treated) were combined and classified as a dose of 0 ppb (means for the two groups were not significantly different ($p>0.10$)). In addition, all pails with fewer than five brood balls recovered were removed from the analysis (a total of four pails from *O. gazella* were removed).

The no-observable-effect level (NOEL) for both *E. intermedius* and *O. gazella* was 64.7 ppb. An LC_{50} could not be calculated based on the number of brood balls formed by the adults, because counting the brood balls prior to emergence disturbs the larvae and emergence of the progeny beetles disturbs the soil obscuring the brood balls.

This study shows that MK-0397 in feces has no effect on the reproduction of two species of dung beetles (*E. intermedius* and *O. gazella*) at concentrations of 64.7 ppb and less.