Environmental Assessment

Doramectin 1% injectable solution for the treatment of parasitic infections in cattle

Pfizer Inc

March 1996

ENVIRONMENTAL ASSESSMENT

TABLE OF CONTENTS

ENVIRONMENTAL ASSESSMENT

Doramectin 1% injectable solution for the treatment
of parasitic infections in cattle

1. DATE: March 3, 1996

2. APPLICANT: Pfizer Inc
(Sponsor #000069)

3. ADDRESS: 235 East 42nd Street
New York, N.Y. 10017

4. DESCRIBE THE PROPOSED ACTION:

a) Requested Approval and Need for the Action

Pfizer Inc is filing a New Animal Drug Application requesting approval for the use of doramectin 1% injectable solution in beef and non-lactating dairy cattle for the treatment and control of a variety of internal and external parasitic infections. Parasitism continues to be a primary cause of production losses in all cattle producing regions of the United States and doramectin 1% injectable will fulfill an unmet need for treatment and control of parasitic diseases caused by various infectious agents.

Doramectin 1% injectable solution would be given to cattle by intramuscular or subcutaneous injection at the recommended dose level of 200 mg doramectin per kilogram of body weight. Each mL of doramectin 1% injectable solution contains 10 mg doramectin, sufficient to treat 110 lb (50 kg) of body weight. Medication would not be given within 35 days of slaughter. Doramectin 1% injectable solution will be used wherever cattle are raised in the U.S., but particularly in Texas, Nebraska, Kansas, Oklahoma, Missouri, South Dakota, Montana, Kentucky, Tennessee and Florida.

b) Locations Where Bulk Drug or Injectable Solution Will be Produced and Types of Environments Adjacent to These Locations.

The bulk drug will be produced at Pfizer's existing manufacturing plant in Nagoya, Japan. The injectable product, a 1% oil solution, will be manufactured at Pfizer's Lee's Summit, Missouri plant.

1) Type of Environment at Nagoya, Japan

LOCATION - The Nagoya, Japan plant is located in an industrial area in the town of Taketoyo in Chita-Gun, Aichi Prefecture, approximately 40 km south of Nagoya, Japan. The plant is constructed on land reclaimed from Kinuura Bay and is bordered by the Bay on the North, East and South. To the West, the plant is bordered by plants operated by the Tokai Carbon Company and the Lubrizol Company for the production of carbon black and lubricating oils

respectively. The nearest dwellings are located approximately 0.6 km west of the plant. The town of Taketoyo population was 37,600 according to a 1991 census. Coordinates of the plant are latitude 34°51'N and longitude 136°56'W.

WEATHER/AIR RESOURCES - The annual precipitation at the Taketoyo town office (approximately 1.5 kilometers west of the plant) is 100 cm to 150 cm. Mean temperatures in summer and winter are about 26°C and 6°C respectively. Degree of air pollution by NOx, SOx or dust is not significant; mean values for 1991 of 0.014 ppm, 0.007 ppm and 0.042 ppm were measured at Taketoyo town which are well below the permit limits set by the national air pollution control law. In the Taketoyo area, no additional restrictions to those of the national law are imposed on the air emissions from the plant facilities. The yearly mean wind velocity is 2.5 m/sec with prevailing winds from the southeast direction in summer and from the northwest direction in winter.

WATER RESOURCES - There is no surface freshwater within 500 m of the plant boundary. The nearest surface freshwater is the Hori River, a small river flowing into Kinuura Bay from the west and the east, the mouth of which is 700 m southwest of the plant. Approximately 80 percent of the plant's water supply is obtained from municipalities (ca. 2,700 m^3/day of industrial water supplied from the prefecture-owned Yahagi Dam which is 30 kilometers northeast of the plant and about 700 m^3/day of potable water from Taketoyo Town); the remainder (ca. 500 m^3/day) is obtained from four on-site wells. Wastewaters from the plant, e.g. fermentation broth filtrates, are pumped to storage tanks at the biological oxidation treatment plant. Wastewaters are blended with more dilute wastes such as floor washings and sanitary sewers at a controlled rate to provide relatively uniform loading to the treatment plant. The effluent from the treatment plant is discharged into Kinuura Bay through the outfall 60 m off the sea wall in compliance with applicable regulations and guidelines. Plant's rain water is collected separately through underground ditches and discharged directly to Kinuura Bay.

LAND RESOURCES - The composition of the reclaimed land that accommodates the Nagoya plant has been determined by means of test borings. The layer from the ground surface to 3 meters in depth is reclaimed soil consisting of yellow-brown sand and gravel with small amounts of silty clay and concrete fragments. The layer from 3 to 17 meters is alluvial marine silt clay with a large amount of shells. The layer from 17 to 30 meters to yellow-brown sand containing a small amount of gravel and serves as the bearing stratum for pile foundations of the plant. The plant site has an elevation of 0.5 m and is surrounded by sea walls to the north, east and south, and to the west is bordered by plants operated by the Tokai Carbon Company and Lubrizol Japan which are also located on the same reclaimed land.

2) Type of Environment at Lee's Summit, MO

LOCATION - The Lee's Summit facility is located on a 103.3 acre site in Lee's Summit, Jackson County, Missouri. The city of Lee's Summit is located approximately 25 miles southeast of Kansas City, MO. Lee's Summit's 1990 population was listed as 47,500 by the U.S. Census Bureau. Local economic indicators in December 1991 indicates that the population is increasing annually by 2,200. The facility is situated on the northern 25 acres of the 103.3 acre site. The remaining property is undeveloped. The site is flanked on its west boundary by State Highway 291. The east boundary is flanked by the west line of the Missouri Pacific Railroad right-of-way. The immediately surrounding areas are zoned for light industrial use. Coordinates of the facility's location are latitude N 38° 53 min 30 sec and longitude W 94°, 22 min and 22 sec. The county coordinates are Section 17, Township 47 North and Range 31.

WEATHER/AIR RESOURCES - Meteorological data for the area are collected at the Kansas City International Airport (approximately 40 miles from the facility). The mean average annual precipitation is 36 inches. During December-February the average high temperature is approximately 38°F, and the average low is approximately 21°F. During June-August, the average high temperature is approximately 86°F, and the average low is approximately 66°F. Prevailing winds in the area are from the south.

The Kansas City five county metropolitan area meets the USEPA federal clean air standard for ground level ozone. The Lee's Summit facility is regulated for air emissions under the Missouri Air Pollution Control Program that is under the authority of the Division of Environmental Quality, Missouri Department of Natural Resources. Particulate emissions are regulated under the Missouri Air Pollution Control Regulation 10 CSR 10-2. The state program incorporates into its regulations: New Source Performance Standard (NSPS), National Emission Standard for Hazardous Air Pollutants (NESHAPS), and National Ambient Air Quality Standards.

Lee's Summit, Missouri is in USEPA Region VII.

WATER RESOURCES - All water used for consumption, process, sanitation, firefighting, and groundskeeping is purchased through the Lee's Summit Water Department. The Lee's Summit Water Department sources 30% of their water from the Kansas City Water District and 70% from the Independence Water District. These districts derive their water both directly from the Missouri River and from deep aquifers located near the Missouri River. The water quality meets the standards for potable water.

There are no sources of potable or public access waters on or near the facility property. The nearest surface water body is a small pond located on the site about 1000 feet south of the facility. The facility is located on top of a watershed that is the approximate intersection of three drainage basins. Ephermeral streams (flowing only during wet periods) are located to the

west, northwest, and south of the property. These streams when filled with water feed into the Cedar Creek basin, East Fork Little Blue River basin, and the Big Creek basin, respectively. The dominant drainage area on the property is that associated with the Big Creek basin. The nearest 100 year flood plain is that associated with Cedar Creek basin and is approximately 1/2 mile from the facility.

The conveyance system for stormwater is separate from that for the process and sanitary sewer system. The wastewater from the process and sanitary sewer system flow to the Little Blue Valley Sewer District (LBVSD) wastewater treatment plant. The discharge of process waste water into the Lee's Summit municipal sewer must meet the conditions and terms set forth in the Industrial User Discharge Permit, #3LB-0496-LS205, issued to the facility by the LBVSD. The LBVSD operates under the direction of the Environmental Protection Agency. All the above are under the Clean Water Act's General Pretreatment Standards 40CFR Parts 403 and Missouri Clean Water Regulations 10CSR 20-6.

Water from the storm water conveyance system is discharged to the drainage basins that are mentioned above. Stormwater collected in the Cedar Creek and East Fork Little Blue basins are discharged to the Little Blue River, a tributary of the Missouri River. Storm water collected in the Big Creek basin is discharged to the South Grand River, a tributary of the Osage River. Discharge of the stormwater is subject to Missouri Clean Water Regulations 10CSR 20-6.200.

LAND RESOURCES - Jackson County, Missouri lies in the Osage Plains and is underlain by a sequence of sedimentary rock of the Paleozoic Pennsylvanian (Missourian series) age totaling more than 2,200 ft in thickness. Borings taken at the Pfizer Lee's Summit site have variously encountered shales, limestones or sandstones immediately below the soil. In borings taken down to a depth of 27-28 ft, a very hard, light gray crystalline limestone has been encountered. Soils on the upland areas of the property have been assigned to the Macksburg silt loam. Soils formed along the slightly concave slopes adjacent to the Macksburg uplands have been assigned to the Sampsel silty clay loam. The recorded thickness of the soil cover from borings ranges from 12 to 25 ft. The upper few feet of the soil cover is typically dark gray to brown silty clay with some organics. The remaining soil layer under this is variably dark gray to brown highly-plastic silty clay. The property is situated on the southwest flank of an anticline that constitutes the upper reaches of three drainage basins. The elevation of the facility is 1053 ft above mean sea level. The elevation of the ground drops southward across the property. Topographical relief on the uplands of the property where the facility is located is relatively low. Total relief across the property within any of the drainage basins is less than 65 ft. The Missouri-Pacific railroad right-of-way and construction along State Highway 291 have created artificial water divides along the west and east property lines.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION:

A. Doramectin

Doramectin is an antiparasitic macrolide produced by *Streptomyces avermitilis*. It belongs to a class of fermentation derived metabolites known as avermectins.

Generic Name: Doramectin

Trade Name: DECTOMAX

Chemical Name: 25-cyclohexyl-5-*O*-demethyl-25-de(1-methylpropyl) avermectin A1a or (2a*E*, 4*E*, 8*E*)-(5'*S*, 6*S*, 6'*R*, 7*S*, 11*R*, 13*S*, 15S, 17a*R*, 20*R*, 20a*R*, 20b*S*)-6'-cyclohexyl-5',6.6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20.20b-dihydroxy-5',6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*, 13*H*, 17*H*-furo-[4,3,2-[*pq*][2.6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-7-yl 2.6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-*a*-L-*arabino*-hexopyranosyl)-3-*O*-methyl-*a*-L-*arabino*-hexopyranoside

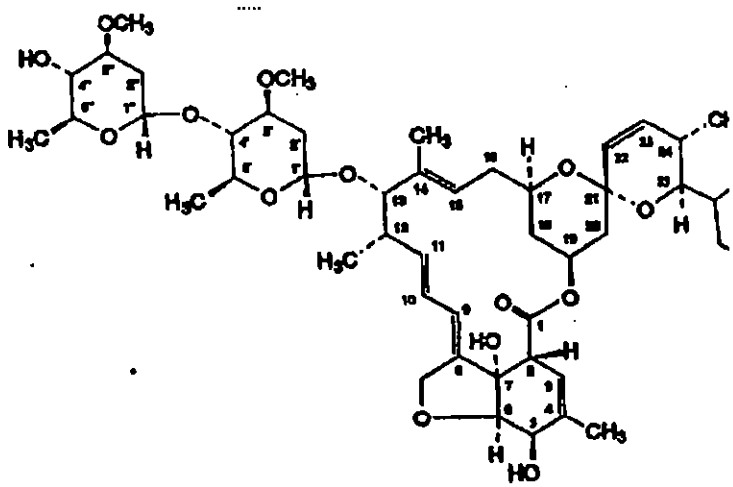
CAS Registry Number: 117704-25-3

Pfizer Code Number: UK-67,994

Molecular Formula: $C_{50}H_{74}O_{14}$

Molecular Weight: 899.13

Structural Formula:



B. Other Injectable Solution Ingredients:

In addition to doramectin, DECTOMAX 1% injectable solution contains 75% sesame oil, 25% ethyl oleate and 0.25% phenol.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

A. From the Sites where Bulk Drug is Produced:

The manufacture of doramectin will be carried out in purpose built fermentation and recovery facilities designed with doramectin containment in mind and to be in compliance with all applicable emissions requirements. The plant is located in Nagoya, Japan and will operate in accordance with local environmental regulations.

1. Production/Processing Overview

Doramectin is fermented in a media consisting of carbohydrates, organic nitrogen sources, fats, fatty acids, oils, mineral salts, miscellaneous inorganic and organic compounds and antifoams in tanks provided with a suitable means of agitation, aeration, temperature control and pH control.

The whole broth is concentrated using conventional filtration, centrifugation or ceramic membrane filtration, then doramectin is extracted from the mycelia concentrate using a suitable solvent at optimal pH. Doramectin dissolved in the solvent may be concentrated by evaporation of a portion of the solvent prior to precipitation through the addition of water, organic salts, and/or inorganic salts. Doramectin precipitate is isolated, redissolved in an appropriate solvent, treated with an adsorbent to remove color prior to crystallization through the addition of water, cooling and/or the addition of inorganic salts. Doramectin crystals may be recrystallized prior to isolation, drying and milling, if necessary.

2. Manufacturing and Occupational Safety

a. Material Safety Data Sheets

The manufacturing site will make available to employees the appropriate detailed Material Safety Data Sheets (MSDS) essentially similar to OSHA Form 20. The MSDS for doramectin and injectable doramectin 10 mg/ml will contain the information shown in the attached examples (Appendix a-1), though the format and local language will vary from one site to another.

b. Hazard Evaluation Studies

Results of acute dermal and ocular irritation studies conducted with albino rabbits indicate that doramectin is neither a primary skin irritant or an ocular irritant:

Of three intact and three abraded rabbit skin sites evaluated, only very slight, non-confluent erythema was apparent at one intact and two abraded sites following a 48 hour exposure to 0.5 g doramectin. No edema was observed and all six sites appeared normal by 72 hours post dose (Appendix c-24).

Instillation of 18.8 mg doramectin to the conjunctival sac caused slight reddening of the conjuntivae, chemosis in two of three rabbits evaluated and iritis in one of three animals. By 48 hours post dose, each treated eye appeared normal (Appendix c-24).

c. Occupational Safety

Steps have been taken to minimize occupational and user exposure to doramectin at Pfizer bulk drug and injectable solution manufacturing sites. The facility at Nagoya, Japan, where doramectin bulk is produced, is equipped with appropriate physical isolation and air handling facilities to minimize worker exposure. Many of the production operations are automated. Worker exposure to doramectin will be monitored by at least semi-annual monitoring of dust levels where doramectin powder is handled. Exposure to solvents will be monitored in compliance with Industrial Safety and Health Law, Article 65. The health of employees will be monitored in accordance with the Industrial Safety and Health Law, Article 66. Pfizer workers at all sites will wear appropriate protective equipment including gowns, gloves and protective masks as circumstances require.

3. Emissions

The substances which could be emitted and/or discharged from Nagoya, Japan are listed along with the respective exposure limits (when available):

Substance Used	Chemical Abstracts Registry No.	TWA[a] ppm	TWA[a] µg/m³
Acetone	67-64-1	1,000,000	2,400,000
Alpha amylase, Rhozyme	N/A	NL	NL
Aluminum oxide	1344-28-1	N/A	10,000
Ammonium sulfate	7783-20-2	NL	NL
Ammonium phosphate mb.	7722-76-1	L	L
Ammonium phosphate db.	7783-28-0	L	L
Ammonium carbonate	1111-78-0	L	L
Ammonium hydroxide	1336-21-6	0,000 as NH_3	5000 as NH_3
Ammonium nitrate soln.	6484-52-2	L	L
Amylase, termamyl	N/A	L	L
Amylglucosidase 200	N/A	L	L
Antifoam Pluronic L-61	9003-11-6	NL	NL
Antifoam BI0-1110	/A	L	L
Antifoam, silicone	N/A	NL	NL
Antifoam Breox FMT-30	N/A	NL	NL
Autolyzed yeast extract	8013-01-2	NL	NL
Bakers yeast	N/A	NL	NL
Betaine hydrochloride	590-46-5	NL	NL
Biotin	58-85-5	NL	NL
Brewers yeast	N/A	NL	NL
Calcium chloride	10043-52-4	NL	NL
Calcium carbonate	1317-65-3	N/A	15000 (Total)
Calcium nitrate	10124-37-5	NL	NL
Calcium hydroxide	1305-62-0	N/A	5000
Calcium oxide	1305-78-8	N/A	5000
Calcium pantothenate	N/A	NL	NL
Canola meal	N/A	NL	NL
Carbon, activated	7440-44-0	N/A	3500
Casein	9000-71-9	NL	NL
Chloine chloride			
Choice white grease	N/A	NL	NL
Cobaltous chloride hex.	7791-13-1	NL	NL
Corn flour	N/A	NL	NL
Corn syrup	8029-43-4	NL	NL
Corn starch	9005-25-8	N/A	15000 (Total)
Cornstep liquor	66071-94-1	NL	NL
Cottonseed meal	68424-10-2	NL	NL
Cottonseed meal	68424-10-2	NL	NL
Cottonseed oil	8001-29-4	NL	NL
Cychohexanecarboxylic acid	98-89-5	NL	NL
Dextrin Hidex 50	9004-53-9	NL	NL
Dextrose	50-99-7	NL	NL
Doramectin	N/A	NL	NL

Substance Used	Chemical Abstracts Registry No.	TWA[a] ppm	TWA[a] µg/m³
Ethanol	64-17-5	1,000,000	1,900,000
Ehtyl acetate	141-78-6	400,000	1,400,000
Ferrous sulfate hept.	7782-63-0	NL	NL
Filteraid	N/A	N/A	N/A
Folic acid	75708-92-8	NL	NL
Fungamyl 1600	N/A	NL	NL
Glucose	50-99-7	NL	NL
Glutamic acid	56-86-0	NL	NL
Heptane	142-82-5	500,000	2,000,000
Hexane	110-54-3	500,000	1,800,000
Hydrochloric acid	7647-01-0	5000[b]	7000[b]
Hydrolyzed soy protein	N/A	NL	NL
Hydrolyzed casein	9000-71-9	NL	NL
Isobutyric acid	79-31-2	NL	NL
Isopropyl alcohol	67-63-0	400,000	980,000
Isovaleric acid	503-74-2	NL	NL
L-isoleucine	73-32-5	NL	NL
L-leucine	61-90-5	NL	NL
L-lysine hydrochloride	657-27-2	NL	NL
L-methinonine	63-68-3	NL	NL
L-tryosine	N/A	NL	NL
L-valine	72-18-4	NL	NL
Lactic yeast	N/A	NL	NL
Magnesium sulfate	7487-88-9	NL	NL
Magnesium sulfate hept.	10034-99-8	NL	NL
Maltose	6363-53-7	NL	NL
Manganese chloride	7773-01-5	N/A	C = 5000 (As Mn)
Methanol	67-56-1	200,000	260,000
Methyl Buteric Acid		NL	NL
Methylene chloride	75-09-2	500,000	1,738,000
Monosodium glutamate	142-47-2	NL	NL
Niacin	59-67-6	NL	NL
NZ amine B	N/A	NL	NL
NZ amine A	N/A	NL	NL
NZ amine B	N/A	NL	NL
NZ amine A	N/A	NL	NL
NZ amine BT	N/A	NL	NL
NZ Amine YTT	N/A	NL	NL
NZ amine YT	N/A	NL	NL
Pentane	109-66-0	1,000,000	2,950,000
Peptonized milk	N/A	NL	NL
Pharmamedica	N/A	NL	NL
Polypropylene glycol	25322-69-4	NL	NL
Polystyrene resin	9003-53-6	NL	NL
Potassium chloride	7447-40-7	NL	NL

Substance Used	Chemical Abstracts Registry No.	TWA[a] ppm	TWA[a] µg/m³
Potassium hydroxide	1310-58-3	N/A	2000[b]
Potassium phosphate	7778-53-2	NL	NL
Potassium phosphate DB	16788-57-1	NL	NL
Potato starch	N/A	NL	15,000
Rapeseed oil	N/A	NL	NL
Rice bran oil	N/A	NL	NL
Sesame oil	8008-74-0	NL	NL
Silicone dioxide	60676-86-0	NL	NL
Sodium chloride	7647-14-5	NL	NL
Sodium hydroxide	1310-73-2	N/A	2000
Sodium lauryl sulfate	151-21-3 AND 51222-39-0	NL	NL
Sodium bicarbonate	144-55-8	NL	NL
Sodium phosphate DB	7558-79-4	NL	NL
Sodium sulfate	7757-82-6	NL	NL
Sodium citrate	18996-35-5	NL	NL
Sodium proprionate	137-40-6	NL	NL
Sodium succinate	150-90-3	NL	NL
Sodium phosphate MB	7558-80-7	NL	NL
Sodium chloride	7647-14-5	NL	NL
Sodium nitrate	7631-99-4	NL	NL
Sodium phosphate DB, anhy.	7558-79-4	NL	NL
Sodium sulfate	7757-82-6	NL	NL
Sodium acetate	127-09-3	NL	NL
Sodium hydroxide	1310-73-2	N/A	2000
Sodium glutamate	142-47-2	NL	NL
Solka floc	9004-34-6	NL	15,000
Soy flower	N/A	NL	NL
Soybean meal	N/A	NL	NL
Soybean flour	N/A	NL	NL
Soybean oil	8001-22-7	NL	NL
starch syrup	N/A	NL	NL
Starch	9005-25-8	N/A	15,000 (Total)
Sucrose	50-20-4	NL	NL
Sulfuric acid	7664-93-9	N/A	1000
Thiamin hydrochloride	67-03-8	NL	NL
Thiamine mononitrate	532-43-4	NL	NL
Torula yeast	N/A	NL	NL
Urea	57-13-6	NL	NL
Wheat starch	N/A	NL	NL
Wheat germ	N/A	NL	NL
Whey	50887-69-9	NL	NL
Whey permeate	N/A	NL	NL
Zinc sulfate hept.	446-20-0	NL	NL

(a) Allowable 8 hour time weighted average exposure according to OSHA Air Contaminants 29 CFR 1910.1000 or limits set by ACGIH.
(b) Ceiling limit
N/A = Not Available
NL = No Limit

4. Nagoya, Japan Site

The Nagoya plant site is located on Kinuura Bay in Taketoyo Town, Japan. This multi-product pharmaceutical manufacturing facility maintains an environmental control program for proper management of liquid and solid wastes and airborne emissions. Treatment and disposal operations include liquid mixing and pretreatment, solid and liquid waste incineration, ventilation and dust collection, vapor condensation and scrubbing.

Solid Wastes

The following are generated during fermentation, concentration and isolation of doramectin.

1. Mycelial solids from the extracted doramectin fermentation broth in a slurry with water, solvents such as methanol and isopropanol, and small amounts of avermectins.

2. Filter aid and carbon cake from the refining process containing water, solvents such as methanol, isopropanol and unrecovered by-products including small amounts of avermectins.

3. Paper and trash generated during the production operations. These solid wastes will be handled in compliance with national requirements of the Environmental Protection Agency Regulations, Article 12 of the Industrial Waste Disposal Control Law and with the Taketoyo Town Environmental Protection Regulations, Articles 20-30.

In order to meet these requirements, all solid wastes will be incinerated under the agreement and Permit of Taketoyo Town. The ash generated from incineration will be landfilled in compliance of an agreement with the Department of Environmental Protection of Aichi Prefecture.

Liquid Wastes

The manufacturing process generates both aqueous and solvent-based streams.

The solvent-based stream is generated in the recovery of solvents used in the product recovery and purification process, such as methanol, isopropanol and hexane. This stream will be destroyed by incineration as certified by the Prefectural Government in compliance with the Environmental Protection Regulations, Article 19.

The aqueous stream consists of the spent fermentation broth filtrate and wash water and contains unconsumed fermentation nutrients, unrecovered by-products and traces of avermectins. This stream will be treated in a chemical pre-treatment unit designed to destroy residual avermectins. The treated stream will receive final biological treatment in a six-stage waste treatment plant.

The effluent from this facility is discharged into Kinuura Bay in compliance with limitations imposed by the Environmental Protection Agreement, with Taketoyo Town, Articles 16-20 and by the National Water Pollution Prevention Law, Article 3.

Air Emissions

Vented air from the fermentation stage will be introduced to a mechanical mist separator to remove possible broth aerosols, prior to venting to the atmosphere. The separated aerosol will be chemically pre-treated and disposed of via the site biological treatment system.

Vent gases from the product recovery process will contain volatile organic compounds such as methanol, isopropanol and hexane and will be controlled as appropriate by condensers. In the product drying area, the air is dust filtered by HEPA filtration to contain any potential product dust. All of these air emissions will be in compliance with the Air Pollution Prevention Law, Article 3; Prefectural Environmental Regulations, Article 19 and the Agreement with Taketoyo Town, Article 16.

The attached statement (Appendix a-2) certifies compliance with all Federal, Prefectural and local emission requirements.

B. From the Site where Injectable Solution will be Produced:

Lee's Summit, Missouri

Doramectin will be compounded/mixed into an injectable solution and packaged for sale at Pfizer Inc's plant for the manufacture of animal health products. The plant is located at One Pfizer Way, Lee's Summit, Missouri and is designed to maintain compliance with all Federal, State and local emission requirements.

The injectable solution manufacturing operation will involve only the compounding/mixing and packaging of doramectin with other ingredients in equipment constructed of non-reactive product contact parts. The ingredients of the injectable solution will be added to a mixing tank in prescribed order and mixed. After the necessary Quality Assurance tests are complete, the injectable solution will be sterile filtered and transferred to bottles via a filling machine. The production of injectable solution will not generate hazardous wastes as defined by the Federal Regulations 40 CFR 261 or by the Missouri Hazardous Waste Management Law 10 CSR 25-4.261.

Solid Wastes

Dry solid waste (such as paper, plastic, glass) generated during the manufacture that are contaminated with doramectin bulk, doramectin injectable, or the excipients will be destroyed by incineration. This waste specifically may include empty fiber and plastic drums, polyethylene drum

liners, empty glass bottles, closures and disposable protective apparel. The incineration process is covered under Federal Regulations 40 CFR 264 or by Missouri Solid Waste Rules 10 CSR 25-7.264.

Liquid Wastes

The manufacturing process generates two liquid waste streams. One stream is oil based, and one is aqueous based. The oil based stream consists of residual doramectin injectable that is drained from the equipment and transfer lines prior to the cleaning procedure. The aqueous stream is generated by equipment and transfer line washings. It consists of water, cleaning agent, and trace amounts of doramectin injectable. The waste streams will be collected and destroyed by incineration as a non-hazardous special waste. The incineration process is covered under Federal Regulations 40 CFR 264 or by Missouri Solid Waste Rules 10 CSR 25-7.264.

Air Emissions

None of the components of manufacture are volatile. Emission of particulate matter during the transfer of the doramectin bulk powder to the compounding vessel is controlled by local ventilation. Air emissions would be subject to the Clean Air Act and the Clean Air Act Amendments codified in 40 CFR Parts 50, 52 and 60, and the Missouri Air Pollution Control Regulation 10 CSR 10-2, the Missouri Department of Natural Resources Air Pollution Program, Division of Environmental Quality. The attached statement (Appendix a-3) certifies compliance with all Federal, State and local emissions requirements.

The 1% injectable product (DECTOMAX) will be manufactured in a new, semi-automated plant located in Lee's Summit, Missouri, which has been specifically designed to minimize worker exposure. Exposure to doramectin will be minimized by means of personnel protective equipment, and by the design of the air handling systems.

During routine manufacturing operations, occupational exposure to doramectin bulk powder will be very short in duration (e.g., approximately 30 minutes or less per production lot of doramectin injection) and well below the 8-hr work exposure limit set by Pfizer.

C. Introduction of Substances as a Result of Use:

1. Doramectin Administration to Cattle

Doramectin will be administered to both pastured and feedlot cattle. Since the latter represent a denser population, they will be used to estimate upper limits for the amount and concentration of doramectin introduced into the environment. The average amount of drug administered to a single animal can be estimated as follows. Feedlot cattle will most commonly be treated shortly after arrival at the feed lot. Assuming the average body weight of

270 kg upon arrival (Environmental Protection Agency, 1974) and a dose level of 0.20 mg/kg, a typically treated animal will receive 54 mg of doramectin.

$$270 \text{ kg} \times 0.20 \text{ mg/kg} = 54 \text{ mg}$$

2. Metabolism and Excretion of Doramectin by Cattle

Doramectin would be introduced into the environment intermittently and in low concentrations through the feces and urine of medicated cattle following administration of the drug parenterally as a single dose at 200 µg/kg body weight. Over a 14 day period following subcutaneous administration of tritiated doramectin at 200 µg/kg to male and female cattle averaging 203 kg in weight, assay of feces and urine accounted for 87% and 1%, respectively, of the dose (Appendix c-1). In feces, 49% of the total dose was recovered as unchanged drug. The maximum mean concentration of unchanged drug in feces was observed 3 days after treatment (319 ppb, 58% of the day 3 residues). In a separate study (CM-92-01), a single major metabolite of doramectin was observed in cattle feces at day 3 post dose, accounting for 14% of the recovered day 3 residues. This metabolite was identified by Fast-Atom Bombardment mass spectrometry as 3"-O-desmethyldoramectin. Two other components, which accounted for 4% and 5% of the recovered day 3 activity, respectively, were likewise identified as 24-hydroxymethyl-3"-O-desmethyldoramectin and 24-hydroxymethyldoramectin. Doramectin and 3"-O-desmethyldoramectin were evaluated against *Daphnia magna*, the aquatic species that was the most sensitive to doramectin of those tested. When EC_{50} values were compared at the 48 hour period in a static toxicity test, the desmethyl metabolite appeared to be approximately 8 times less toxic (Appendices c-19 and c-20). Nevertheless, a conservative approach is to assume that the entire administrated dose is excreted as the equivalence of unchanged drug.

3. Concentration of Doramectin in Excreted Cattle Wastes

A feedlot animal typically produces 22 kg of wet waste per day (Environmental Protection Agency, 1974) and over the course of a typical 136 day stay in the feedlot (Feedstuffs, 1988) would produce a total of 2992 kg wet waste:

$$22 \text{ kg wet waste/day} \times 136 \text{ days} = 2992 \text{ kg wet waste}$$

A worst case estimate assumes that each animal will be treated once. Therefore, the average maximum concentration of drug plus metabolites in the excreted wet waste would be 18 ppb:

$$\frac{54 \text{ mg drug excreted}}{2992 \text{ kg waste}} = \frac{0.018 \text{ mg}}{\text{kg}} = 18 \text{ ppb}$$

4. Concentration of Doramectin in Aged Feedlot Wastes

Fresh cattle excreta contains about 80% water by weight (Ensminger, 1976), whereas after aging on the feedlot, moisture content is reduced to about 25-40% (Environmental Protection Agency, 1974; Sweeten and Withers, 1990). Assuming an average moisture content of 30% in aged feedlot waste and no degradation of doramectin residues in the manure, the concentration of doramectin residues would be increased by a factor of 2.7 (0.80/0.30) over that expected in wet waste, giving maximum expected concentrations in aged feedlot waste of approximately 0.049 mg/kg or 49 ppb (0.018 mg/kg x 2.7).

5. Potential Concentration of Doramectin in Soil Amended with Feedlot Wastes

Use of feedlot manure containing doramectin as fertilizer would result in introduction of the drug into the soil. The resulting concentration of drug in soil can be estimated from the concentration of drug in aged manure and the rate of application of aged manure to soil.

Manure is incorporated into the top 15 cm of soil at a rate of 5-20 tons aged waste/acre/year (Ensminger, 1976; Sweeten and Withers, 1990). At a density of 1.5×10^3 kg/m^3, the top 15 cm of soil weighs about 9.1×10^5 kg/acre; therefore, using an average rate of incorporation of 15 tons (13.6 metric tons) manure/acre/year, use of aged manure containing 49 ppb doramectin residues would result in a maximum concentration in soil of only about 0.73 ppb drug residue:

$$(0.049 \text{ mg/kg})(13.6 \times 10^3 \text{ kg/acre}) = 6.66 \times 10^2 \text{ mg/acre}$$

$$(6.66 \times 10^2 \text{ mg/acre}) \div (9.1 \times 10^5 \text{ kg/acre}) = 7.3 \times 10^{-4} \text{ mg/kg or } 0.73 \text{ ppb}$$

This is a worst case estimate, which assumes treatment of all animals and no degradation of doramectin in the excreta prior to incorporation into soil.

6. Amount of Drug Used and Introduced into the Environment

a. Quantity

It is estimated that use of doramectin for the therapy of parasitic infections of cattle could result in up to approximately 2.7 metric tons being used and introduced into the environment annually. This estimate is based on the amount of drug needed to medicate a single animal and the number of animals likely to be medicated over the period of a year.

The 1994 USDA survey indicates that approximately 34.9×10^6 beef cows and approximately 31.3×10^6 calves and stockers were on pasture and approximately 25×10^6 cattle were processed through feedlots. Survey information collected for the southwestern U.S. (Section 6.C.6b) indicates that during the second and fourth quarters of the year, approximately 20% of the cattle on pasture were treated with ivermectin while approximately 10% of the population were treated in each remaining quarter. Assuming that ivermectin use is a close proxy of the maximum doramectin usage and further assuming a similar rate of treatment across the U.S., approximately 21×10^6 beef cows and 19×10^6 calves and stockers would be treated over the entire year. Furthermore, if as many as 25% of feedlot cattle were treated an additional 6×10^6 cattle would be treated over the entire year. Assuming a dose level of 0.2 mg/kg and average body weights for beef cows, calves/stockers and feedlot cattle at time of treatment of 432 kg, 145 kg and 270 kg, respectively, animals would receive 86.4, 29 and 54 mg doramectin, respectively. Therefore, treatment of the number of cattle indicated above would result in use and excretion of approximately 2,700 kg doramectin:

86.4 mg/beef cow $\times 21 \times 10^6$ beef cows $= 1.814 \times 10^9$ mg or 1,814 kg

29 mg/calf-stocker $\times 19 \times 10^6$ calves-stockers $= 551 \times 10^8$ mg or 551 kg

54 mg/feedlot cow $\times 6 \times 10^6$ feedlot cattle $= 324 \times 10^8$ mg or 324 kg

Total = 2,689 kg or approximately 2.7 metric tons

b. Pattern of Use

Cattle pasturing patterns were examined and ivermectin usage was determined to better understand the introduction of residues into the pasture environment as a result of use. The objective was to determine the spatial and temporal use of ivermectin among pastured cattle so that the proportion of dung pats containing residues could be established. Several veterinarians that practice deworming and rely principally upon ivermectin provided input to help determine what proportion of a given herd may be expected to receive treatment and the likelihood that treatment of adjacent herds occurs simultaneously.

Section 8.C.1 (below) addresses a concern that use of doramectin in pastured cattle may adversely affect dung dependent arthropods and degradation of dung in pastures. Dung beetles were identified specifically as insects that might

be threatened. Information provided in Section 8.C.1 leads to the conclusion that non-native (exotic) dung beetles introduced and established in the southern states and in Hawaii are the only species that would potentially be threatened. Therefore, data collection was limited to the southern states where exotic dung beetles are established and where significant numbers of cattle are kept on pastures. Drug usage was focused on ivermectin because it is the only avermectin approved for use in the U.S. It is believed that introduction of doramectin would be unlikely to increase overall usage of avermectins. Thus, it is assumed that current ivermectin usage is a close proxy of the maximum doramectin usage.

A survey focused on two regions, the Southwest U.S. and Southeast U.S. where data exist that track ivermectin usage. States that comprise each region are listed below:

Southwest	Southeast
Arizona	Alabama
Arkansas	Florida
New Mexico	Georgia
Oklahoma	Louisiana
Southern California	Mississippi
Texas	South Carolina
	Maryland
	North Carolina
	Virginia

A usage tracking study (Doane, 1992-1994) was used to conduct the regional survey. This is a quarterly syndicated mail survey conducted by an independent market research firm which specializes in the agriculture industry. The survey monitors animal health product usage of producers in the U.S. and is widely used by animal health companies and others to understand markets.

The key objective of the survey was to accurately project the number of animals treated with ivermectin products over 3 years (1992-1994). A secondary objective was to measure seasonal product usage. Thus seasons in this study are defined to be months with more consistent weather patterns (Dec-Jan-Feb, Mar-Apr-May, Jun-Jul-Aug, Sep-Oct-Nov). The sample size was approximately 2,000 cattlemen each quarter.

The above survey was supplemented by a survey of approximately 300 cattle producers commissioned by Pfizer, Inc in 1991 and designed to accurately project ivermectin usage patterns in various regions of the U.S. on a monthly basis.

An additional survey (Pfizer, 1995) was conducted to determine in detail ivermectin treatments during peak usage periods in three local areas, each of approximately 50 square miles in size, located in high density cattle rearing counties of Texas and Florida. Information was obtained by monitoring

ivermectin purchases over key 3 month periods and by interviewing key practicing and extension service veterinarians.

Key Pastured Cattle Areas:

The highest concentrations of pastured cattle (e.g. beef cows) are located in the "Cattle Belt" which runs through the midwest United States. The top 15 pastured cattle states are listed in the table below. These states account for 70% of all pastured cattle. Texas is the top pastured cattle state and accounts for nearly 17% of pastured cattle in the U.S. Florida ranks number 10 and accounts for 3.2% of all pastured cattle.

Table 1. Key Pastured Cattle States

Rank	State	1994		
		Inventory (000's)	Percent	Cumulative Percent
1	Texas	5,800	16.7	17
2	Missouri	2,230	6.4	23
3	Nebraska	1,920	5.5	29
4	Oklahoma	1,842	5.3	34
5	South Dakota	1,598	4.6	39
6	Montana	1,478	4.3	43
7	Kansas	1,473	4.3	47
8	Kentucky	1,155	3.3	50
9	Tennessee	1,130	3.3	54
10	Florida	1,093	3.2	57
11	Iowa	1,075	3.1	60
12	North Dakota	930	2.7	63
13	Arkansas	926	2.7	65
14	Alabama	862	2.5	68
15	Colorado	820	2.4	70

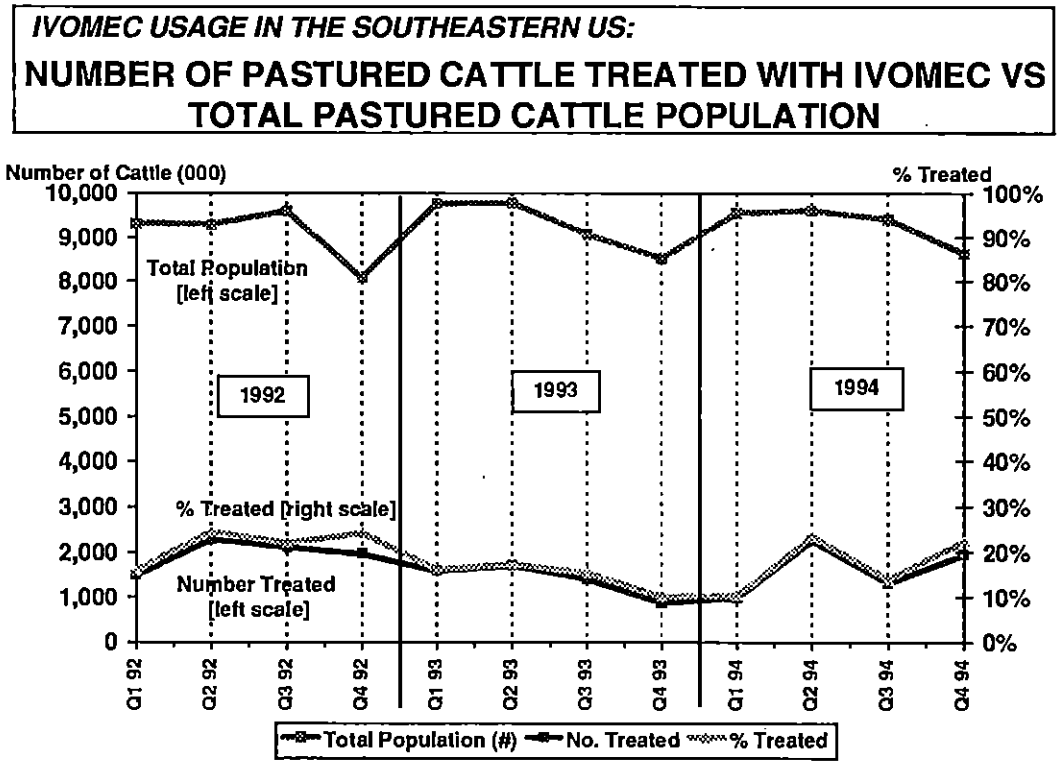
The cattle population in the survey areas of the southern United States fluctuated around 30 million head with some seasonal variation from 1992 through 1994. The largest concentration of pastured cattle exists during the January to July time period with the second quarter typically a time of peak numbers. This is because most southwest operations have a spring calving season while many southeast operations calve in the fall. Thus, cattle on pasture peak in the spring (2Q) as spring calves are born in the southeast and fall calves mature in the southwest. Also contributing to peak numbers during this time are cattle from cold-winter areas which are moved to the southwest for winter grazing.

A. Regional Survey:

Chart 1 (below) compares the total number of cattle on pasture each quarter in the Southeast survey region during 1992-1994 with the total number and percent treated with ivermectin.

The number of ivermectin treatments per quarter remained fairly constant from quarter to quarter; percent of cattle treated fluctuated less than 10 percentage points within each year. However, in 1994, usage varied considerably from quarter to quarter with percentage of cattle treated with ivermectin peaking at greater than 20% in the second and fourth quarters. Still, less than 25 percent of the cattle on pasture were treated in any single quarter.

Chart 1. Southeastern survey region. Total pastured cattle population and number treated (left scale) with ivermectin by quarter for 1992-1994. Percent treated (right scale).

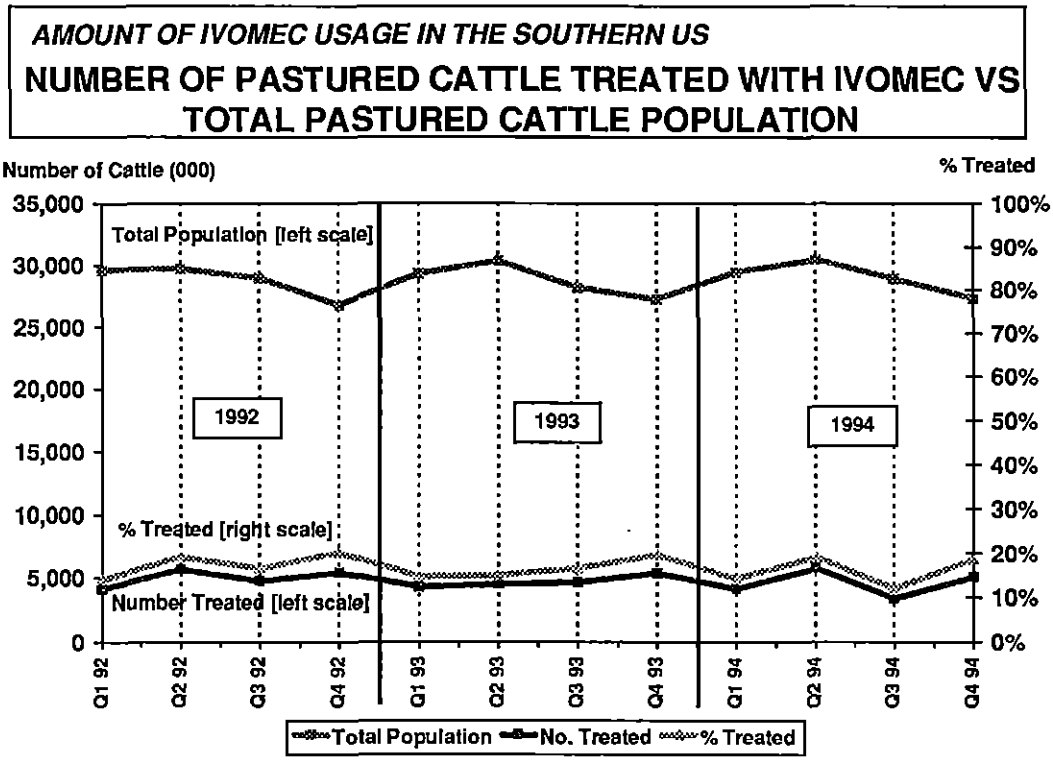


SOURCE: DOANE USAGE TRACKING STUDY

Chart 2 (below) compares the total number of cattle on pasture each quarter in the Southwest survey region during 1992-1994 with the total number and percentage treated with ivermectin.

The Southwest region accounts for more than double the cattle population as the Southeast region. As in the Southeast, the total cattle on pasture population tends to peak in the second quarter yet ranges around 20 million head. Ivermectin treatments in 1992 and 1994 follow the expected peaking pattern in the second and fourth quarters, however, 1993 treatments were more constant. During the entire 3 year period, ivermectin treatments in the Southwest region did not exceed 20% in any quarter.

Chart 2. Southwestern survey region. Total pastured cattle population and number treated (left scale) with ivermectin by quarter for 1992-1994. Percentage treated (right scale) also shown. Due to larger populations, the left scale is different than Chart 1.



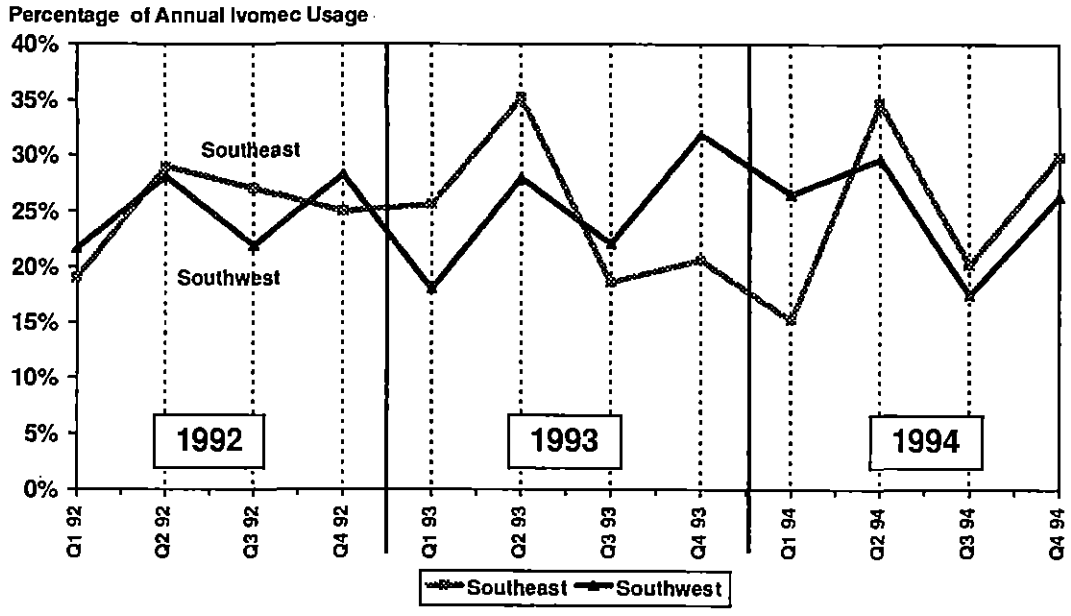
SOURCE: DOANE USAGE TRACKING STUDY

Chart 3 (below) shows the distribution of annual ivermectin treatments for both the Southeast and Southwest survey regions per quarter from 1992-1994. The Southwest region treatments are more concentrated in the second and fourth quarters. For example, in 1994, 30% of annual ivermectin treatments occurred in the second quarter and 25% occurred in the fourth quarter. The treatment patterns in the Southeast region are somewhat more erratic.

Chart 3. Southeast and Southwest survey regions combined. Ivermectin treatments per quarter on a percentage basis.

AMONG IVOMEC-TREATED CATTLE IN THE SOUTHERN US:

DISTRIBUTION OF ANNUAL IVOMEC TREATMENTS ACROSS QUARTERS BY AREA



SOURCE: DOANE USAGE TRACKING STUDY

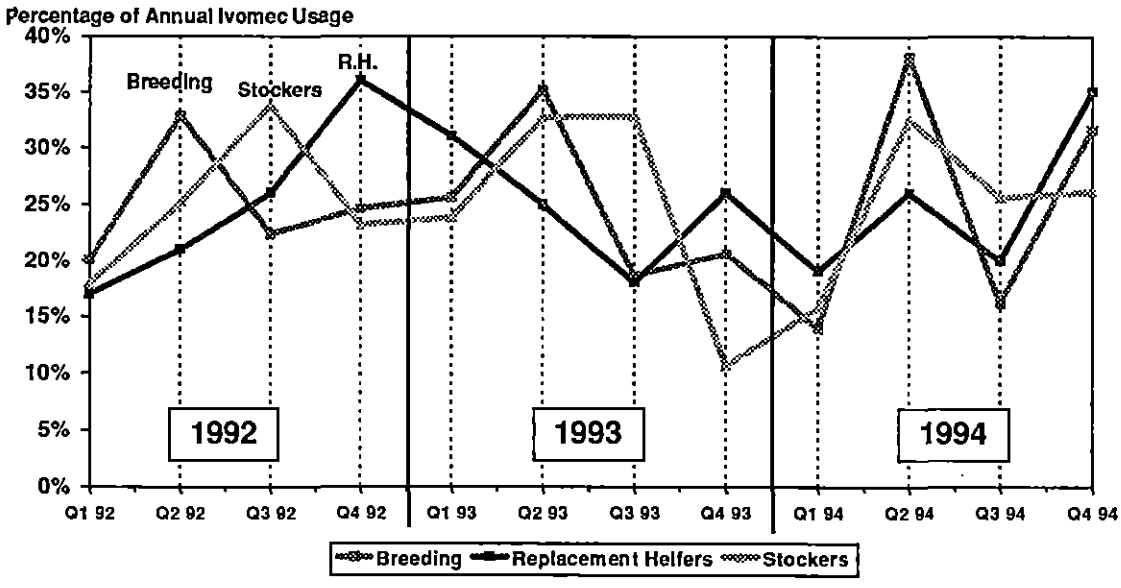
Chart 4 (below) shows the distribution of annual ivermectin treatments for the Southeast region by type of cattle from 1992-1994. Each cattle type represents a different size category: breeding herd (average weight 432 kg), replacement heifers (cows held back to calve in the future, average weight 300 kg), and stockers (calves being grown for processing, average weight 145 kg).

Although the total treatment distribution of ivermectin is fairly constant throughout the year, when analyzed by type of cattle, definite patterns of treatment emerge. Generally, the breeding herd is most often treated in the 2nd quarter, stockers are treated most often in the 3rd quarter, and replacement heifers are treated most often in the 4th quarter. When combined, the three different peaks result in the flatter distribution of total treatments.

Chart 4. Southeastern survey region. Percentage of herd treated with ivermectin when separated into different types of cattle by quarter for 1992-1994.

AMONG IVOMEC-TREATED CATTLE IN THE SOUTHEASTERN US:

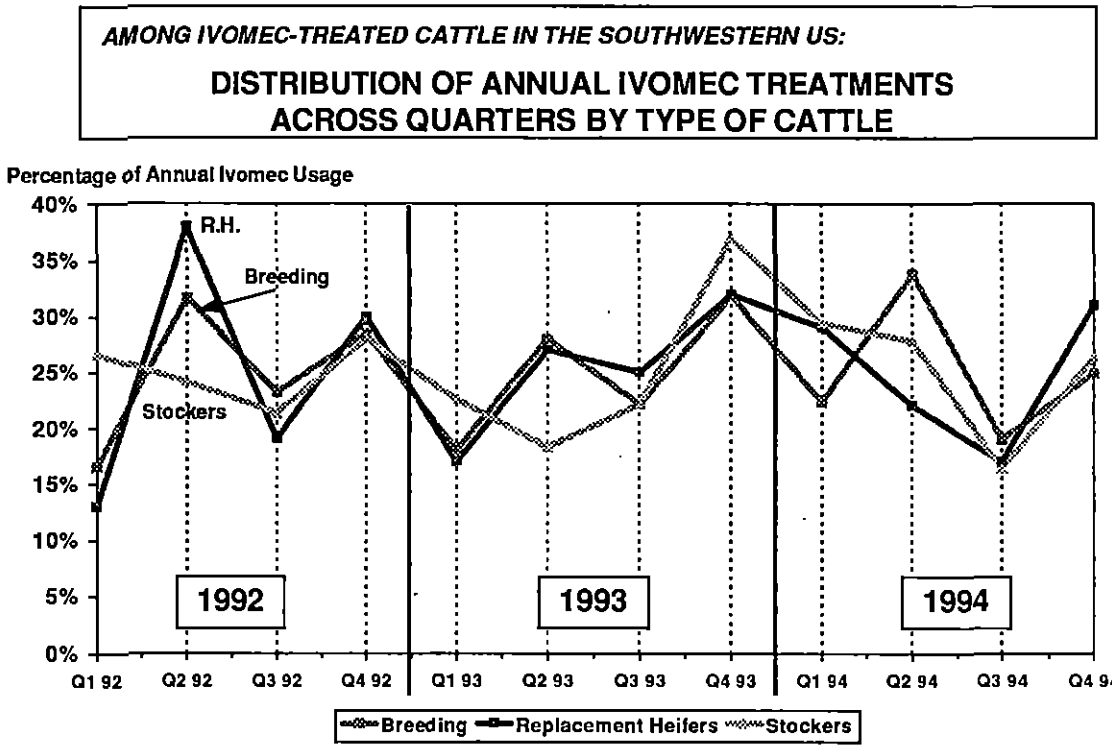
DISTRIBUTION OF ANNUAL IVOMEC TREATMENTS ACROSS QUARTERS BY TYPE OF CATTLE



SOURCE: DOANE USAGE TRACKING STUDY

Chart 5 (below) shows the distribution of annual ivermectin treatments for the Southwest region for 1992-1994 for cattle differentiated as in Chart 4. Peaks of highest usage occur in the second and fourth quarters. When analyzed by type of cattle, treatment peaks tend to occur at the same time, thus for each group of cattle, the peak usage periods are the second and fourth quarters.

Chart 5. Southwestern survey region. Percentage of herd treated with ivermectin when separated into different types of cattle by quarter for 1992-1994.



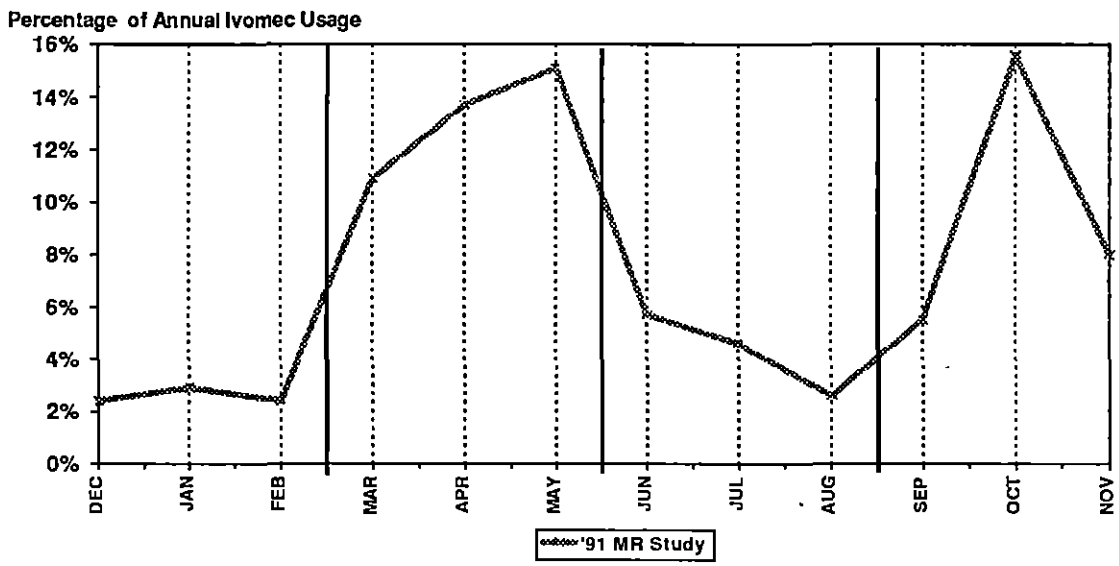
SOURCE: DOANE USAGE TRACKING STUDY

Chart 6 (below) shows the monthly distribution of total ivermectin treatments for all pastured cattle in the combined Southeastern and Southwestern survey regions over a one year period. This 1991 survey of 300 cattlemen provides a clearer picture of the monthly distribution of ivermectin usage among cow-calf producers across the entire southern region. According to this study, peak usage occurs in the March to May time period with the ultimate peak in May; a secondary peak time is October.

Chart 6. Southeast and Southwest survey regions. Monthly distribution during 1991 of total ivermectin treatments for all pastured cattle.

AMONG IVOMEC-TREATED CATTLE IN THE SOUTHERN US:

DISTRIBUTION OF ANNUAL IVOMEC TREATMENTS BY MONTH AMONG COW/CALF PRODUCERS



SOURCE: 1991 PFIZER CUSTOM MARKET RESEARCH STUDY

Conclusion: The regional survey across the Southeastern and Southwestern U.S. indicates that on the basis of the total number of pastured cattle treated with ivermectin, peaks occur in the second and fourth quarters of the year. However, percentage treated per quarter tends to remain below 20% of the total cattle population, even during peak times.

B. Local Surveys

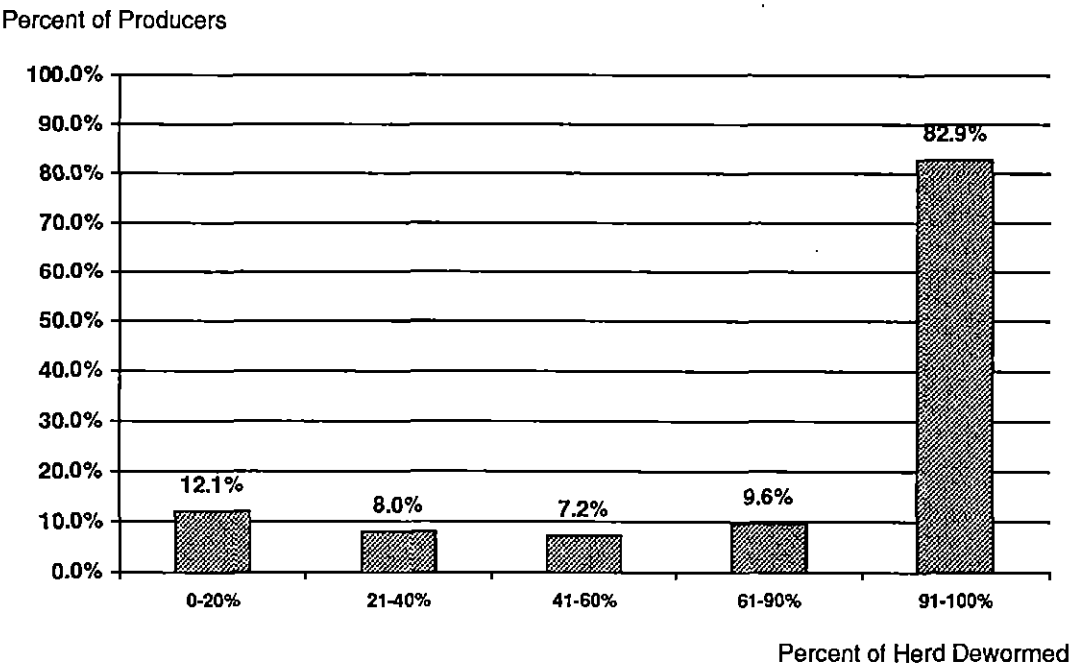
The Texas survey profiled operations during March-May, 1995 in Matagorda county in Southeastern Texas, one of the top 100 beef cow counties according to the 1992 USDA survey. The county contains 449 cow-calf operations and approximately 37,500 beef cows. The average cow-calf

herd is 84 head and the stocking rate is approximately 9 acres per cow-calf pair.

Interviews revealed the following: The seasonal peak of cow-calf pairs occurs in March-May; calves range from 1-7 months in age. A significant amount of deworming occurs at this time. Producers tend to treat all cows. Some also treat all calves and others treat only fall born calves. Operators tend to treat as many cows per day as possible. Large operations require at least 4-5 days for treatment of all cattle.

This information confirms results of the 1991 Pfizer survey. Chart 7 (below) from this latter survey illustrates that over 80% of cow-calf producers in the southern U.S. deworm 91-100% of their cattle. Since some producers deworm at least part of their herd more than once per year, total percentages shown below exceed 100%.

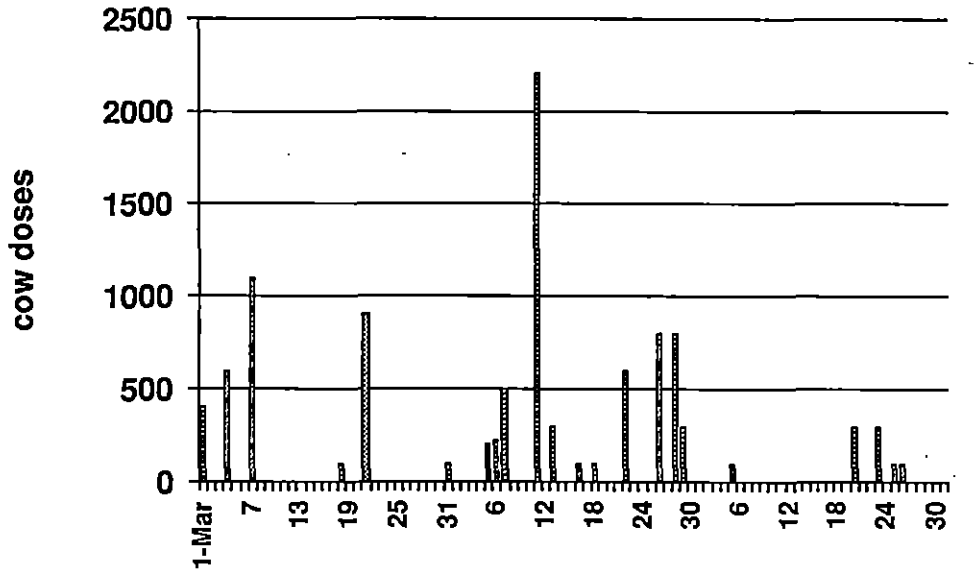
Chart 7. Percent of cattle herd dewormed by cow-calf operators in the southern U.S.[1]



[1]Source: 1991 Pfizer market research study

Chart 8 (below) tracks daily sales of ivermectin by a practicing veterinarian from March-May, 1995 and illustrates that product was purchased and presumably used fairly evenly throughout this time frame. In agreement with comments provided by the extension veterinarian who was interviewed, the purchase data suggest the unlikelihood that a number of adjacent herds were treated simultaneously.

Chart 8. Daily sales of ivermectin purchased through a large veterinary practice in Matagorda county, Texas from March-May, 1995.



1. Over the 3 months, daily sales totaled enough to dose 10,225 cows or 27% of Matagorda county's beef cow population.
2. Sales over the 3 months came from 19 individual cow-calf operations.
3. The large spike in sales on April 11 was accounted for primarily by one operation.

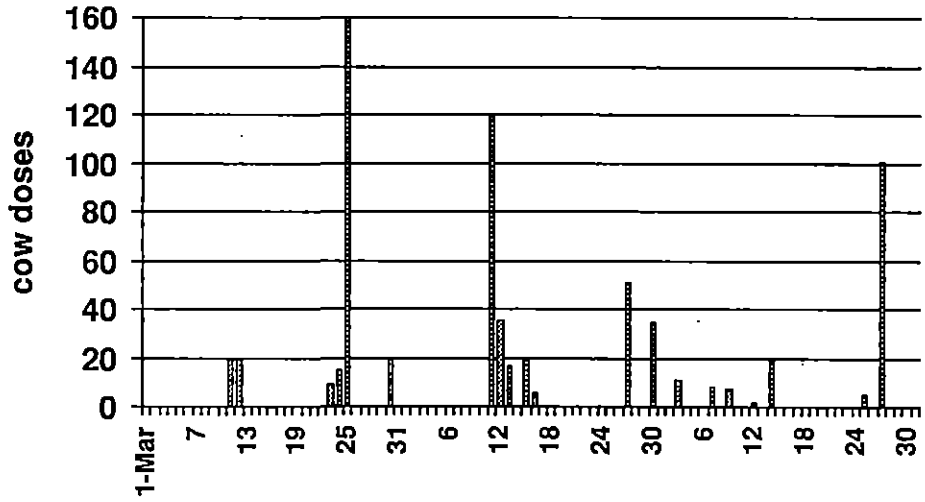
In Texas, operators were advised by both the practicing and extension veterinarians to delay worming until late May or early June in order to rid cattle of inhibited *Ostertagia* spp. Explanations offered for treatments being spread over 3 months include factors such as weather, particularly when using the pour-on formulation. Others include the need to schedule deworming around other farming and non-farming activities as well as entrenched husbandry habits dictating time of treatment.

The Florida survey profiled operations during March-May, 1995 in Marion county in west-central Florida. The county contains 697 cow-calf operations and approximately 25,400 beef cows. The average cow-calf herd is 36 head at a stocking density of approximately 7.2 acres per cow-calf pair.

Interviews revealed the following: The seasonal peak of cow-calf pairs occurs in March-May (numbers of animals, not pounds of beef). Peak deworming activities occur during this period and also in September-November when cattle are treated for liver fluke and/or roundworms. Operators tend to treat all cows and older calves. Treatment of larger herds (2000-3000 head) requires at least 1 week's time.

Chart 9 (below) which monitored sales (and presumably use) of ivermectin in Marion county, Florida, shows that purchase occurred over the entire 90 day period. As indicated by the extension veterinarian who was interviewed, the data suggest that neighboring herds were not treated simultaneously but randomly over a period of 90 days. Principal reasons for the spread offered by the extension veterinarian included problems in scheduling contracted crews to handle animals and differences in breeding and calving seasons from one operator to the next.

Chart 9. Daily sales of ivermectin through a large veterinary practice in Marion County, Florida from March-May, 1995.



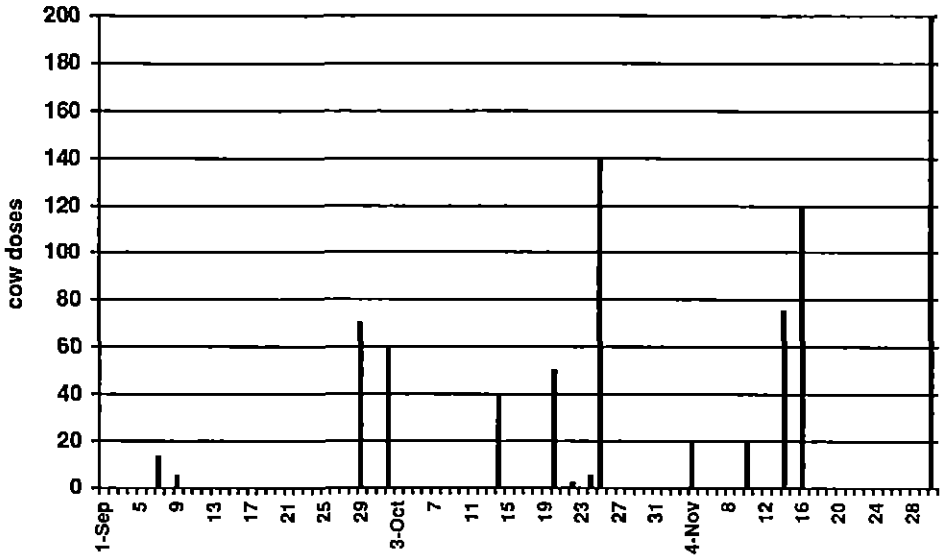
1. Over the 3 months, daily sales totaled enough to dose 679 cows or 3% of Marion county's beef cow population.
2. Sales over the 3 months came from 27 individual cow-calf operations.
3. The high spike seen on March 25 was accounted for by two operations; each buying enough for 80 cows.

Purchases of ivermectin were also monitored during September-November, 1994, in Desoto county in southwestern Florida, one of the top 100 beef cow counties according to the 1992 USDA survey. The county contains 305 cow-calf operations and approximately 39,000 beef cows. The average cow-calf herd is 128 head at a stocking rate of approximately 6.3 acres per cow-calf pair.

Interviews revealed the following: Calving occurs during the September-November time period, and numbers are moving close to the seasonal peak. Deworming activities are high; operators tend to treat all animals and in the shortest time possible. A maximum of 200-250 animals are treated per day, so large operators of 8000 or more head may require several weeks for treatment of all individuals.

Ivermectin purchase, and presumably treatment is spread out over the 3 month period as shown on Chart 10 (below). According to the practicing veterinarian, principal reasons for treatment being spread out include differences in time of breeding and calving among producers as well as a desire among some operators to delay treatment until cooler fall weather arrives.

Chart 10. Daily sales of ivermectin through a large veterinary practice in Desoto county, Florida from September -November, 1994.



1. Over the 3 months, daily sales totaled enough to dose 820 cows or 2% of Desoto county's beef cow population.
2. Sales over the 3 months came from 14 individual cow-calf operations.
3. The sales figures are low from this clinic because it relies almost solely on consulting services rather than retail sales.

Conclusions: A survey of large practices in Texas and Florida indicates that considerable deworming activities take place during the calving periods of March-May and September-November. Based on veterinary testimony, operators treat nearly all their cattle at one time to a maximum rate of 200-250 per day. Also based on veterinary testimony and daily sales (and presumably use), treatment of local herds under the care of a single veterinarian occurs throughout the 3 month period rather than in a more compressed time period. In Matagorda county Texas, 19 operators (4%) purchased sufficient ivermectin over 90 days to treat 27% of the county beef cow population. In contrast, in two Florida counties surveyed, 4-5% of the operators purchased sufficient ivermectin over 90 days to treat 2-3% of their respective counties beef cow populations. The survey suggests that herds in adjacent pastures would not be treated simultaneously; therefore, over a season, the total number of pats containing residues in a pasture or adjacent pastures would be a small percentage of total pats, and pats containing residues would be limited to areas traversed by treated herds for the first 2-3 weeks post dose.

7. Number of Acres Affected

Acreage used for grazing and for disposal of feedlot wastes would be exposed to doramectin residues.

Each feedlot animal would produce about 2,992 kg (2.99 tons) of wet waste or 1108 kg (1.1 tons) of aged waste during a 136 day fattening period. Medication of one-half the total cattle population (56 million) would produce a total of about 62 million tons of aged waste annually:

$$1.1 \text{ tons/animal/year} \times 56 \times 10^6 \text{ animals} = 61.6 \times 10^6 \text{ tons/year}$$

At an application rate of 13.6 metric tons of aged manure per acre, this manure would be dispersed over about 4.6 million acres:

$$62 \times 10^6 \text{ tons} \div 13.6 \text{ tons/acre} = 4.6 \times 10^6 \text{ acres.}$$

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

A. Summaries of Doramectin Environmental Fate Studies

1. Aqueous Solubility

The solubility of doramectin in water is 25 ppb at 25 ± 0.01°C. A full report summary is presented in Appendix c-2.

2. Physical-Chemical Properties

Dissociation Constant: The doramectin molecule contains neither a basic nor an acidic functional group and consequently does not protonate or dissociate over the range of pH 5 to pH 9.

Ultraviolet-Visible Absorption Spectrum: Doramectin shows absorption within the wavelength range between 200 to 800 nm. An absorption peak occurs at 244 nm, with shoulders at 238 and 253 nm. A plot of the UV-visible spectrum at pH 7 is presented in Appendix c-3. The spectrum does not change significantly at pH 5 or 9.

Melting Temperature: The average melting temperature of doramectin is 160.5-162.2°C.

Vapor Pressure: Thermogravimetric analysis suggests that doramectin has a very low vapor pressure and is non-volatile. When compared with pyrene, which has a reported vapor pressure of 7×10^{-7} torr at 20°C, the estimated vapor pressure of doramectin is $<7 \times 10^{-7}$ torr.

A full report summary of these physical-chemical properties is presented in Appendix c-3.

3. Octanol-Water Partition Coefficient

The octanol-water partition coefficient, K_{ow}, for doramectin is 25,787; log K_{ow} is 4.41. A full report summary is presented in Appendix c-4.

4. Soil Sorption and Desorption

A soil sorption and desorption test was conducted using three different soils: Texas clay loam (TXCY); California clay loam (CACY); and Mississippi silty clay loam (MSCY). The distribution coefficients, K_d, determined from the Freundlich adsorption isotherms, were 70.8 (TXCY), 234 (CACY), and 562 (MSCY), with corresponding K_{oc} values of 7520, 13300, and 86900, respectively, indicating strong sorption of doramectin to all three soil types. It was calculated that at a solution:soil ratio of 5:1, 93.4% of doramectin will sorb to TXCY soil, 97.9% will sorb to CACY, and 99.1% will sorb to MSCY. A full report summary is presented in Appendix c-5.

5. Fecal Sorption and Desorption

Fecal sorption and desorption of doramectin was measured using feces collected from 300 kg steers fed a nonmedicated ration of corn silage plus mineral mix. The distribution coefficient, K_d, determined from the Freundlich adsorption isotherm, was 15,600, with a corresponding K_{oc} value of 34,100, indicating strong sorption of doramectin to cattle feces. A full report summary is presented in Appendix c-6.

6. Soil Column Leaching

A soil column leaching study of ^{14}C-doramectin was conducted to estimate the mobility of doramectin in two soils: Thoresby loamy sand and Alconbury sandy clay loam. Leachate from both soil columns contained no detectable ^{14}C-radioactivity (<1.2% of applied, limit of detection). Most of the applied ^{14}C-radioactivity (89.4-97.7%) was retained in the top 5 cm of the columns, with radioactivity in lower sections below the limit of reliable measurement (<3% of applied). A full report summary is presented in Appendix c-7.

7. Aquatic Photodegradation

Doramectin underwent rapid photolysis in dilute aqueous solution, with a calculated rate constant of 0.16 hours^{-1} and a corresponding half-life of 4.45 hours. ^{14}C-photodegradate analysis revealed at least 10 minor polar

degradation products, none of which individually accounted for more than 10% of the applied radioactivity. A full report summary is presented in Appendix c-8.

8. Aerobic Biodegradation in Soil

Aerobic biodegradation of doramectin in soil was assessed using three different soils: Ohio clay loam, Illinois silt loam, and North Dakota loam. Mineralization of ^{14}C-doramectin to CO_2 did not occur to any appreciable extent (3-4% $^{14}CO_2$ in 72 days). Analysis of soils for unchanged doramectin and metabolites by extraction and HPLC analysis at termination of the study (day 72) revealed that doramectin had been transformed to metabolites in all three soils. The amounts transformed were 42.2%, 53.5% and 55.6% for the Ohio, Illinois, and North Dakota soils, respectively. The estimated time to 50% biotransformation for these soils was 79, 62, and 61 days, respectively. One breakdown product accounted for more than 10% of the total applied radioactivity in a single soil, Illinois silt loam (range 12.7-13.8%) and was identified as the 8-α-hydroxy analog of doramectin. A full report summary is presented in Appendix c-9.

B. Potential Concentration and Fate of Doramectin Residues in Environmental Compartments

Use of doramectin could result in introduction of residues into four specific environments as follows: 1) sites where cattle are treated, 2) sites where cattle waste is disposed, 3) areas receiving runoff from such sites, and 4) ground water below such sites. Doramectin would not be expected to partition into the atmosphere because of its high molecular weight, high melting point and low vapor pressure.

1. Potential Release of Doramectin from Cattle Feedlot Waste to Rainfall Runoff

Only insignificant amounts of doramectin are expected to partition into surface waters in runoff from a feedlot due to the strong sorption of drug to cattle feces (Appendix c-6). An estimate of the maximum concentration of doramectin in rainfall runoff can be made using the concentration of drug in feedlot manure and the feces/water partition coefficient. For a worst case consideration, assume simultaneous treatment of all cattle in a feedlot with each treated head receiving 54 mg doramectin (see Section 6.C.1). Further, assume 100% of the dose is excreted as parent drug during the following 14 days, with a rainfall event occurring on day 14. If a typical animal produces 22 kg of wet waste per day, 308 kg will be produced over the 14 days during which drug is excreted. The concentration of drug in manure from treated cattle is 54 mg/308 kg = 0.18 mg/kg. The concentration of doramectin in surface water equilibrated with the doramectin-containing manure, C_w, can be calculated using the relationship

$$C_w = C_m/K_d$$

where C_m is the concentration of doramectin in manure
and K_d is the feces/water partition coefficient

The feces/water partition coefficient for doramectin is 15,600 (Appendix c-6). The maximum concentration of doramectin in equilibrated surface runoff is therefore (0.18 mg/kg)/15,600 = 1.2×10^{-5} mg/kg or 12 ppt. Runoff from rainfall events occurring at later times after drug administration will contain even less, as the concentration of doramectin residues in manure will have decreased by further dilution with fresh manure. For example, at the end of a 136 day stay, the maximum concentration of drug in aged manure is only 49 ppb (Section 6.C.4); C_w would therefore be only 3.1×10^{-6} mg/kg or 3.1 ppt ((0.049 mg/kg)/15,600). Residues in any runoff would be further diminished by sorption to soil during the runoff event and dilution into the receiving pond or lake.

The calculated concentration of doramectin in feedlot surface runoff water can be used to estimate the amount of doramectin that could be transported to the aquatic environment during a rainfall event. Assuming that a three inch rainfall event produces one inch of runoff, the total amount of doramectin lost in solution in the runoff from each acre can be determined for the worst case situation just described as follows:

Amount removed = (volume of runoff per acre)(concentration in runoff)
= (1/12 acre-ft)(1.233×10^6 L/acre-ft)(1.2×10^{-5} mg/L) = 1.2 mg

In a feedlot with a stocking density of 200 head/acre and assuming all of the animals were treated with doramectin, this would represent only 0.011% of the total drug residues:

[1.2 mg ÷ (54 mg/head x 200 head)] x 100 = 0.011%

Therefore, in this worst case example, 1.2 mg doramectin/acre would be carried in surface runoff at a concentration of 12 ppt, representing only 0.011% of the residues expected in fresh feedlot manure. The level would be even lower in a runoff event from aged feedlot manure.

2. Fate of Doramectin in Waste-Amended Soil

The innate biodegradability of doramectin in soil has clearly been shown by demonstration that the drug undergoes biotransformation to approximately 14 quantifiable metabolites which collectively account for as much as 56% of residues extracted from soil at 72 days (Appendix c-9). The estimated time for transformation of 50% of doramectin to metabolites in three different soils was 61, 62 and 79 days. Although the kinetics of doramectin degradation in soils cannot be predicted from the studies conducted and are likely to be complex, first order kinetics have been found applicable for describing degradation of a variety of chemicals present at very low (e.g.,

ppm) concentrations (Alexander and Scow, 1989) and will be used to describe the degradation of doramectin in soil.

The concentration, C_t, of doramectin in soil at any defined time after its application to soil can be determined by the following equation assuming the initial drug concentration (C_0) in soil and the depletion half life are known:

$$C_t = C_0 e^{-kt}$$

Depletion rate constants (k) can be calculated from the estimated times (t) to 50% biotransformation by converting the above equation to logarithms and rearranging:

$$\log C_t = \log C_0 - kt/2.3$$

$$k = \frac{(2.3)(\log 2)}{t} = \frac{0.693}{t}$$

Time to 50% Biotransformation (days)	k ($Days^{-1}$)
61	0.01136
62	0.01117
79	0.00877

If the initial concentration of doramectin in manure-amended soil is 0.73 ppb (Section 6.C.5) and assuming a time to 50% transformation of 79 days, the most conservative value obtained from soil biodegradation studies, 0.03 ppb will remain in the soil 365 days after application (log C = log 0.73 -[0.00877 x 365/2.3] = -1.53; C = 0.0296 ppb). The table below indicates that a maximum concentration of approximately 0.76 ppb doramectin residues in soil is reached after application of manure to the soil two times with a 365 day interval:

Number of successive reapplications	Concentration (ppb) of doramectin residues in soil
0	0.73
1	0.0296 + 0.73 = 0.7596
2	0.0308 + 0.73 = 0.7608
3	0.0309 + 0.73 = 0.7609

Thus, annual field application of aged manure containing doramectin residues would not be predicted to lead to increasing concentrations of drug in soil.

3. Potential Concentration of Drug in Surface Runoff from Waste-Amended Soil

Doramectin sorbs tightly to soils, with soil/water partition coefficients or sorption coefficients (K_d) ranging from 70.8 to 562 for three soils with varying properties; corresponding sorption coefficients expressed on an organic carbon basis (K_{oc}) are 7,520 - 86,900 (Appendix c-5). Chemicals with K_{oc} values greater than 1000 are essentially immobile in soils (Kanega, 1980; Hamaker and Thompson, 1972) and therefore not expected to leach into ground water or move into surface water. Furthermore, any doramectin residues in surface waters would be expected to rapidly decline as low concentrations of the drug in aqueous solution are degraded within a matter of hours by sunlight. Aqueous solutions of 1 ppm doramectin exposed to simulated sunlight were degraded to numerous minor metabolites with a half-life of 4.45 hours (Appendix c-8). Consequently, it is unlikely that more than inconsequential trace concentrations of doramectin would ever be present in solution in streams or ponds.

Estimates of the amount of doramectin that might enter surface waters after feedlot waste is applied to agricultural soils can be made from the doramectin soil/water partition coefficients determined in the soil sorption/desorption study (Appendix c-5). The concentration of doramectin in equilibrated surface water (C_w) can be calculated using the relationship

$$C_w = C_s/K_d$$

where C_s is the concentration of doramectin in waste-amended soil
and K_d is the soil/water partition coefficient

The lowest K_d value for the three soils tested, 70.8, will be used to estimate the maximum surface water concentration. The maximum doramectin concentration in soil amended with aged manure is 0.76 ppb or 7.6×10^{-4} mg/kg (Section 7.B.2). Therefore, $C_w = (7.6 \times 10^{-4}$ mg/kg)/70.8 = 1.07×10^{-5} mg/kg or 11 ppt. This is the maximum concentration that would be found in surface water that has equilibrated with the doramectin-amended soil; this would be diluted as the surface water mixes with water in a receiving pond, lake or stream and would decline further as the doramectin is rapidly degraded by sunlight.

The calculated maximum concentration of doramectin in surface runoff water from waste-amended soil can be used to estimate the amount of doramectin that could be transported to the aquatic environment during a rainfall event. Assuming that a three inch rainfall event produces one inch of runoff, the total amount of doramectin lost in solution in the runoff from each acre can be determined as follows:

Amount removed = (volume of runoff per acre)(concentration in runoff)
= (1/12 acre-ft)(1.233×10^6 L/acre-ft)(1.1×10^{-5} mg/L)
= 1.13 mg/acre

This represents only 0.17% of the total doramectin residues applied (666 mg/acre, Section 6.C.5).

In a 40 acre watershed draining to a 2.5 acre receiving pond with an average depth of 2.5 feet, the maximum concentration of doramectin residues in the pond receiving runoff would be only 5.9 ppt:

$$(1.13 \text{ mg/acre} \times 40 \text{ acres}) \div (2.5 \text{ acre} \times 2.5 \text{ ft} \times 1.233 \times 10^6 \text{ L water/acre-ft})$$
$$= 45.2 \text{ mg} \div 7.7 \times 10^6 \text{ L} = 5.87 \times 10^{-6} \text{ mg/L or 5.9 ppt}$$

This maximum estimated concentration in the receiving pond assumes none of the doramectin residues are adsorbed by soil during runoff. Susceptibility of doramectin to degradation by sunlight in aqueous solution would further reduce this concentration in the pond to even lower levels.

4. Potential Leaching of Drug into Ground Water from Waste-Amended Soil

As noted above, the strong sorption of doramectin to soils and to cattle manure indicates that it will be essentially immobile in waste-amended soils and therefore will not leach into ground water. The predicted immobility of doramectin was verified in a soil column leaching study using ^{14}C-doramectin and two representative soils (Appendix c-7). With a rainfall equivalent of 50 cm passing through the columns, no appreciable leaching was observed. In fact, all of the ^{14}C-radioactivity recovered (89 - 98%) was found in the top 5 cm of the columns, with lower segments and leachates containing no detectable ^{14}C radioactivity (<3% and <1.2% of the applied radioactivity, respectively). This observation is consistent with an estimate of doramectin's leaching potential based on calculation of its relative mobility (R_f) using the following equation (Helling and Turner, 1968; Environmental Protection Agency, 1982; Hamaker, 1975):

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

Where K_{oc} = soil sorption coefficient relative to organic carbon content

%OC = organic carbon content (= %organic matter/1.7)

d_s = density of soil solids

θ = pore fraction of the soil

Using the lowest K_{oc} value measured for doramectin in the soil sorption and desorption study (7,520; Appendix c-5), $\theta = 0.5$ and additional soil properties corresponding to the two soils that were used in the soil column leaching study (Appendix c-7), R_f values can be calculated as follows:

Thoresby Loamy Sand: $d_s = 1.38$; %OC = %OM/1.7 = 1.2/1.7 = 0.71

$$R_f = \frac{1}{1 + (7520)(0.71/100)(1.38)(1/0.5^{2/3} - 1)} = 2.26 \times 10^{-2}$$

Alconbury Sandy Clay Loam: $d_s = 1.04$; %OC = 2.7/1.7 = 1.59

$$R_f = \frac{1}{1 + (7520)(1.59/100)(1.04)(1/0.5^{2/3} - 1)} = 1.35 \times 10^{-2}$$

These values indicate the distance in cm that the bulk of applied doramectin could move through these soils for every cm of water percolating through the soil. The 50 cm rainfall equivalent used in the soil column leaching study would then be expected to move the doramectin only 0.68-1.13 cm (50 cm x R_f), consistent with the results obtained. To extrapolate to field conditions, if half the volume from a 25.4 cm (10 in) rainfall percolates to the water table, the applied doramectin will move only 0.17-0.29 cm (0.5 x 25.4 cm x R_f); even 10 times this amount of rainfall (i.e., 100 inches) would not lead to significant movement of doramectin though the soil.

Given the low concentration of doramectin in soil following repeated application of cattle feedlot manure (0.76 ppb; Section 7.B.2), the low concentration in surface water equilibrated with waste-amended soils (11 ppt; Section 7.B.3), the very high K_{oc} values, and the susceptibility of doramectin to biotransformation in soil, doramectin is not expected to leach into ground water to any significant extent.

5. Potential Mobility and Degradation of Doramectin in Dung Pats Deposited in Fields

Doramectin present in dung pats of pastured cattle would be tightly sorbed to the excreta (Appendix c-6) and would not be expected to leach from the dung pats into the soil or into surface run-off. As discussed in Section 7.B.1, the average concentration of drug residue in fresh manure from treated cattle over the 14 day period post-treatment when the drug is excreted is 0.18 mg/kg. The feces/water partition coefficient (K_d) of 15,600 will limit concentrations in equilibrated surface water to only 12 ppt (Section 7.B.1). This water can permeate into soil around or beneath the dung pats or flow over the soil surface; in either case, any drug residues will partition from the water to the soil, depleting the waterstream of residues. Once in the soil, doramectin will be subject to biotransformation to minor metabolites (Section 7.B.2) and will be gradually depleted from the soil environment. Likewise, the susceptibility of doramectin to biodegradation and photodegradation will reduce levels of residues in the dung pats. Rates of degradation will likely depend upon various climatic and environmental parameters, as has been reported for ivermectin (Halley et. al., 1989). Disruption of dung pats by weather, i.e. freeze-thaw cycles and rainfall, as well as the activity of vertebrates, i.e. trampling by livestock and foraging by mammals and birds, will tend to disperse the dung and any associated residues into the soil, where biodegradation will continue.

6. Summary of Fate of Doramectin Residues in Environmental Compartments

Maximum expected concentrations of doramectin residues in various environmental compartments as estimated in scenarios outlined above are summarized as follows:

Compartment	Maximum Expected Concentration	EA Section
Wet feedlot wastes (136 days, 80% moisture)	18 ppb	6.C.3
Aged feedlot wastes (136 days, 30% moisture)	49 ppb	6.C.4
Surface runoff, fresh (14 day) feedlot wastes	0.012 ppb	7.B.1
Surface runoff, aged (136 day) feedlot wastes	0.003 ppb	7.B.1
Waste-amended soil, first application	0.73 ppb	6.C.5
Waste-amended soil, reapplication	0.76 ppb	7.B.2
Surface runoff, waste-amended soil	0.011 ppb	7.B.3
Receiving pond, 40 acre watershed	0.0059 ppb	7.B.3
Ground water	Insignificant	7.B.4

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

A. Summaries of Studies of Doramectin Effects on Non-Target Organisms: Terrestrial Species

1. Soil Microbes

Minimum inhibitory concentrations of doramectin for five representative soil microorganisms, measured by agar dilution, were: *Clostridium perfringens*, 40 mg/L; *Nostoc*, 60 mg/L; *Aspergillus flavus*, 600 mg/L; *Pseudomonas aeruginosa*, 800 mg/L; and *Chaetomium globosum*, 800 mg/L. A full report summary is presented in Appendix c-10.

2. Seed Germination and Root Elongation

Seeds of 3 species of monocotyledons and 3 species of dicotyledons were exposed to varying concentrations of doramectin to determine effects upon germination and root elongation. No observable effect concentrations (NOEC) and lowest observable effect concentrations (LOEC) are as follows:

	% Germination[a]		Root Elongation[a]	
Species	NOEC (mg A.I./kg)	LOEC (mg A.I./kg)	NOEC (mg A.I./kg)	LOEC (mg A.I./kg)
Corn	840	>840	840	>840
Cucumber	840	>840	840	>840
Perennial ryegrass	6.6	>6.6	1.6	3.3
Soybean	990	>990	990	>990
Tomato	840	>840	840	>840
Wheat	57	>57	57	>57

[a] The NOEC and LOEC values were based on statistical analysis of percent germination and root elongation data collected at test termination. Morphological abnormalities were not used to define the NOEC and LOEC values.

Perennial ryegrass was the most sensitive of the 6 species exposed to doramectin, with an NOEC of 1.6 mg A.I./kg and an LOEC of 3.3 mg A.I./kg, based on the effects observed on root elongation. A full report summary is presented in Appendix c-11.

3. Seedling Growth

Two studies were conducted to determine effects of doramectin on growth of seedlings of 3 species of monocotyledons and 3 species of dicotyledons. Shoot length, shoot dry weight and root dry weight were monitored. In the first study, summarized in Appendix c-12, all 6 species were evaluated by exposing seedlings to doramectin-coated silica sand. The no observable effect concentration (NOEC) for soybean was 980 ppm and the NOEC for tomato appears to be between 53-130 ppm. A NOEC for cucumber was not assigned, but reductions in root weights of up to 45% were observed, starting at 33 ppm, the lowest concentration tested in the definitive test, although the reductions were not statistically significant. Monocotyledons showed non-dose related effects and were retested in a second study, summarized in Appendix c-13. In this study, seedlings were exposed to varying levels of doramectin added to the aqueous nutrient solution or to a

single level of drug applied to silica sand. No significant effects were noted except for increases in root dry weight for corn at the lowest and highest solution concentrations tested, and these observations were judged not to be meaningful. Reductions in ryegrass shoot length of 15% at 3.7 ppb and 11% at 45 ppb, and in shoot weights of 23% and 29% at the same respective doses in nutrient solution, were observed. However, doramectin applied to sand at 47 ppm did not elicit the same response. Therefore, NOECs of 45 ppb for drug solution, the highest concentration tested, and 47 ppm for drug applied to sand were established for corn, wheat and perennial ryegrass for each of the criteria measured.

4. Earthworms

No mortality was observed in the earthworm *Eisenia foetida* exposed to 1000 ppm doramectin in an artificial soil for 28 days. The 28 day LC_{50} is therefore > 1000 ppm. Based on weight gain, the most sensitive criteria monitored, the NOEC was 2 ppm and the LOEC was 4 ppm. A full report summary is presented in Appendix c-14.

5. Immature Dung Beetles and Horn Flies

The LC_{90} of doramectin for hornfly (*Haematobia irritans*) larvae in cattle feces is approximately 3 ppb; the NOEC for larvae development or emergence of adults from the puparium is 2.4 ppb. The LC_{50} and LC_{90} of doramectin for immature dung beetles (*Onthophagus gazella*) are approximately 12.5 ppb and 38.2 ppb, respectively; concentrations up to 250 ppb had no effect upon number of brood balls produced by mating pairs. A full report summary is presented in Appendix c-15.

6. Invertebrate Colonization and Disintegration of Dung Pats in Pasture

Dung pats deposited by pastured cattle or constructed of bulked dung collected 4, 32 or 64 days after doramectin treatment degraded at rates equivalent to nontreated controls. Numbers of larval and adult dung beetles (*Aphodius* spp. and *Sphaeridium* spp.) were equivalent in pats from control and treated animals. Larvae of dung feeding flies, mainly *Ravinia* spp., *Neomyia cornicina* and *Musca autumalis* were reduced in pats from treated cattle. Predatory beetles, primarily larval *Sphaeridium* spp. and adult *Staphylinidae* were also reduced at 4 days but not at 28 days. Full report summaries for these studies are presented in Appendices c-16 and c-17.

B. Summaries of Studies of Doramectin Effects on Non-Target Organisms: Aquatic Species

During conduct of aquatic toxicity studies, loss of chemical was noted, likely due to sorption of doramectin to containers and particulate matter and/or photolysis of doramectin in aqueous solution. For evaluation of effects on the green alga *Selenastrum capricornutum*, measured concentrations were about 65% of nominal at initiation of the definitive study; however, rapid loss

of doramectin from solution during this test to levels below the limit of detection precluded determination of actual exposure concentrations. For *Daphia magna* and fish toxicity studies, test chemical recovery ranged from approximately 40% to 57% of nominal concentrations. Measured concentrations at test initiation and test termination for these latter studies were in close agreement and, therefore, the initial and final measured values have been averaged to provide an exposure concentration.

1. Freshwater Algae

No NOEC of doramectin for the freshwater green alga *Selenastrum capricornutum* could be determined due to rapid loss of chemical from solution. However, results of a preliminary 96-hour range-finding test at nominal drug concentrations of 1.0, 0.10, 0.010 and 0.0010 mg/L indicate that doramectin is not acutely toxic to *S. capricornutum*. A full report summary is presented in Appendix c-18.

2. *Daphnia magna*

Acute toxicity of doramectin, 3"-O-desmethyldoramectin and 8-α-hydroxydoramectin for the water flea *Daphnia magna* was measured under static conditions. The 48 hour EC_{50} concentrations and NOECs are as follows:

	EC_{50}	NOEC
Doramectin	0.10 ppb	0.025 ppb
3"-O-desmethyldoramectin	0.84 ppb	0.16 ppb
8-α-hydroxydoramectin	1.1 ppb	0.39 ppb

Full report summaries are presented in Appendices c-19, c-20 and c-21.

3. Bluegill Sunfish

Acute toxicity of doramectin for bluegill sunfish (*Lepomis macrochirus*) was measured under static conditions. The 96 hour LC_{50} is 11 ppb and the NOEC is 2.3 ppb. A full report summaryis presented in Appendix c-22.

4. Rainbow Trout

Acute toxicity of doramectin for rainbow trout (*Onchorhynchus mykiss*) was measured under static conditions. The 96 hour LC_{50} is 5.1 ppb and the NOEC is 2.5 ppb. A full report summary is presented in Appendix c-23.

C. Potential Effects of Doramectin Usage on Non-Target Organisms

1. Terrestrial Species

a. Soil Dwelling

As discussed above under Sections 6.C.5 and 7.B.2, the maximum estimated concentration (MEC) of doramectin residues in soil is 0.76 ppb. This concentration could only occur when cattle manure containing doramectin residues had just been mixed into soil, assuming no degradation of doramectin had taken place in the manure, and accounts for the very small residual amount of drug that may remain from previous annual fertilizations. This maximum estimated concentration in soil is not expected to have an adverse effect on non-target, soil dwelling terrestrial species. Minimum inhibitory concentrations of doramectin were 40 ppm or above for soil microorganisms tested, more than 5×10^4 times the soil MEC. The NOEC for earthworms was 2 ppm, a level that exceeds the soil MEC by 2.6×10^3 times; no lethal effects were observed for earthworms at concentrations up to 1000 ppm, 1.3×10^6 times the soil MEC. Seed germination or root elongation for six different species of agricultural crop seeds were affected only at concentrations of 3.3 ppm or greater, 4.3×10^3 times the soil MEC. Seedling growth of the dicotyledons tomato and soybean was not affected at concentrations of 53-980 ppm, between 7×10^4 and 1.3×10^6 above the 0.76 ppb maximum estimated doramectin soil concentration. Although cucumber showed some reduction in root weights at 33 ppm and above, these reductions were not statistically significant and occurred at concentrations at least 4.3×10^4 times the soil MEC. In monocots (corn, ryegrass and wheat), no suppressive effects on seedling growth were observed when doramectin was applied to the sand support medium at 47 ppm, 6.2×10^4 times the MEC for soil. Furthermore, although some reductions in ryegrass shoot length and shoot weights were observed, no statistically significant adverse effects were observed on monocots when doramectin was incorporated into the nutrient solution at 45 ppb, 59 times the soil MEC and 4×10^3 times the 11 ppt MEC for doramectin in undiluted soil surface runoff (Section 7.B.3), which would correspond to maximum interstitial water concentrations to which seedlings would be exposed. Importantly, the tight binding of doramectin to soil and its extremely low water solubility will limit doramectin availability to plants to such an extent that residues are not expected to affect plant growth. Moreover, the susceptibility of doramectin residues to degradation prior to and following land application will result in exposure of terrestrial species to drug residues at concentrations likely to be significantly below the maximum estimated soil concentration. Such exposures will be transient as doramectin residues further degrade in the soil environment. Therefore, doramectin residues in soils are not expected to affect plant growth.

b. Dung Dwelling

Dung-dwelling arthropods are sensitive to doramectin. Studies in which immature stages of the horn fly *Haematobia irritans* and dung beetle *Onthophagus gazella* were exposed to fresh cattle dung spiked with doramectin (Appendix c-15), indicated that actively feeding larvae were affected by the doramectin-containing dung in a laboratory environment. The LC_{90} value for hornfly larvae in cattle feces is approximately 3 ppb; the NOEC for larvae development or emergence of adults from the puparium is 2.4 ppb. The LC_{50} and LC_{90} of doramectin for immature dung beetles are approximately 12.5 ppb and 38.2 ppb, respectively; concentrations up to 250 ppb had no effect upon number of brood balls produced by mating pairs. Studies conducted with pastured cattle (Appendices c-16 and c-17) showed that in dung pats deposited or constructed of bulked dung collected 4, 32 or 64 days after doramectin treatment, numbers of larval and adult dung beetles (*Aphodius* spp. and *Sphaeridium* spp.) were equivalent in pats from control and treated animals. Larvae of dung feeding flies, mainly *Ravinia* spp., *Neomyia cornicina* and *Musca autumalis* were reduced in pats from treated cattle. Predatory beetles, primarily larval *Sphaeridium* spp. and adult *Staphylinidae* were also reduced at 4 days but not at 28 days, probably due to the absence of flies upon which they feed at the early time point rather than any drug effect.

Ecology of Dung Beetles in the U.S.: Concern has been expressed that use of avermectins in pasture cattle in the U.S. may adversely affect dung dependant arthropods (Schmidt, 1983) and dung beetles have been identified specifically as insects that may be threatened (Ridsdill-Smith, 1993). A discussion concerning the ecology of dung beetles follows, e.g. beetle geographic and temporal distribution, mobility, dung preference and breeding period. From this, beetle species will be identified whose breeding populations could be threatened by exposure to doramectin residues in dung pats (Section 6.C.6, Doramectin potential use survey). This information will be used in developing a hazard assessment (Section 8.C.4).

The name "dung beetle" is applied to soil dwelling beetles of the order Coleoptera. These scavengers feed upon decomposing organic matter and are prime contributors to the breakdown of organic wastes (Halffter and Edmonds, 1982). Most species have a similar life cycle: small numbers of eggs are laid in organic matter such as dung on which larvae pupate and emerge as winged adults when environmental conditions, particularly soil temperature and moisture are favorable (Ratcliffe, 1991). Larvae tend to ingest whole dung and derive nutrition mainly from the fiber content while adults derive nutrition principally from the microorganisms and organic colloids suspended in dung.

The beetle family Scarabaeidae contains three subfamilies with species that can utilize cattle dung as a food source: the subfamily Aphodiinae (aphodids), the subfamily Geotrupinae (geotrupids) and subfamily

Scarabaeinae (scarabs). The former 2 subfamilies contain the principal dung feeders of northern latitudes while scarabs are most prevalent in subtropical and tropical regions (Fincher, 1981). Dung feeding aphodids tend to live freely in the dung as either larvae or adults while geotrupids and scarabs either build nests in the dung or visit pats as adults and transport dung to nests or chambers at or below the soil surface.

Most dung beetles are strong fliers with diel flight activity dependant on temperature, relative humidity and solar radiation (Landin, 1968). Dung is located by olfactory stimulation and beetles tend to reach dung on the wing by flying toward the wind. Cattle dung pats are visited immediately after defecation and several authors have counted over 1000 specimens in a single pat within two hours of defecation (Woodruff, 1973). Monographs have been published that detail the distribution of Scarabaeidae in Florida (Woodruff, 1973) and Nebraska (Ratcliffe, 1991). Both monographs additionally indicate the distribution of described species throughout the U.S.

Subfamily Aphodiinae: Beetles in this subfamily are usually small (< 5mm) and without distinctive markings. Adults do not relocate food from the original dung deposit as do many Geotrupinae and most Scarabaeinae (Anderson and Loomis, 1978). Adults and larvae live freely in pats and actively burrow and tunnel in them, but their presence has not been shown to impact rate of dung degradation (Anderson et. al., 1984). About 300 species have been described from North America north of Mexico (Woodruff, 1973) and of these, 210 are of the genus *Aphodius* (Gordon, 1983) and 41 of the genus *Ataenius* (Woodruff, 1973). Members of the genus *Aphodius* have a 2-6 month temporal distribution, however, many species that reproduce during the fall or winter months do so only in the more southern reaches of their habitats. Over half the *Aphodius* species are found in the eastern U.S. with elliptical shaped niches extending west and south of mid-New York and Pennsylvania into the Great Lakes region, the mid U.S. south of the Great Lakes to Texas and Oklahoma or along the eastern seaboard through Florida and occasionally the Gulf Coast. Over 40% of the Eastern species utilize deer dung as their exclusive or principal food source. A further 20% prefer decaying animal matter and less than 20%, referred to as generalists, will utilize cattle dung from pastures (Gordon, 1983). Generalist feeders do not appear to be exclusively dependant on cattle dung and will also use dung from deer, fox, pack rat, gopher and tortoise (Woodruff, 1973).

Nearly half the *Aphodius* species are found in the midwestern and western states and most are associated with the burrows of rodents. They have a temporal distribution from February-March or September-December (Gordon, 1983). Additionally, a group of 11-12 *Aphodius* species native to Europe have been introduced accidentally into the U.S. over the last 70 years and all but three species are now distributed broadly from New Jersey to California and from Illinois and Minnesota to Texas (Hanski, 1991). Introduced species tend to dominate aphodid assemblages in bovine dung pats on pasture (e.g. Cervenka, 1986) and prefer cattle dung but can utilize

dung from sheep, elk, moose, swine and possibly ground squirrels (Gordon, 1983, Fincher et. al., 1986).

Seventeen native *Aphodius* species are described from Florida and many are also found in other states along the eastern seaboard or gulf coast. Additionally, five of the group of 11-12 accidentally introduced species are also prevalent in Florida, particularly in cattle dung but also are found in the dung of deer and other species (Woodruff, 1973). The rapid rate of spreading of *Aphodius* species was recently documented by workers who trapped three accidentally introduced species in Durango, Mexico that earlier had been documented to occur only as far south as Florida (Lobo, 1994).

Thirty-five species of Scarabaeidae including 11 species of *Aphodius* were captured in traps set in open pastures and woodlands in east-central Texas (Fincher et. al., 1986). Three accidentally introduced species were noted, including large numbers of *A. lividus*, captured in both habitats during spring and fall months. In Minnesota, Scarabaeidae were collected from fresh cattle dung in pasture and woodlands from sites in the northern and central parts of the state (Cervenka and Moon, 1991). A total of 23 species were identified including 12 *Aphodius*, 7 of which were members of the accidentally introduced group.

Forty-one species of *Ataenius* from the subfamily Aphodiinae are found in the U.S., mostly along the eastern seaboard and gulf coast states; 30 species are found in Florida. Worldwide, species are most abundant in tropical and subtropical regions. Feeding preferences of beetles described in the U.S. vary considerably from cow dung to dung of fox, deer, pack rats and squirrels to leaf mold and humus to decomposing plants and animals (Woodruff, 1973). Life cycles of most species are not well understood and larval stages of many species have not yet been identified. Adults have been captured in all months of the year suggesting that breeding may occur year round.

Subfamily Geotrupinae: Beetles of this subfamily are generally robust in appearance, typically 10-30 mm in length and usually dull in color. Adults relocate food from the source and provision the young, usually in subterranian burrows, some to depths of 8-10 feet. Life cycles require 1-2 years or more for completion. The subfamily consists of four tribes, two of which occur in the U.S. Most species are secretive in nature, seldom found in abundance and spend most of their lives deep in burrows. They tend to be observed most frequently in spring and summer months during the day. Species have been observed feeding on fungi, rotting plants and vertebrate dung including human. Few seem to have a preference for cow dung but the feeding habits and preferences of many species are unknown (Howden, 1955 and 1964).

The genus *Geotrupes* from the tribe Geotrupini are mostly coprophagous species in Europe but North American species have a more varied food selection (Hanski, 1991). Dung from horses and various livestock including

cattle is used along with fungi and decomposing plants (Woodruff, 1973). Examples of species that utilize cattle dung include *G. blackburnii* and *G. egeriei*. Both are distributed broadly east of the Rocky Mountains. Larval development requires 3-6 months or more depending on temperature and humidity and can occur year round, especially between January and November.

Subfamily Scarabaeinae: Most members of this subfamily are coprophagous as adults and larvae. Adults range in size from 2-25 mm, may be brightly colored and possess conspicuous protuberances such as horns. The subfamily consists of five tribes, three of which contain about 75 species native to the U.S. (Woodruff, 1973). The remaining tribes are probably represented only by a few species that have been introduced into the U.S. All scarabaeinae provision their nests with food, mainly dung for the larval stage.

Three nest building groups are recognized (Fincher, 1986). The "endocoprids" nest inside dung pats where they carve out dung balls into which eggs are inserted. "Telecoprids" or "ball rollers" form dung balls at the pat and roll them away for burial below the soil surfaces. "Paracoprids" dig tunnels beneath dung pats which are then provisioned with dung for use by larvae.

The tribe Scarabaeini of the subfamily Scarabaeinae contain the ball rolling species or "tumble bugs." Four genera are found in the U.S., the most widely known belonging to the genus *Canthon* with 19 species represented. *Canthon* spp. are broadly distributed throughout the U.S., i.e. *C. chalcites* occurs throughout the eastern states, *C. ebenus* and *C. imitator* throughout the arid central U.S. from Texas to South Dakota, *C. pilularius* in all regions east of the Rocky Mountains and *C. praticola* and *C. probus* throughout the southern and western states (Woodruff, 1973; Ratcliffe, 1991).

Most Scarabaeini are found in open pasture and tend to be associated with the habitat or placement of large animals such as livestock (Woodruff, 1973). Beetles trapped in east central Texas over a two year period revealed a high proportion of Scarabaeini particularly in open pasture rather than wooded pasture (Fincher, et. al, 1986). Most species feed on dung from horse or cattle but adults also feed on fungi and carrion (Woodruff, 1973). Adults and larvae (where known) are active during spring and summer months. Under favorable conditions, Scarabaeini adult populations may exceed several hundred per dung pat and are capable of removing significant amounts of dung from pastures.

The tribe Coprini of the subfamily Scarabaeinae is represented in the U.S. by four genera. Most species have a shiny, metallic appearance. Three species of the genus *Ateuchus* are found in the U.S., mainly in the northeast, gulf coast states and Florida. While they are coprophagous and will utilize the feces of woodchuck, pack rat and cats, they are also attracted to rotting fungi and carrion. Two species of the genus *Dichotomius* are

found in the U.S.: *D. colonicus* in the west from southern Arizona to Mexico and *D. carolinus* throughout the eastern U.S., south of New York and east of west Texas. The latter is one of the more significant native species in terms of ability to disperse cattle dung. The number of *D. carolinus* typically found in one field was estimated as capable of burying 21 pounds of air dried dung while excavating 126 pounds of soil (Linquist, 1933). This species has been identified most frequently in late spring and early summer as well as in September and October (Woodruff, 1973).

Nine species of the genus Copris occur in the U.S., i.e. *C. fricator*, *C. minutus* and *C. inemarginatus* are commonly found in cow dung throughout their geographical regions of distribution (Ratcliffe, 1991; Woodruff, 1973). Although usually associated with dung, adults will also utilize carrion. The first two species mentioned above occur broadly throughout the eastern U.S., south of Maine and east of Texas while the latter has been identified only in Florida. Temporally, adults have been collected mostly in late spring and early summer but also in other months in smaller numbers in the more southern reaches of their habitat.

Nine species of the genus *Phanaeus* occur in the U.S. and four of these, i.e. *P. difformis*, *P. igneus*, *P. triangularis* and *P. vindex* are commonly found in cow dung. The last mentioned species is distributed broadly across the eastern U.S. from Massachusetts south through Florida and west to west Texas, Oklahoma and Kansas (Blume and Aga, 1978). *P. igneus* occurs most frequently along the eastern seaboard from Virginia to Louisania (Woodruff, 1973). *P. difformis* occurs throughout much of Texas, Oklahoma and Kansas. *P. triangularis* occurs sporadically in the southeastern U.S. with more abundant populations in west Texas and southern Arizona. All *Phanaeus* species are notable dung buriers but none appear to be dependant upon cow dung, and rather seem to prefer dung from humans, dogs or pigs (Blume and Aga, 1978). Numbers of adult *Phanaeus* spp. trapped in southern Georgia on various days between mid July and mid October were compared with temperature and precipitation. Few beetles were trapped in July, probably because June was dry and egg hatching and larval development was prevented. Beetles were trapped in high numbers in late August and late September, probably because of rain in late July and early September that permitted eggs to hatch and larvae to develop (Stewart, 1967).

The tribe Onthophagini is represented in the U.S. by only one genus, *Onthophagus* and by approximately 39 species. Most are coprophagous, but additionally, adults consume fungi, carrion and decaying vegetable matter (Woodruff, 1973). A number of species have been commonly observed in cow dung but it has not been determined whether any species, including those more recently introduced into the U.S. (*O. gazella*, *O. taurus*, *O. depressus)*, feed exclusively on bovine dung. Several species of *Onthophagus* are found throughout much of the U.S. such as *O. hecate* and *O. pennsylvanicus*. Others such as *O. knausi* and *O. orpheus* as well as *O. pseudorpheus* are found in the central U.S.; species such as *O. striatulus*

floridanus and *O. depressus* are known only from a single region (Florida, Georgia).

The introduction of dung beetle species native to southern Europe, southern Africa or the Indian subcontinent of Asia that bury significant quantities of cattle dung for use as a larval food source has been recommended for dispersal of pasture dung pats (Marsh and Campling, 1970), for improving pastures by accelerating nutrient recycling, for improving soil stability and permeability (Anderson and Loomis, 1978) and for controlling pestiferous flies (Fincher, 1981). Fincher (1990) points out that although there are many native species of dung-burying scarabs associated with cattle dung, there are very few species that can bury significant amounts of dung from a cowpat within a few days after deposit. Since the early 1970s, a number of exotic species of dung beetles have been released in the United States. The species of beetles and states where establishment has been reported are listed in Table 1. From this, it is apparent that exotic species have become established within the region encompassing the southeastern states, the southcentral states of Texas and Oklahoma and the southwest including southern California. A number of introduced species are also present in Hawaii. Wider movement appears to be limited because the beetles are intolerant to soil temperature and soil moisture extremes.

O. gazella, an introduced species native to Africa, is found predominantly in open grassland on sandy soil. It undergoes several breeding cycles per year and is ranked as the most efficient producer of offspring among the Onthophagini (Bornemissza, 1970; Blume and Aga, 1978). This species was introduced into Hawaii in 1970 (Bornemissza, 1970) and subsequently into the southern and western states Texas, California, Georgia, Arkansas and Mississippi beginning in 1972 (reviewed by Fincher et. al, 1983). This species was observed to efficiently bury cow dung (Bornemissza, 1970) and its introduction was aimed at enhancing utilization of grazing pastures by controlling dung fouling and breeding of dung dependant nuissance flies. *O. taurus*, a related species accidently introduced into Florida, was hoped to accomplish the same in southeastern states (Fincher and Woodruff, 1975). By 1983 a survey showed rapid spread of both species east and west from points of introduction. For instance, following Texas introductions, *O. gazella* spread 12-32 km 1-2 years after release and four years after release was present in all 18 counties south of the release area. After three more years, beetles were found south of the Rio Grande River (Fincher et. al., 1983). For *O. taurus*, surveys conducted 4-6 years after it was initially observed in the Florida panhandle revealed beetle migration north through Georgia to the Tennessee border, northeast to the North Carolina border and northwest to Mississippi and Alabama (Fincher et. al, 1983). The most recent surveys published in 1986 and 1990 indicate that *O. gazella* is now present from southern California through Arizona, New Mexico, Oklahoma and Texas and east to Florida and north to Georgia (Fincher, 1986; Fincher, 1990). *O. taurus* has spread west to mid Louisiana and north and east to Tennessee and North Carolina (Fincher et. al., 1983).

The seasonal distribution of dung feeding scarabs was monitored monthly over two years in two niches (open pasture and wooded pasture) in central Texas (Fincher et. al., 1986). In both habitats, adult *Onthophagus* spp. i.e. *O. medorensis, O. tuberculifrons, O. gazella, O. oklahomensis* and *O. pennsylvanicus* emerged beginning in March and peak numbers were observed in March (*O. tuberculifrons*) or May-September (others). This occurred in a year (1979) when temperatures and rainfall during early spring were considered normal. When early spring was abnormally cold (1980) adults did not emerge until July and peaks occurred from July to October. In a second survey conducted in 1980, adult *O. gazella* were not observed until September and October in west Texas (Schmidt, 1983). *O. gazella* has also been observed in June and July in southern Oklahoma, in October in southern Arkansas, and in August in Alabama, Georgia and Florida (Hunter and Fincher, 1985). *O. taurus* and *O. depressus* have been captured from March-October in Florida (Fincher and Woodruff, 1975; Woodruff, 1973).

The tribe Oniticellini is represented in the U.S. by 2-3 genera, each consisting of one species. The tribe appears to be closely related to the Onthophagini, particularly in their paracoprid nesting behavior. *Oniticellus* (*Liatongus*) *imilitaris* and *O. cinctus* are native to South Africa and Sri Lanka, respectively, and were introduced into Hawaii (Fincher, 1986). Probably the most important species from this tribe is *Euoniticellus intermedius*, a species native to Africa and introduced into California and Texas to further assist in control of dung breeding pest flies and for dispersal of dung pats on pasture (Blume, 1984; Fincher, 1990). *E. intermedius* was introduced to compliment the cattle dung dispersing activities of *O. gazella*. The former is diurnal and the latter is nocturnal. *E. intermedius* is able to feed and oviposit under conditions of low soil moisture as would often be the case during the daylight hours in central Texas from May-June and September when beetles are active and breeding (Blume, 1984).

The tribe Onitini is represented in the U.S. by a single species, *Onitis alexis*. This large species is native to the arid Sahel region of Africa and was deemed a suitable candidate for introduction into arid pasture lands of the southwest and southern California. Adults are large (25 mm) and robust and considered extremely efficient at dung burial, since a single pair are capable of burying one quarter of a cow dung pat (Halffter and Edmonds, 1982). *O. alexis* is active during the drier, warmer months, i.e. June-September. Each female constructs 2-3 nests in her lifetime, each containing about 50 eggs.

Conclusions: Information provided in this section supports the conclusion that species of dung beetles native to the U.S. will not be threatened by use of doramectin in pastured cattle, and therefore need not be included in the hazard assessment that will follow. This is principally because native species do not appear to be dependant upon cattle dung as an exclusive food source. Moreover, the habitat of many native species is widespread and includes regions of the country with relatively few pastured cattle, e.g. the northeastern states. Also, the breeding period of most native species extends from Spring through Fall and is not necessarily limited nor

coincidental with periods of high drug use. Taken collectively, considerable segments of the native population would not encounter residues and attendant survival risks because they either do not feed on cattle dung or their reproductive period includes times of the year when fewer cattle are excreting residues. Beetle populations not exposed to residues would compensate for any decrease in reproductive potential among native populations feeding on dung from treated cattle.

Conversely, introduced (exotic) dung beetles, on the other hand, could be at risk because they appear to be dependant on cattle dung; however, it is not clear that this point has been thoroughly investigated. The hazard assessment to follow will focus on regions of the country where introduced beetles are documented to be present and where significant numbers of pastured cattle reside, i.e. the southern U.S. Hawaii was excluded because it does not contain significant numbers of pastured cattle, although it does contain introduced beetle species. Likewise, other regions of the U.S. mainland were excluded even where cattle populations are high because introduced dung beetles are not present.

Potential Effects of Doramectin Treatment on Dung Degradation: Concern has been raised, i.e. Strong, 1992, that treatment of cattle with avermectins (such as doramectin) might delay the degradation of dung pats on pasture due to the insecticidal activity of residues excreted in dung. Although treatment of pastured cattle with doramectin showed no effect on rate of dung pat degradation (Appendices c-16 and c-17), it may not be possible to extrapolate results from the site of these studies to other parts of the country or to more extended pasture areas. To provide a broader perspective, literature describing effects of avermectins on dung fauna and dung degradation has been reviewed. This information will be considered in relationship to exposure to doramectin residues in a hazard assessment (Section 8.C.4).

TABLE 1. Species of Exotic Dung Beetles Known to be Established in the U.S.

State	Species	Reference
California	*Onitis alexis*	Anderson & Loomis, 1978
	Onthophagus taurus	Anderson & Loomis, 1978
	Onthophagus gazella	Anderson & Loomis, 1978
	Euoniticellus intermedius	Fincher, 1990
Hawaii	*Onthophagus gazella*	Bornemissza, 1970
	Canthon humectus	Fincher, 1986
	Copris incertus	Fincher, 1986
	Onthophagus incensus	Fincher, 1986
	Onthophgagus sagittarius	Fincher, 1986
	Onthophagus binodis	Fincher, 1986
	Onthophagus nigriventris	Fincher, 1986
	Liatongus militaris	Fincher, 1986
	Oniticellus cinctus	Fincher, 1986
	Onitis alexis	Fincher, 1986
Texas	*Onthophagus gazella*	Fincher, 1986
	Euoniticellus intermidius	Fincher, 1986
Georgia	*Onthophagus gazella*	Fincher, et. al., 1983
	Onthophagus taurus	Fincher, et. al, 1983
	Onthophagus depressus	Fincher, 1990
Louisiana	*Onthophagus gazella*	Fincher, et. al., 1983
	Onthophagus taurus	Fincher, et. al., 1983
Florida	*Onthophagus taurus*	Fincher & Woodruff, 1975
	Onthophagus gazella	Hunter & Fincher, 1985
	Onthophagus depressus	Fincher, 1990
Mississippi	*Onthophagus taurus*	Fincher, et. al., 1983
	Onthophagus gazella	Hunter & Fincher, 1985
Alabama	*Onthophagus taurus*	Fincher, et. al., 1983
	Onthophagus gazella	Hunter & Fincher, 1985
Oklahoma	*Onthophagus gazella*	Hunter & Fincher, 1985
Arkansas	*Onthophagus gazella*	Hunter & Fincher, 1985
Arizona	*Onthophagus gazella*	Fincher, 1990
New Mexico	*Onthophagus gazella*	Fincher, 1990
South Carolina	*Onthophagus taurus*	Fincher, et. al., 1983

The first study to examine the possibility of a relationship between the insecticidal activity of avermectins excreted in dung and rate of dung pat degradation was conducted in west Texas in 1980 (Schmidt, 1983). The study compared cattle treated intramuscularly with ivermectin at 200 µg/kg to nontreated cattle and was conducted in September and October when adult *O. gazella* were active in the region. Based on emergence of adults from dung pats, several groups of non-targeted insects were greatly reduced in numbers including Sphaeroceridae, Sepsidae, *Gymnodia* spp. and parasitic wasps. Beetles were observed to be equally numerous in pats from treated and untreated cattle. Dung pats artificially formed from bulk dung collected from treated and non-treated cattle appeared to disintegrate at the same rate, based on visual inspection of the pats.

A number of studies were conducted in Europe, Africa and Australia after workers in the UK (Wall and Strong, 1987) reported that the dung from calves administered an experimental ivermectin slow release bolus would not support the development of some dung breeding arthropods and degraded at a much slower rate than pats formed from the dung of nonmedicated calves. In this study, pats formed from bulk dung collected every 10-20 days after placement of the boluses showed major differences in numbers of Coleoptera and Diptera larvae and adults compared to controls through 100 days. By this time, control pats had largely disintegrated, but pats from treated cattle were largely intact, based on relative differences in wet weight of the pats. The same authors published a later article (Strong and Wall, 1988) describing an additional segment of the above study. Pats formed from bulk dung containing ivermectin added at 0.5, 0.25 and 0.125 ppm were placed on pasture and subsequently examined for dung inhabiting arthropods. After 33 days on pasture, equal numbers of Scarabaeidae larvae were collected from nonmedicated pats and those containing 0.125 ppm ivermectin; no larvae were found in pats containing 0.25 or 0.5 ppm drug. After 70 and 121 days on pasture, all pats including nonmedicated controls were almost devoid of insects except for dipteran pupae.

Other studies conducted in the UK include those of Scottish workers who surveyed invertebrates found in formed pats which were placed on pasture after addition of ivermectin at concentrations of 2, 1 and 0.5 ppm compared with no medication (McCracken and Foster, 1993). Pats containing all concentrations of ivermectin markedly reduced fly larvae, e.g. Muscidae, but had little effect upon adult *Aphodius* beetles (five species) and unspeciated *Aphodius* larvae.

Two studies conducted in 1987, one near London (Jacobs et. al., 1988) and the other near Glasgow (McKeand et. al., 1988) and a third study conducted over two grazing seasons (1988, 1989) near Southampton (Wratten et. al., 1993) measured the disintegration of natural fecal pats in continuously grazed paddocks. Groups of cattle received either no medication or ivermectin pour-on at 500 µg/kg or ivermectin subcutaneous injection at 200 µg/kg after 3, 8 and 13 weeks on pasture. This is a regimen often

recommended in the UK for anthelmintic prophylaxis. In the third study, an additional group was administered an ivermectin bolus that delivered 50-80 µg/kg/day for 90 days (first year) and 45-80 µg/kg/day for 120 days (second year).

In the first study, pour-on dosing began in May and calves grazed the same paddocks until October. The following March, after removing sheep that had grazed the pasture over the winter, paddocks were systematically searched for cow dung pats. No pats were found where nonmedicated or ivermectin treated calves had grazed, although evidence existed of former dung pats. In the second study, cattle also received ivermectin pour-on formulation beginning in April. No differences in degradation rates were observed, based on diameter, depth and wet weight of dung pats over a nine week observation period beginning one week after administration of the third dose in June.

The objective of the third study mentioned was to evaluate the impact of ivermectin use on dung degradation and pasture quality. Several of the conclusions reached by the authors have been challenged by other workers (Holter et. al., 1994), i.e. criteria employed in measuring dung pat disappearance, organic content of paddock soil and earthworm numbers. Accepting these points of criticism and focusing only upon results at the whole paddock level, ivermectin treatment did not appear to adversely impact on pasture utilization because there was no evidence of dung build up in the pasture and no evidence that the pasture had to be selectively grazed to avoid rank forage.

In a study conducted in May 1993 near Bristol (Strong and Wall, 1994) , the number of insects colonizing formed pats constructed of dung from nonmedicated cattle were compared to pats from cattle injected once with ivermectin or moxidectin at 200 µg/kg. Treatment had no effect on pat colonization by adult aphodid beetles or upon egg laying. However, dung from ivermectin treated cattle inhibited larval development of aphodid beetles and cyclorrhaphous dipteran flies for 7-14 days after treatment, respectively. Moxidectin treatment had no effect on insect larval development.

In Denmark, the insect colonization and/or disintegration of formed dung pats was investigated (Madsen et. al., 1988 and 1990). Pats were formed from bulk dung collected from cattle following subcutaneous administration of ivermectin at 200 µg/kg. Larvae of aphodian beetles were inhibited by dung collected one day after treatment while pupae and larvae of dipteran nematocera and cyclorrhapha were inhibited for 1-10 days and 30 days, respectively, after treatment. In both studies, pats formed from dung collected one day after injection and placed on composted soil in flower pots or on pasture degraded more slowly than controls, based on visual observations or decreases in percent organic matter. Pats formed 20 days after injection also degraded more slowly based on the latter criteria but not pats collected 30 days post dose.

Similar results were obtained in another Danish study in which cattle received ivermectin by subcutaneous injection at 200 µg/kg or via the pour-on formulation at 500 µg/kg (Sommer et. al., 1992). As before, larvae of aphodian beetles were inhibited by dung collected 1-2 days after treatment with either formulation. Larval development of nematocerin Diptera were not inhibited at any time point, but cyclorrhaphian Diptera were inhibited in dung collected for 13-14 days after administration of the pour-on and for 28-29 days after subcutaneous injection. Rate of degradation of pats formed from dung collected for one to two days after ivermectin administration was reduced relative to controls after 45 days on pasture, on the basis of organic matter remaining in the pats. Similar studies were conducted in Spain with formed pats to determine the effect of intramuscularly or subcutaneously administered ivermectin treatment at 200 µg/kg on insect development (Wardhaugh and Rodriguez-Menendez, 1988; Lumaret et. al., 1993). In the first mentioned study, feeding larvae of the dipteran fly *Orthelia cornicina* were inhibited in dung collected for 32 days post treatment. Ninety percent of larvae *Copris hispanus* were inhibited in development in dung collected three days post dose and 20% were inhibited in dung collected after 16 days. Sublethal effects were noted as a result of adult *C. hispanus, B. bubalus* and *Onitis belial* beetles feeding on dung from recently treated cattle. Effects included suppressed feeding activity, reduced ovipositing rates and egg viability. In the second study, larval development of the dipteran fly, *Neomyia cornicina* was prevented when exposed to dung collected 10 days post dose. Development of larval *E. fulvus* beetles was prevented by exposure to dung collected 1 but not 10 days post dose; however, larvae exposed to dung collected 10 days post dose took longer to develop than beetles exposed to control dung.

In Zimbabwe, a study was conducted in January-March, 1991 during the rainy season to measure ivermectin effects on dung burial activity and development of beetles (Sommer et. al., 1993b). Pats formed from dung of nonmedicated cattle or those treated subcutaneously at 200 µg/kg were placed on soil or pitfull traps to monitor beetle activity. Ivermectin treatment had no effect on dung burial activity or upon numbers of brood masses produced by the dominant species, *Diastellopalpus quinquedens*. However, only 28% of larvae developed in pats formed from dung collected two days after treatment, compared to 90-94% development in pats from dung collected 8 and 16 days after treatment.

Studies with formed and natural dung pats were conducted in Germany (Schaper and Liebsich, 1991; Barth et. al., 1993). In the first mentioned study, development of various larval Diptera (muscids, sepsids, sphaerocerids) were reduced in pats formed from dung collected from cattle for several weeks after treatment. Dung was collected weekly beginning after the second of two injections given at 5 week intervals. Scarabaeidae (species not differentiated) development was not inhibited, nor was there any difference in rate of degradation of dung pats between treated and control groups. The second study monitored insect invasion and rate of

degradation of natural pats that were voided on pasture 21, 70 and 119 days after cattle were administered ruminal devices that released ivermectin continuously at a daily rate of 58-71 µg/kg for 120 days. Samples taken at 3, 7, 14 and 28 days after pats were voided revealed statistical reductions in numbers of coleopteran and dipteran larvae at all time points. Based on measurement of surface area and organic matter content (as a percentage of dry weight), pats from control and treated cattle degraded at statistically equivalent rates, although numerically, pats from ivermectin treated cattle generally had a larger surface area for the first 60-90 days.

In several studies cited above, the persistence of ivermectin in formed dung pats was measured by HPLC quantitation. Danish workers (Sommer et. al., 1992; Sommer and Steffansen, 1993a) formed pats from dung collected 1-2 days after administering the pour-on formulation at 500 µg/kg or subcutaneously injecting at 200 µg/kg. Pats placed on pasture in Denmark in August or in Tanzania at the end of the rainy season (May-June) showed no decrease in residue concentrations after 45 and 14 days, respectively. In contrast, Spanish workers (Lumaret et. al., 1993) found the mean concentration of ivermectin in pats formed 2, 4, 7 or 10 days after subcutaneous injection of cattle at 200 µg/kg to decrease below the level of detection (20 µg/kg wet weight) within six days. Formed pats were placed on pasture in southern Spain in the spring during a hot, dry period.

In Western Australia (Ridsdill-Smith, 1988), dung collected from cattle treated subcutaneously with abamectin at 200 µg/kg was toxic for larvae of the introduced dung beetle, *O. binodis*. Inhibition was 100% one week post dose and approximately 50% at two and four weeks. At eight weeks, survival of larvae exposed to manure from abamectin-treated cattle was equivalent to those exposed to manure from cattle treated with levamesole hydrochloride. Survival of adult beetles was not impacted by abamectin treatment, but brood ball production was reduced by 70 and 50%, one and two weeks post dose, respectively, and was normal by four weeks post dose.

In winter rainfall regions of Australia such as the southern portion of Western Australia, *O. binodis* produces two generations per year and the native species, *O. ferox* produces one generation (Ridsdill-Smith, 1993). Breeding initiates in September when temperatures rise and ceases by November when dung quality deteriorates. This breeding period does not coincide with times recommended for avermectin treatment of weaned cattle, but it would coincide with treatment of dairy cattle replacement heifers. Any impact on beetle abundance would depend on concentration and duration of residues excreted.

In south-central Australia (Wardhaugh and Mahon, 1991) higher numbers of adult *O. australis* and *O. pexatus* were found in dung from cattle treated three days and 25 days previously with abamectin (subcutaneously at 200 µg/kg) compared to dung from untreated cattle. An examination of pats from treated cattle revealed more dung beetle tunneling, suggesting a

greater degree of dung burial. This suggests that beetles from treated pats were spending more time in the dung. Moreover, pats formed three days after treatment and recovered after six weeks of field exposure had significantly less residual dry weight than untreated pats. Pats from treated cattle had nearly disintegrated compared to pats from untreated cattle.

Conclusions: Information provided in this section reveals that larval development of dung dependant dipteran and coleopteran species is impacted to varying degrees by avermectin treatment of cattle. Studies in which dung was collected following avermectin treatment, formed into artificial pats and placed on pasture for insect colonization were useful in determining the relative sensitivity of insect groups to avermectins and the duration of insecticidal activity exerted by various drug formulations. In general, larval development of cyclorrhaphan diptera was inhibited for the longest period of time followed by nematoceran diptera, scarabaeinan beetles and finally aphodian beetles. Studies in which known concentrations of ivermectin were added to formed pats provided only limited additional information. For example, ivermectin concentrations between 0.5-2 ppm had no effect on aphodids but markedly reduced fly larvae. In another study, scarabaeidan larvae (presumably aphodids) were unaffected by 0.125 ppm ivermectin, while larval development was inhibited by 0.25 and 0.5 ppm drug. Where two or more dosage forms were compared in the same study, the bolus inhibited development for the longest period of time followed by the injectable formulation. The pour-on formulation was the least inhibitive. The persistence of avermectin in dung pats appears to be influenced by climate with drug disappearing most rapidly under hot, dry conditions. Since insects colonize pats immediately after defecation and find them much less attractive after 1-2 days, drug persistence probably has little impact on pat colonization except for beetle species that have been observed to preferentially colonize dung from avermectin treated cattle.

Studies to determine if avermectins impact rate of dung pat degradation have not yielded consistent results probably because the design of studies has varied considerably and measured variables have not been standardized. Of six studies that monitored breakdown of natural dung pats or those formed from bulk dung, three studies showed that avermectin treatment resulted in an effect while three did not. Authors have pointed out that several criteria employed to quantitate parameters were not sensitive enough to readidly distinguish differences in pat sizes between different treatment groups. For example, in studies conducted with natural pats, detecting significant differences in surface areas between treatment groups is difficult because of their irregular shape and the large standard error attendant in computing surface areas. Also, measurement of dry weight of dung organic matter is preferable to measurement of total dry weight of dung because mineral soil, which is heavy, may be added to the latter by earthworms. Interestingly, the authors who originally called attention to this phenomena (Wall and Strong, 1987), simply photographed dung pats and measured their wet weight.

Three studies have been conducted to monitor degradation under conditions that simulate normal grazing practices. Over one or two seasons, no delay in rate of dung degradation was noted nor was there a buildup of dung in the paddocks nor ungrazed forage due to fouling of pastures. Thus, under conditions approximating the normal grazing environment, treatment of cattle with avermectins does not appear to lead to accumulation of dung in pasture.

Hazard Assessment: This section considers whether or not the use of doramectin in pastured cattle threatens exotic dung beetles that have been introduced into the southern United States. The assessment considers information provided in previous sections as follows: 1) the toxicity of doramectin for dung beetles, specifically, the EC_{50} and EC_{90} of doramectin for the introduced species, *O. gazella* (Section 8.A.5), 2) the excretion of doramectin by cattle (Section 6.C.2), 3) the ecology of dung beetles (Section 8.C.1b), 4) the spatial and temporal introduction of doramectin residues into the southern U.S., regionally and locally (Section 6.C.6b).

When adult pairs of *O. gazella* were exposed to fresh cattle feces containing measured concentrations of doramectin, the number of viable progeny were reduced compared to nonmedicated controls at concentrations of 16 ppb or higher. At concentrations of 4 ppb and less, progeny were not reduced compared to controls. The EC_{50} and EC_{90} were calculated to be approximately 12.5 and 38.2 ppb, respectively. In a similar experiment (Doherty et. al., 1994), *O. gazella* progeny were reduced by 40% and by 95%, respectively, by abamectin incorporated in dung at concentrations of 4-8 ppb. In contrast, moxidectin reduced progeny only when incorporated into dung at concentrations in excess of 250 ppb.

When doramectin was administered to cattle subcutaneously, nearly 90% of the total dose of 200 µg/kg was eliminated in feces within 14 days. By day 14 unchanged drug in feces averaged 133 ng/g, which exceeds the EC_{90} for *O. gazella* and suggests that larval development would be inhibited in dung pats voided in pastures over this time. Similar results were obtained with ivermectin (Roncalli, 1989) where *O. gazella* larvae failed to develop in dung pats voided on pastures by cattle treated subcutaneously at 300 µg/kg 7 and 14 days earlier but not after 21, 28 or 35 days.

Many species of dung beetle native to the U.S. were precluded from the hazard assessment because they do not utilize cattle feces as a food source to any significant extent and, therefore, would not be exposed to drug. This would include most members of the subfamily Aphodiinae and nearly all Geotrupinae. Among the former, only the group of 11-12 accidentally introduced *Aphodius* spp. are commonly found in cow dung. However, these species would not be threatened because they are very broadly distributed throughout the U.S. including regions such as the northeast where only modest numbers of beef cattle are reared. If local beetle populations were disrupted, their rapid spreading rate (as recently documented by Lobo, 1994) would ensure repopulation of depleted areas.

Moreover, pasture studies to date suggest that aphodids are not very sensitive to doramectin (Appendix c-16) or to ivermectin (Madsen et. al., 1988 and 1990; Strong and Wall, 1994; Sommer et. al., 1992). For example, dung collected from cattle treated 4 days earlier with doramectin had no effect on numbers of adults or larvae. Dung collected from cattle treated with ivermectin inhibited larval development for only 1-2 days except for one study where inhibition for 1-2 weeks was reported.

In the subfamily Geotrupinae, 5 *Geotrupes* spp. are associated with cattle dung (Fincher, 1990) but none are dependant upon it as an exclusive food source (Hanski, 1991) and, therefore, would not be threatened by use of doramectin in beef cattle. Moreover, geotrupid species found most frequently in regions supporting large populations of pasture cattle breed most months of the year (January-November), including periods when fewer cattle would be treated.

Approximately 10 genera of the subfamily Scarabaeinae native to the U.S., and representing 3 tribes (Scarabaeini, Coprini and Onthophagini), have been associated with cow dung (Fincher, 1990). However, none would appear to be threatened by use of doramectin because alternative food sources are readily available, e.g. dung from large livestock such as horse in the case of Scarabaeini and a variety of mammals including rats, dogs, cats and pigs in the cases of the others. Breeding has been observed during all seasons, including winter, except in the northern most niches. Therefore, any reduction in populations during periods of more frequent drug use should be offset by reproduction during periods when drug use is less frequent, i.e. summer months. Moreover, when the density of egg laying adults in dung pats is reduced, beetles compensate by producing more brood balls per pat, resulting in more progeny (Fincher, 1994).

Further discussion will focus on introduced species of beetles which could be at risk because they appear to be dependant upon cattle dung. With the exception of Hawaii, they have been documented only from the southern states. This region contains a large beef cattle population which serves as a source of dung for beetles and also a target for treatment with doramectin.

The southwest survey area contains 3 of the top cattle producing states (Texas, Oklahoma, Arkansas), collectively accounting for 25% of the total U.S. population. The southeast survey area contains 2 of the top cattle producing states (Florida, Alabama) which collectively accounts for 6% of the total U.S. population. Survey information was collected quarterly and monthly on a regional basis and daily on a local basis to understand the spatial and temporal use of ivermectin which serves as a proxy for doramectin usage.

The regional survey indicated that from 1992-1994, no more than 20-25% of the total pastured cattle population were treated with ivermectin per quarter in the southeastern or southwestern regions, respectively. Viewed conversely, 75-80% of the total population from both regions were untreated

in any given quarter. Amongst ivermectin treated cattle in the southwest region, 25-30% were dosed in either the second or fourth quarters. Temporal distribution was more erratic in the southeast; nevertheless, the largest percentage of treatments were administered in the second quarter. When treatments were broken out according to one of three weight classes (breeding herd, replacement heifers, stockers), the southwest region showed peak treatments for each weight class in the second and fourth quarters. In the southeast, the breeding herd was treated mainly in the second quarter, replacement heifers in the fourth quarter and stockers in the third quarter. When combined, the treatment peak for each weight class resulted in a flatter distribution of total treatments. The monthly tracking survey conducted in 1991 across the entire southern U.S. revealed that over 40% of the total doses were administered in the spring quarter and over 30% in the fall quarter. Most doses were administered in the months of May and October (16% each).

An additional survey was conducted in one Texas and two Florida counties with high beef cow populations to profile temporal drug use at the local level. Specifically, the survey utilized sales records during the March-May and September-November periods to profile treatment patterns among clients from individual veterinary practices and to assess the liklihood that adjacent herds would be treated simultaneously. Practicing and extension veterinarians were also interviewed to further confirm treatment practices.

Sales information and interview comments were in good agreement and confirmed that March-May and September-November were not only seasonal peaks for numbers of cow-calf pairs but also the periods of most frequent ivermectin use. Comments indicated that most operators simultaneously treated most cows and many calves. An entire herd would usually be treated in one week or less (200-250 cattle per day). Recognizing that the county survey tracked purchase rather than use, it is nevertheless reasonable to assume that drug was administered soon after purchase. Therefore, sales figures and veterinarian comments indicate that cattle were treated throughout the entire 3 month period, strongly suggesting that in a limited geographic region such as a county, individual herds would be treated in a randomized fashion rather than a number of adjacent herds treated all at once. Reasons for a more randomized treatment pattern tended to center on scheduling issues such as availability of labor, the need to work around other farming and non-farming tasks and delays caused by adverse weather.

Based on information presented earlier, it is reasonable to assume that dung in pastures voided by treated cattle would be unsuitable for beetle development for 2 weeks post dose. Pastures containing dung with residues would likely be scattered randomly throughout the county rather then concentrated within a contiguous area. Since beetles visit and utilize only freshly voided dung (Fincher, 1981), pats containing residue which were voided earlier would not be a threat to beetle survival. Even if such pats did not readily degrade, they would not be a threat to beetles nor to pasture utilization because they would be finite in number.

Periods of peak ivermectin usage during March-May do not coincide with periods of peak reproductive activity among exotic beetles introduced in Texas. Surveys cited earlier indicated that *O. gazella* is active in central and east Texas in May-September when spring rainfall is normal and July-September in a drier year (Fincher et. al., 1986). In west Texas, this species was reported to be active in September-October (Schmidt, 1983). *E. intermedius*, another introduced species, was observed in central Texas in May-June and in September (Blume, 1984).

In Florida, the introduced species *O. taurus* and *O. depressus* are active and reproducing from March-October (Fincher and Woodruff, 1975; Woodruff, 1973). *O. gazella* has been observed in Florida in August and is probably active and reproducing through October (Hunter and Fincher, 1985). Therefore, the breeding season for introduced beetles only partially overlaps periods in which ivermectin is used more frequently, i.e. March-May in central Florida and September-November in southern Florida. However, from June-September, when beetles are reproducing, ivermectin monthly use averages only 3-6% of the yearly total, indicating that most pastures would not contain drug residues.

About one-half of the studies conducted in the U.S., Europe and Africa to assess impact of avermectin treatment on rate of dung pat degradation showed that degradation of pats from treated cattle was significantly delayed. In studies where effects on degradation were observed, pats were fenced off from cattle and other vertebrates to prevent trampling or disruption by foraging activities. Studies that simulated normal pasturing practices where vertebrates were not separate from dung pats showed no accumulation of dung on pasture, suggesting that pat disruption leads to dung dispersion.

Nevertheless, there may be pasturing situations where pats are dropped in less accessable areas and, therefore, remain largely undisturbed, e.g. woodland pastures. In these cases, dung dispensing insects may be absent from pats dropped by treated cattle for 2-3 weeks post dose.

Overall conclusions: Dung pats dropped by cattle for 2 weeks after doramectin treatment likely contain sufficient drug to prevent development of some species of dung beetles. Under certain conditions, the dung pats may also require significantly longer periods of time to degrade. However, many factors appear to be involved in dung degradation and under conditions that simulate actual pasture use, avermectins including doramectin have not been shown to adversly impact grazing efficiency of pasture.

It appears unlikely that native dung beetles would be adversely impacted by use of doramectin. Many species simply do not utilize cattle dung as a food source, or if they do, they also utilize other sources of dung. Some species of dung beetles e.g. aphodids do not appear to be very sensitive to avermectins including doramectin, and if impacted it is only for a few days

after treatment. Exotic species appear to be more sensitive to avermectins and also appear to be the most dependant on cattle dung as a sole food source. In the U.S., these species are found only in Hawaii and the southern states. In the latter region, they are active and breeding from approximately May through October, with peaks from June-August. Native species, in contrast, are often distributed over much larger habitats and tend to be active and breeding most months of the year except for winter months in the more northern reaches of their habitats. Dung beetles are winged insects and are strong fliers. Where they have been tracked, they are capable of covering considerable distances and have expanded their niches by 50-80 km per season in Australia (Lee, 1979) and up to 32 km per year in the U.S. (Fincher et. al., 1983).

Tracking ivermectin use (which serves as a proxy for doramectin use) revealed that no more than 20-25% of pastured cattle in southern states where exotic beetles occur are treated per quarter. A monthly maximum of 16% of the number of treatments given yearly are in May and in October. Treatment of individual herds occurs randomly across each county. Although each operator treats essentially all cows and many calves, the probability of simultaneous treatment of a block of adjacent herds is remote. Therefore, although exotic beetles that ingest dung from cows voided within 2 weeks of treatment may be impacted, sufficient dung from nontreated cattle is available locally to prevent extinction of the species or even significant disruption of local populations. Further, breeding activities of exotic beetles are most prevalent in June-August and during this period drug use accounts for only 3-6% of total annual usage, thus providing additional insurance that insect populations would not be unfavorably impacted. Since drug is excreted for only a finite period after treatment, any pasture with recently treated cattle will contain only a limited number of fecal pats containing significant drug residues. Such pats are a threat to beetles for only a day or two after they are voided because beetles visit only fresh pats.

2. Aquatic Species

The potential exposure of aquatic organisms to doramectin is expected to be intermittent, since it depends upon rain runoff from cattle feedlot wastes or soil fertilized with cattle manure containing drug residues; and short-lived, since the concentration of doramectin in water would decline as the drug sorbed to suspended particulates and was degraded by photolysis and transformed by microorganisms. The maximum estimated concentration of doramectin in undiluted surface runoff from a cattle feedlot is 0.012 ppb, or 12 ppt, under worst case considerations (Section 7.B.1); such runoff is directed to retention facilities and therefore not expected to impact on surface water habitats. The maximum estimated concentration in runoff from waste-amended soil is 0.011 ppb, or 11 ppt (Section 7.B.3), although this maximum estimated level would be transient due to the susceptibility of doramectin residues to microbial degradation. Runoff from waste-amended soils may enter ponds or streams, where it would be immediately diluted into the receiving water body. As little as a one-to-ten dilution of the runoff into the receiving water body would reduce maximum doramectin levels to the 1

ppt range. Levels of doramectin would be further reduced by the sorption of any free doramectin to organic matter in the receiving water body, as well as by photolysis. However, even maximum levels of 1 ppt are not expected to have untoward effects on non-target aquatic organisms. For the water flea, *Daphnia magna*, the aquatic species that was most sensitive to doramectin of those tested, the NOEC of 25 ppt is significantly greater than the maximum concentration that might be found in a surface water receiving body. The desmethyl and 8-α hydroxy analogs of doramectin, the principle excretion and soil biodegradation metabolites, were also evaluated against *Daphnia magna* and were found to be 8 to 11 times less toxic than doramectin (Appendices c-20 and c-21). Finally, the doramectin NOECs for bluegill sunfish and rainbow trout of 2.3 and 2.5 ppb, respectively, are more than 2×10^3 times higher than the 1 ppt maximum expected surface aquatic concentration. In summary, exposure of aquatic organisms to doramectin is expected to be intermittent and transient, with only very low levels likely to be found in surface waters due to the tight binding of doramectin to organic matter, its extremely low water solubility, and its susceptibility to degradation and to photolysis. Therefore, doramectin use is not expected to impact aquatic organisms.

9. USE OF RESOURCES AND ENERGY

Manufacturing doramectin bulk and injectable solution will require amounts of resources and energy similar to those required to produce and formulate other fermentation-derived antiparasitics for use in animal health. Disposal of wastes generated from production will not require use of unusual amounts of energy or natural resources.

No effects are anticipated upon endangered or threatened species nor upon properties listed in or eligible for listing in the National Register of Historic Places.

10. MITIGATION MEASURES

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. The high value of the drug per unit weight makes it unlikely that significant quantities would be disposed of casually. Other than the withdrawal time and environmental safety, including instructions for proper disposal of drug containers which is specified on the label and repeated below, no mitigation measures are necessary:

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

11. ALTERNATIVES TO THE PROPOSED ACTION

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. Therefore, alternatives to the proposed action do not need to be considered.

12. LIST OF PREPARERS

The following are all members of the staff of Pfizer Central Research Division:

Daniel P. Brannegan, M.A.

Manager of Environmental Health and Safety
M.A. in Organic Chemistry
8 years experience in laboratory studies; 9 years experience in present position.

Larry R. Chappel, Ph.D.

Assistant Director
Animal Health Product Development
23 years experience in R&D on animal health drugs.

Catherine P. Reese, Ph.D.

Principal Research Investigator, Environmental Safety
Animal Health Product Development
11 years experience in R&D on animal health drugs.

The following individuals are members of Pfizer's Animal Health Operations and International Manufacturing Division:

Richard H. Bartel, B.S., P.E.

Environmental Affairs Supervisor, Lee's Summit plant.
B.S. in Chemical Engineering; 31 years experience in chemical manufacturing and environmental engineering.

Carol A. Eilers

Laboratory Quality Control, Lee's Summit plant.
13 years experience in Quality Control

John Landon

Marketing Manager, Anthelmintics
North American Animal Health Division
15 years experience in Market Research

N. Nishimura

Engineering Manager, Nagoya plant
Degree in Chemical Engineering
26 years experience with Pfizer, 4 years as Engineering Manager

Jan Short

Manager, Market Research
North American Animal Health Division
10 years experience in Market Research

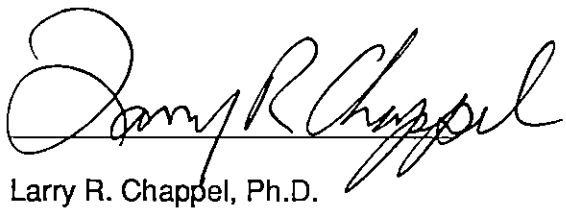
The following individual reviewed portions of Section 6.C.6: Dung Beetle Ecology and Potential Effects of Doramectin Treatment on Dung Degradation:

G. T. Fincher, Ph.D.

Research Entomologist
Food Animal Protection Research Laboratory, USDA, ARS
College Station, TX 77845

13. CERTIFICATION

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate and complete to the best of his knowledge.



3,4,96

Larry R. Chappel, Ph.D.
Assistant Directer
Animal Health Product Development
Pfizer Central Research
Pfizer Inc

Date

14. REFERENCES

Alexander, M. and K. M. Scow. 1989. Kinetics of biodegradation in soil, pp. 243-269. In: B.L. Sawhney and K. Brown, eds. Reactions and movement of organic chemicals in soils. Soil Sci. Soc. Amer. Special Publication No. 22. SSSA, Madison, WI.

Anderson, J.R. and E.C. Loomis, 1978. Exotic dung beetles in pasture and rangeland ecosystems. California Agriculture, Feb. 1978, p. 31-32.

Anderson, J.R., R.W. Merritt and E.C. Loomis, 1984. The insect free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *J. Econ. Entomol.* 77:133-141.

Barth, D., E.M. Heinze-Mutz, R.A. Roncalli, D. Schluter and S.J. Gross, 1993. The degradation of dung produced by cattle treated with an ivermectin slow release bolus. Vet. Parasitol. 48:215-227.

Blume, R.R. 1984. *Euoniticellus intermedius* (*Coleoptera:Scarabaeidae*): Description of adults and immatures and biology of adults. *Environ. Entomol.* 13:1064-68.

Blume, R.R. and A. Aga, 1978. Observations on ecological and phylogenetic relationships of *Phanaeus difformis* Leconte and *Phaneus vindex* Macleay (Coleoptera:Scarabaeidae) in North America. The Southwestern Entomologist 3:113-120.

Bornemissza, G.F. 1970. Insectary studies on the control of dung breeding flies by the activity of dung beetle *Onthophagus gazella* F. (Coleoptera:Scarabaeinae). *J. Aust. Ent. Soc.* 9:31-41.

Cervenka, V.J., 1986. A survey of insects associated with bovine dung in Minnesota. MS Thesis, Masters of Science, University of Minnesota.

Cervenka, V.J. and R.D. Moon, 1991. Arthropods associated with fresh cattle dung pats in Minnesota. *J. Kansas Entomol. Soc.* 64:131-145.

Doane Market Research. 1992-1994. Anthelmintic Tracking Study. Twin Oaks Office Center, St. Louis, MO.

Doherty, W.M., N.P. Stewart, R.M. Cobb and P.J. Keiran, 1994. *In vitro* comparison of the larvaicidal activity of Moxidectin and Abamectin against *Onthophagus gazella* (F.) (Coleoptera:Scarabaeidae) and *Haematobia irritans exigua* De Meijere (Diptera:Muscidae). J. Aust. Ent. Soc. 33:71-74.

Ensminger, M.E. 1976. Beef Cattle Science. The Interstate Printers and Publishers Inc., Danville, IL.

Environmental Protection Agency. 1974. Development document for effluent limitations. Guidelines and new source performance standards - Feedlots point source category Document PB - 238 651 EPA44011-74-004-a.

Environmental Protection Agency. 1982. Chemical fate test guidelines: soil thin-layer chromatography (CS-1700). EPA-560/6-82-003. U.S. EPA, Washington, DC. 11.

Feedstuffs, October 31, 1988, p. C-1.

Fincher, G.T. 1981. The potential value of dung beetles in pasture ecosytems. *J. Ga. Entomol. Soc.* 16:316-333.

Fincher, G.T. 1986. Importation, colonization and release of dung-burying scarabs. *Misc. Pub. Ento. Soc. Am.* 61:69-76.

Fincher, G.T. 1990. Biological control of dung-breeding flies: pests of pastured cattle in the United States. p. 137-151. In D.A. Rutz and R.S. Patterson (eds.), Biocontrol of Arthropods Affecting Livestock and Poultry. Westview Press, Boulder, CO.

Fincher, G.T. 1994. Food Animal Protection Research Laboratory, USDA, ARS College Station, Texas, Personal Communication.

Fincher, G.T. and R.E. Woodruff. 1975. A European dung beetle, *Onthophagus taurus* Schreber new to the U.S. (Coleptera:Scarabaeidae). *Coleopts Bull.* 29:349-350.

Fincher, G.T., T.B. Stewart and J.S. Hunter III. 1983. The 1981 distribution of *Onthophagus gazella:* Fabricius from releases in Texas and *Onthophagus taurus* Schreber from and unknown release in Florida (Coleptera:Scarabaeidae). *Coleopts Bull.* 37:159-163.

Fincher, G.T., R.R. Blume, J.S. Hunter III and K.R. Beerwinkle. 1986. Seasonal distribution and diel flight activity of dung feeding scarabs in open and wooded pasture in East-Central Texas. *Southwestern Entolomologist Suppl.* 10:1-35

Gordon, R.D., 1983. Studies on the genus *Aphodius* of the United States and Canada (Coleoptera:Scarabaedae) VII. Food and Habitat; Distribution; Key to Eastern Species. Proc. Entomol. Soc. Wash. 85:633-652.

Halffter, G. and W.D. Edmonds, 1982. The nesting behavior of dung beetles (Scarabaeinae). Instituto de Ecologia Mexico, D.F. pp. 176.

Halley, B.A., T.A. Jacob and A.Y.H. Lu, 1989. The environmental impact of the use of ivermectin: environmental effects and fate. Chemosphere 18:1543-63.

Hamaker, J. W. 1975. The interpretation of soil leaching experiments, pp. 115-133. In: R. Haque and V. H. Freed, eds. Environmental dynamics of pesticides. Plenum Press, NY.

Hamaker, J. W. and J. M. Thompson. 1972. Adsorption, pp. 49-144. In: C. A. I. Goring and J. W. Hamaker, eds. Organic chemicals in the soil environment, Vol. 1. Marcel Dekker, NY.

Hanski, I., 1991. North Temporate Dung Beetle, in "Dung Beetle Ecology", I. Hanski and Y. Cambefort, Eds. Princeton University Press, Princeton, N.J.

Helling, C. S. and B. C. Turner. 1968. Pesticide mobility: determination by soil thin layer chromatography. Science 162:562-563.

Holter, P., L. Strong, R. Wall, K. Wardhaugh and R. Herd, 1994. Viewpoint: Effects of ivermectin on pastureland ecology. Vet. Rec. 211-212.

Howden, H.F., 1955. Biology and Taxonomy of North American Beetles of the Subfamily Geotrupinae with Revision of the *Genera Bolbocerosoma, Eucanthus, Geotrupes* and *Peltotrupes* (Scarabaeidae). Proc. U.S. Nat. Museum, 104:151-319.

Howden, H.F., 1964. The Geotrupinae of North and Central America. Memoirs of the Entomol. Soc. Canada 39:3-92.

Hunter, J.S. III and G.T. Fincher. 1985. Five new state records for the Afro-Asian dung beetle *Onthophagus gazella* (Coleoptera:Scarabaeidae). *J. Entomol. Sci.* 20:24-25.

Jacobs, D.E., J.G. Pilkington, M.A. Fisher and M.T. Fox, 1988. Ivermectin therapy and degradation of cattle feces. Vet. Res. 123:400.

Kanega, E. E. 1980. Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. Ecotox. Environ. Safety 4:26-38.

Landin, B., 1968. The diel flight activity of dung beetles (Coleoptera, Scarabaeidae); a study of the influence of environmental factors with particular reference to the light. Opuscula Ent. Suppl. 32:1-172.

Lee, B. 1979. Dung beetles to the rescue. New Scientist, 5 April, 46-47.

Linquist, A.W., 1933. Amount of dung buried and soil excavated by certain Coprini (Scarabaeidae). J. Kansas Entomol. Soc. 6:109-125.

Lobo, J.M. 1994. A southern distribution for the introduced dung beetle *Aphoduis* (*Otophorus*) *haemorrhordalis* (L. 1758). J. New York Entomol. Soc. 102:385-386.

Lumaret, J.P., E. Galante, C. Lumbreras, J. Mena, M. Bertrand, J.L. Bernal, J.F. Cooper, N. Karidi and D. Crowe, 1993. Field effects of ivermectin residues on dung beetles. J. Appl. Ecology. 90:428-436.

Madsen, M., J. Gronvold, P. Nansen, 1988. Effects of treatment of cattle with some anthelmintics on the subsequent degradation of their dung. Acta. vet. scand. 29:515-517.

Madsen, M., B. Overgaard Nielsen, P. Holter, O. C. Pedersen, J. Brochner Jespersen, K.-M. Vagn Jensen, P. Nansen and J. Gronvold, 1990. Treating cattle with ivermectin: effects on the fauna and decomposition of dung pats. J. Appl. Ecol., 27:1-15.

Marsh, R. and R.C. Campling, 1970. Fouling of pastures by dung. Commw. Agric. Bur. Herbage Abstr. 40:123-130.

McCracken, D.I. and G. N. Foster, 1993. The effect of ivermectin on the invertebrate fauna associated with cow dung. Environ. Toxicol. Chem. 12:73-84.

McKeand, J., K. Bairden and A.-M. Ibarra-Silva, 1988. The degradation of bovine faecal pats containing ivermectin. Vet. Rec., 122:587-588.

Pfizer Inc, 1995. Ivermectin usage patterns in exotic dung beetle environments. Pfizer Inc. Cattle Business Group. Survey on file.

Ratcliffe, B.C., 1991. The Scarab Beetles of Nebraska Bulletin of the University of Nebraska State Museum, Vol. 12, pp. 333.

Ridsdill-Smith, T.J., 1988. Survival and reproduction of *Musca vetustissima* Walker (Diptera:Muscidae) and a scarabaeine dung beetle in dung of cattle treated with avermectin B1. J. Aust. Ent. Soc. 27:175-178.

Ridsdill-Smith, T.J. 1993. Effects of avermectin residues in cattle dung on dung beetle (Coleoptera:Scarabaeidae) reproduction and survival. *Vet. Parasitol.* 48:127-137.

Roncalli, R.A. 1989. Environmental aspects of use of ivermectin and abamectin in livestock: effects on cattle dung fauna, pp. 173-181. "Ivermectin and Abamectin" W.C. Cambell ed. Springer-Verlag, New York.

Schaper, R. and A. Liebisch, 1991. Einflus eines systemisch wirkenden Antiparasitikums (Ivermectin) auf die Dungfauna und den Dungabbau der Rinder bei Weidhaltung. Tieraerztl. Umsch., 46:12-18.

Schmidt, C.D. 1983. Activity of an avermectin against selected insects in aging manure. *Environ. Entomol.* 12:455-457.

Sommer, C., B. Steffansen, B. Overgaard Nielsen, J. Gronvold, K.-M. Vagn Jensen, J. Brochner Jespersen, J. Springborn and P. Nansen, 1992. Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: concentrations and impact on dung fauna. Bull. Entomol. Res., 82:257-264.

Sommer, C. and B. Steffansen, 1993a. Changes with time after treatment in the concentrations of ivermectin in fresh cow dung and in cow pats aged in the field. Vet. Parasitol. 48:67-73.

Sommer, C., J. Gronvold, P. Holter, M. Madsen and P. Nansen, 1993b. Dung burial activity and development of ivermectin exposed *Diastellophalpus quinquedens* in a field experiment. Entomol. Exp. Appl. 66:83-89.

Stewart, T.B., 1967. Food preferences of coprophagous beetles with special references to *Phaneus* spp. J. Georgia Entomol. Soc. 2:69-77.

Strong, L., 1992. Avermectins: a review of their impact on insects of cattle dung. Bull. Entomol. Res., 82:265-274.

Strong, L. and R. Wall, 1988. Ivermectin in cattle: non-specific effects on pastureland ecology. Aspects Appl. Biol., 17:231-238.

Strong, L., and R. Wall, 1994. Effects of ivermectin and moxidectin on the insects of cattle dung. Bull. Entomol. Res. 84:403-409.

Sweeten, J.M. and R. E. Withers, Jr. 1990. Feedlot manure drops in Texas. Beef. October, p. 54-55.

Wall, R. and L. Strong, 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. Nature, 327:418-421.

Wardhaugh, K.G. and R.J. Mahon, 1991. Avermectin residues in sheep and cattle dung and their effects on dung beetle (Coleoptera:Scarabaeidae) colonization and dung burial. Bull. Entomol. Res. 81:33-339.

Wardhaugh, K.G. and H. Rodriguez-Menendez, 1988. The effects of the antiparasitic drug, ivermectin, on the development and survival of the dung-breeding fly, *Orthellia cornicina* (F.) and the scarabaeine dung beetles, *Copris hispanus*, L., *Bubas bubalus* (Oliver) and *Onitis belial* F. J. Appl. Entomol., 106:381-389.

Woodruff, R.E., 1973. Arthropods in Florida and Neighboring Land Areas. Volume 8, The Scarab Beetles of Florida. Florida Dept. Agriculture and Consumer Services.

Wratten, S.D., M. Mead-Briggs, G. Gettinby, G. Ericsson and D.G. Baggott, 1993. An evaluation of the potential effects of ivermectin on the decomposition of cattle dung pats. Vet. Rec. 133:365-71.

Appendix a-1

Material Safety Data Sheets

Pfizer Inc
Central Research
Eastern Point Road
Groton, Connecticut 06340
Emergency Telephone: 203 441-4100

May, 1994
[supercedes Sept. 1991]

MSDS #0132

Doramectin

[UK-67,994]

SECTION I: *PHYSICAL DATA*

Appearance:	White powder
Melting Point:	165-167°C
Molecular Weight:	899
Description:	Doramectin is a broad spectrum antiparasitic agent for cattle and swine. Doramectin is nearly insoluble in water, but freely soluble in most polar organic solvents.
Chemical Family:	Avermectin/antiparasitic agent for cattle and swine.

SECTION II: *FIRE AND EXPLOSION HAZARD*

Doramectin should not present a fire hazard. If doramectin is involved in a fire, the latter may be suppressed with any appropriate extinguishing medium, including water. Care should be taken to prevent runoff of doramectin contaminated fluids into water sources.

Doramectin is rated as a severe explosion hazard. The minimum explosion concentration is 0.025 oz/fk^3 and the minimum spark ignition energy is 0.40 joules. Doramectin is very sensitive to electrical ignition. Areas where dust could be generated should contain explosion relief vents, explosion suppression systems, or an oxygen deficient environment. All conductive elements of the system should be bonded and grounded.

SECTION III: *HEALTH HAZARD INFORMATION*

Doramectin is orally active against parasites in cattle in doses as low as 200 micrograms/kg. In 90 day safety evaluation studies, the no observed effect level was 0.1 mg/kg/day in dogs. Mydriasis was noted at higher doses, and anorexia, tremors, and ataxia occurred at 2 mg/kg/day. The no observed effect level in rats after 90 days was 2 mg/kg/day. There was no evidence of mutagenic potential in a standard battery of tests for genetic toxicity. In a multi generation study in rats the no effect level was 0.3 mg/kg/day. Doramectin was not teratogenic in rats and mice at levels up to 6.0 mg/kg/day or in rabbits at doses up to 0.75 mg/kg/day. Developmental abnormalities were seen in the rabbit at 3.0 mg/kg/day – a level that was also maternally toxic. A related drug is known to produce birth defects in laboratory animals.

Doramectin has been tested for skin and eye irritation and it is not an irritant to intact or abraded rabbit skin, and is not an ocular irritant to rabbit eyes.

SECTION IV: *FIRST AID INFORMATION*

Ingestion: In the event of ingestion of doramectin (solid or liquid solutions), summon medical attention immediately.

Inhalation: Personnel who have inhaled doramectin should be removed to fresh air and observed by medical personnel.

Skin/Eye Contact: Skin contacted with doramectin should be washed thoroughly with water. Contaminated clothing should be removed. If any effects are observed, medical attention should be sought.

SECTION V: *REACTIVITY DATA*

Bulk doramectin is light sensitive and should be stored in the dark. Stability is enhanced by storage below 4°C. The material is moderately stable under acidic or basic conditions and generally strong acid/base conditions are required for appreciable decomposition.

SECTION VI: *SPILL OR LEAK PROCEDURE*

Spills of doramectin should be collected (scooped or swept) into appropriate recovery containers. Personnel involved in clean-up of spills, particularly solids, must wear respiratory protections, gloves and eye protection. Spills and liquids contaminated with doramectin should not be flushed into collection systems which lead to fresh or salt water sources.

SECTION VII: *PRECAUTIONARY INFORMATION*

When handling doramectin, normal protective measures which minimize personnel exposure should be employed. Gloves, respiratory protection, eye protection, and appropriate clothing should be worn when handling doramectin. Wear gloves and eye protection when handling the material in a fume hood.

issued by: D. P. Brannegan

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

Pfizer Inc
Central Research
Eastern Point Road
Groton, Connecticut 06340
Emergency Telephone: 203 441-4100

May, 1994
[supercedes May, 1992]

MSDS #0175

DECTOMAX ® Injectable

(Doramectin 1.0%, UK-67,994)

SECTION I: *PHYSICAL DATA*

Appearance:	Amber oil
Composition:	Solution of Doramectin, 10 mg/ml in 25% ethyloleate and 75% sesame oil, 0.25% phenol. Doramectin is nearly insoluble in water, but freely soluble in most polar organic solvents.
Chemical family:	Avermectin/antiparasitic agent for cattle and swine.

SECTION II: *FIRE AND EXPLOSION HAZARD*

Injectable doramectin 10 mg/ml should not present a fire hazard. If Injectable doramectin 10 mg/mL is involved in a fire, the latter may be suppressed with any appropriate extinguishing medium, including water. Care should be taken to prevent runoff of doramectin contaminated fluids into water sources.

Injectable doramectin 10 mg/ml should be handled in a manner which prevents exposure to heat sources and open flames. Standard precautions to minimize static charge buildup should be employed.

SECTION III: *HEALTH HAZARD INFORMATION*

Doramectin is orally active against parasitics in cattle in doses as low as 200 micrograms/kg. In 90 day safety evaluation studies, the no observed effect level was 0.1 mg/kg/day in dogs. Mydriasis was noted at higher doses, and anorexia, tremors, and ataxia occurred at 2 mg/kg/day. The no observed effect level in rats after 90 days was 2 mg/kg/day. There was no evidence of mutagenic potential in a standard battery of tests for genetic toxicity. In a multi generation study in rats the no effect level was 0.3 mg/kg/day. Doramectin was no teratogenic in rats and mice at levels up to 6.0 mg/kg/day or in rabbits at doses up to 0.75 mg/kg/day. Developmental abnormalities were seen in the rabbit at 3.0 mg/kg/day – a level that was also maternally toxic. A related drug is known to produce birth defects in laboratory animals.

Doramectin has been tested for skin and eye irritation and it is not an irritant to intact or abraded rabbit skin, and is not an ocular irritant to rabbit eyes.

Injectable doramectin 10 mg/ml is a solution of doramectin prepared for direct administration. As such the health hazards of the injectable formulation are far less than the bulk active ingredient, doramectin.

SECTION IV: *FIRST AID INFORMATION*

Ingestion: In the event of ingestion of Injectable doramectin, 10 mg/ml summon medical attention immediately.

Inhalation: Personnel who have inhaled mists or fine sprays of Injectable doramectin 10 mg/ml should be removed to fresh air and observed by medical personnel.

Skin/Eye Contact: Skin contacted with Injectable doramectin 10 mg/mL should immediately be washed thoroughly with water. Contaminated clothing should be removed. If any effects are observed, medical attention should be sought.

SECTION V: *REACTIVITY DATA*

Injectable doramectin 10 mg/ml is light sensitive and is packaged in amber bottles. Stability is enhanced by storage below 4°C. The material is moderately stable under acidic or basic conditions and generally strong acid/base conditions are required for appreciable decomposition.

SECTION VI: *SPILL OR LEAK PROCEDURE*

Spills of Injectable doramectin 10 mg/ml should be collected (use of absorbent materials) into appropriate recovery containers. Personnel involved in clean-up of skills, should wear respiratory protection, gloves and eye protection. Spills and liquids contaminated with Injectable doramectin 10 mg/ml should not be flushed into collection systems which lead to fresh or salt water sources. All wastes from spills and cleanup of Injectable doramectin 10 mg/ml should be disposed of by incineration.

SECTION VII: *PRECAUTIONARY INFORMATION*

When handling Injectable doramectin 10 mg/ml normal protective measures which minimize personnel exposure should be employed. Gloves, eye protection, and appropriate clothing should be worn when handling Injectable doramectin 10 mg/ml.

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

issued by: D. P. Brannegan

Appendix a-2

Certification of Compliance - Bulk Manufacturing Site

Nagoya Plant
5-gochi,Taketoyo-cho,Chita-gun,Aichi 470-23, Japan
Tel . 0569(72)2111 Telex 456-3620 PTNPT J
Fax. 0569(72)6891

Pfizer ファイザー

Pfizer Pharmaceuticals Inc.

Attachment #2

Date : November 22, 1995

TO WHOM IT MAY CONCERN :

This is to certify that to the best of our knowledge, Pfizer's manufacturing plant at Taketoyo, Aichi Prefecture, Japan is in compliance with all applicable national and local occupational safety and emissions requirements and is expected to remain in compliance when Doramectin is produced at the site.

Akihiro Etoh
Vice-President, Manufacturing & Distribution
Pfizer Pharmaceuticals Inc.
Nagoya Plant

Appendix a-3

Certification of Compliance - Injectable Product Manufacturing Site

January 3, 1996

This is to certify that when the Doramectin 1% solution is produced, the Pfizer Inc plant at Lee's Summit, Missouri will be in compliance with all applicable federal, state, and local emissions and occupational safety requirements, and is expected to remain in compliance.

Frank R. LaPietra
Director of Operations

Appendix b

Data Summary Charts

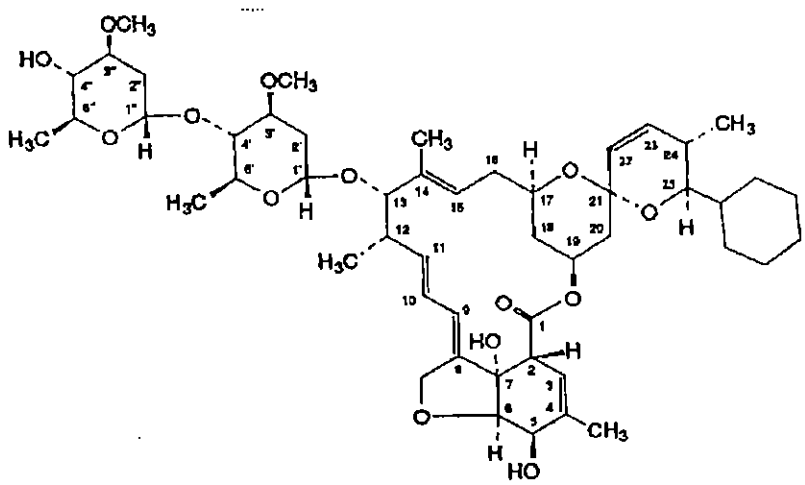
APPENDIX b

DATA SUMMARY CHARTS

PHYSICAL-CHEMICAL AND ENVIRONMENTAL FATE DATA

Generic Name: Doramectin

Structural Formula:



Molecular Formula: $C_{50}H_{74}O_{14}$

Molecular Weight: 899.13

Solubility in Water: 25 ppb

n-Octanol Water Partition Coefficient: 25,787

Vapor Pressure: Non-volatile

Dissociation Constants: The doramectin molecule contains neither a basic or acidic functional group and consequently it does not protonate or dissociate over the range of pH 5 to pH 9.

Ultraviolet-Visible Absorption Spectrum: Peak at 244 nm with shoulders at 238 and 253 nm.

Melting Temperature: 160.5 - 162.2°C

Soil Sorption:

Soil Type	Kd	Koc
Texas Silty Clay Loam	70.8	7,520
California Clay Loam	234	13,300
Mississippi Silty Clay Loam	562	86,900

	Kd	Koc
Fecal Sorption: Cattle feces	15,600	34,100

Photodegradation: Half-life (hours) 4.45

Biodegradation in Soil:

Soil Type	Estimated Time to 50% Biotransformation (days)
Ohio Clay Loam	79
Illinois Silt Loam	62
North Dakota Loam	61

ACUTE AND SUBACUTE TOXICITY STUDIES

TERRESTRIAL ORGANISMS

ORGANISM	ENDPOINT
Soil Microbes	Minimum Inhibitory Concentration (µg/ml)
Clostridium perfringens	40
Aspergillus flavus	600
Pseudomonas aeruginosa	800
Nostoc	60
Chaetomium globosum	800
Crop Seeds	NOEC for Seed Germination and Root Elongation (ppm)
Corn	840
Cucumber	840
Soy Bean	990
Tomato	840
Perennial Ryegrass	1.6
Wheat	57
Crop Seedlings	NOEC For Survival, Root Weight, Shoot Weight, Shoot Length and Abnormal Appearance (ppm)
Corn	0.045 (solution), 47 (sand coating)
Cucumber	not assigned but ≤470
Soybean	980
Tomato	53-130
Perennial Ryegrass	0.045 (solution), 47 (sand coating)
Wheat	0.045 (solution), 47 (sand coating)
Earthworms	28 day LC_{50} > 1000 ppm LOEC, weight gain 4 ppm NOEC, weight gain 2 ppm

ACUTE AND SUBACUTE TOXICITY STUDIES

Dung Dwelling Insects	LC_{90} (ppb)
Hornfly	3
Dung beetle	55

AQUATIC ORGANISMS

		ENDPOINT	
ORGANISM	LC_{50}	NOEC	LOEC
Freshwater Algae	---	ND*	---
Water flea (Daphnia)	0.10 ppb	0.025 ppb	0.066 ppb
Bluegill sunfish	11 ppb	2.3 ppb	7.1 ppb
Rainbow trout	5.1 ppb	2.5 ppb	7.6 ppb

*Could not be determined in a definitive test; preliminary test indicated no acute toxicity at initial concentrations up to 1.0 ppm.

Appendix c-1

Excretion of Doramectin by Medicated Cattle

Report Summary: EXCRETION OF DORAMECTIN BY MEDICATED CATTLE

Study Number: 1535N-60-89-010

Test System: Excreta obtained from medicated cattle

Summary of Experimental Design: A mixed-sex group of cattle, two male castrate and two female cattle, with a mean weight of 203 kg each received a single subcutaneous injection of [^{3}H] doramectin in a proposed commercial formulation of 75:25 sesame oil:ethyl oleate at 200 µg/kg. Collections of feces and urine were made over 24 hour periods for 14 days after dosing to assess the percentage of the dose excreted, and the concentration of unchanged drug in excreta. A day 3 post dose sample of feces derived from cattle injected with [^{3}H]doramectin in an aqueous micelle formulation was utilized for an assessment of the metabolic profile of radioactivity (Study: CM-92-01).

For the determination of total radioactivity, urine samples were assayed in replicate by liquid scintillation counting. Fecal samples were combusted in triplicate to yield tritium-labeled water which was trapped and assayed by liquid scintillation counting. The concentrations of unchanged doramectin in feces were determined by high performance liquid chromatographic analysis of solid phase extracts of the drug. The profile of drug and metabolites in feces was characterized by a liquid chromatographic gradient system employing radiochemical detection.

Summary of Results: By day 14, an average of 87% of the dose was recovered in feces (Table 1), and 0.90% in urine. No tritium water was detected in lyophiles from any fecal samples, thus demonstrating metabolic stability of the radiolabel in cattle. The concentration of total residues in pooled feces peaked at day 5 with a mean of 562 ppb, and declined thereafter to a level on day 14 of 239 ppb (Table 2). The percentage of the fecal residues which is unchanged drug was 49% overall (Table 1). The maximum mean concentration of unchanged drug in feces (319 ppb; 58% of the total sample residues and 5.0% of the total dose) was observed on day 3. The profile of radioactivity in bovine feces collected at day 3 post dose (Study: CM-92-01) consisted of unchanged drug as the major component, and lower concentrations of metabolites identified as: 24-hydroxymethyl-3"-O-desmethyldoramectin, 24-hydroxymethyldoramectin, and 3"-O-desmethyldoramectin.

Table 1

Excretion of [^{3}H] Doramectin and Total Radiolabelled Residues
in Feces of Cattle Following SC Administration of [^{3}H]Doramectin
200 µg/kg, SID x 1

Experiment No. 1535N-60-89-010

		Percent of Dose															
		Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Unchanged Drug	Males	2.8	3.4	4.9	4.6	4.3	4.3	3.5	1.8	2.8	3.5	4.0	2.9	2.5	2.6	1.9	49.8
	Females	1.8	6.3	1.9	5.3	3.3	3.0	2.3	2.3	3.7	3.7	3.9	3.3	3.7	1.1	1.9	47.5
	Mean	2.3	4.9	3.4	5.0	3.8	3.7	2.9	2.1	3.3	3.6	4.0	3.1	3.1	1.9	1.9	49.0
Total Residues	Males	4.8	7.4	7.9	9.1	8.1	9.1	7.1	6.9	5.6	5.8	5.4	3.8	2.9	3.4	3.6	91.0
	Females	3.1	11.3	4.0	7.2	6.3	6.4	4.1	5.4	6.6	5.7	5.4	4.2	4.9	3.0	2.8	80.5
	Mean	4.0	9.4	6.0	8.2	7.2	7.8	5.6	6.2	6.1	5.8	5.4	4.0	3.9	3.2	3.2	86.8

Table 2

Concentration of [^{3}H] Doramectin and Total Radiolabelled Residues
in Feces of Cattle Following SC Administration of [^{3}H] Doramectin
200 µg/kg, SID x 1

Experiment No. 1535N-60-89-010

Equivalents of [^{3}H] UK-67,994
(ng/g)

		Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Unchanged Drug	Males	214	245	373	377	368	363	362	151	257	260	291	216	214	179	136
	Females	33	309	90	260	185	162	225	191	210	234	221	175	217	74	129
	Mean	124	227	232	319	277	263	294	171	234	247	256	196	216	127	133
Total Residues	Males	366	535	608	740	686	773	725	583	513	436	390	287	250	237	257
	Females	170	551	191	358	351	350	396	446	373	357	304	223	288	208	220
	Mean	268	543	400	549	519	562	561	515	443	397	347	255	269	223	239
Percent Drug to Total Residues		46	42	58	58	53	47	52	33	53	62	74	77	80	57	56

Appendix c-2

Aqueous Solubility of Doramectin

Report Summary: AQUEOUS SOLUBILITY OF DORAMECTIN

Study Number: 2438-1088-6131-700

Test System: Column generator

Summary of Experimental Design: Accurate determination of the low solubility of doramectin proved to be difficult, but was accomplished by the column generator method. The determination was carried out in triplicate. Each column consisted essentially of a water-jacketed vertical glass tube packed with a solid support of diatomaceous earth particles. The solid support was coated with doramectin by drawing a solution of doramectin in acetone up into the column, allowing it to drain, and evaporating the residual acetone. Purified water was then pumped through the column with a peristaltic pump. Samples of effluent were analyzed periodically for doramectin by a specific high performance liquid chromatography assay, until a constant concentration was reached. The temperature was maintained at 25 ± 0.01°C and the columns were protected from light.

Calculations: The experimentally determined solubility was used to estimate the octanol-water partition coefficient (K_{ow}), the bioconcentration factor (BCF), and the soil sorption coefficient (K_{oc}) of doramectin by means of standard equations:

$\log K_{ow} = 5.00 - 0.67 \log S$, where S is in ppb	equation (1)
$\log BCF = 2.791 - 0.564 \log S$, where S is in ppm	equation (2)
$\log K_{oc} = 3.64 - 0.55 \log S$, where S is in ppm	equation (3)

In order to test whether the correlations above apply to compounds like doramectin, the same calculations were carried out for a very closely related compound (abamectin, avermectin B_{1a}), and the results were compared with the known, experimentally determined values.

Summary of Results: Based on the measured concentrations shown in Table 1, the solubility of doramectin in water was determined to be 25 ppb.

Table 2 shows values calculated by means of equations (1), (2), and (3) for doramectin and for abamectin, and experimentally determined values for abamectin. Comparison of calculated and experimental values for abamectin indicates that equation (1) can provide a useful estimate for the octanol-water partition coefficient of avermectins, whereas the calculated bioaccumulation factor is too high by several orders of magnitude and the calculated soil sorption constant is about an order of magnitude too high. Thus, what can be projected for doramectin with a reasonable degree of confidence is that it will largely partition into octanol in preference to water.

Table 1. Analytical results for the water solubility determination of doramectin at 25°C.

Time after test Initiation (hour)	Concentration of doramectin in µg/L Replicate A	B	C	Mean	Standard Deviation
1		3400[a]		3400[a]	NA
24		800[a]		800[a]	NA
48	55	58	52	55	3.0
69	32	28	31	30	2.2
79	26	26	25	26	0.6
93	16	18	17	17	1.0
96	27	36	33	32	4.8
102	24	18	19	21	2.9
116	33	16	24	24	8.3
165	24	20	23	23	2.2
Mean Equilibrium Concentration[b]				25 µg/L	5.2

[a] Only one sample analyzed
[b] Mean concentration values from 69 to 165 hours were used in the calculation of the water solubility before rounding to two significant figures.

Table 2. Correlation of Aqueous Solubility with Partitioning Properties.

Property	Doramectin	Abamectin (Calc'd)	Abamectin (Found)
Solubility	25 ppb	--	7.8 ppb
K_{ow}	11,571	25,252	9,900
BCF	4,950	9,547	52
K_{oc}	33,200	63,001	4,760

Appendix c-3

Physical-Chemical Properties of Doramectin

Report Summary: PHYSICAL-CHEMICAL PROPERTIES OF DORAMECTIN

Dissociation Constant: The doramectin molecule contains neither a basic nor an acidic functional group and consequently, it does not protonate or dissociate over the range of pH 5 to pH 9.

Ultraviolet-Visible Absorption Spectrum: The absorption spectrum of doramectin in 1:1 methanol: aqueous buffers at about 20-24°C was determined in triplicate with a diode array spectrophotometer. Doramectin shows absorption within the wavelength range between 200 to 800 nm. An absorption peak occurs at 244 nm, with shoulders at 238 and 253 nm. The average molar absorptivities at these three wavelengths at pH 7 and their relative standard deviation (% RSD) for triplicate determinations are listed below:

Wavelength nm	Molar Absorptivities average, (L/mol-cm)	% RSD
238	28900	3.6
244	31800	3.6
253	20400	3.7

A plot of the UV-visible spectrum at pH 7 is attached (Figure 1). The spectrum does not change significantly at pH 5 or 9.

Melting Temperature: Samples of doramectin and of a melting point reference standard were heated in a digital melting point apparatus. The temperature was raised at a constant rate of about 0.4°C/min., and the temperatures were noted at which changes were observed in either material. The melting point determinations for the sample and melting point standard were carried out in triplicate and duplicate, respectively. The doramectin replicates melted within a range of 160.4-162.2°C, with an average melting temperature of 160.5-162.2°C. The melting point standard (Thomas Scientific, Melting Point Standards, #6428-F12) was run simultaneously and melted at 163.0-165.5°C, in acceptable agreement with the specified value of 165.5-166.5°C.

Thermogravimetric Analysis: A sample of doramectin was heated in a commercial thermal gravimetric analysis instrument (Perkin-Elmer Differential Scanner Calorimeter, model DSC-4), which continuously and accurately monitored the weight of the sample. The determination was run in triplicate. Doramectin exhibited an average 3.09% loss of weight up to a temperature of about 150°C, corresponding to a water content of 3.03% as determined by the Karl Fischer method. There was no further loss of weight until decomposition started at about 255°C. Results indicate that doramectin has a very low vapor pressure and is non-volatile. A copy of the TGA plot is attached (Figure 2).

An additional study was conducted in which 5 mg samples of doramectin and pyrene (for which the vapor pressure has been reported as 7×10^{-7} torr at 20°C) were examined for weight loss at a severe challenge condition of 100°C for 20 hours under nitrogen. Doramectin did not lose any significant weight beyond solvated water, whereas 15% of the pyrene was lost by volatilization. Although thermogravimetric analysis is not a validated method for determining vapor pressure, and the reported value cannot be considered as definitive, evidence from related chemicals and physical-chemical characteristics, including the thermogravimetric proprties of doramectin, indicate that the compound would not be expected to volatilize under environmental conditions. When compared to pyrene, the estimated vapor pressure of doramectin is $< 7 \times 10^{-7}$ torr.

FIGURE 1

UV-VIS ABSORPTION SPECTRUM OF DORAMECTIN

pH 7.0 Buffer

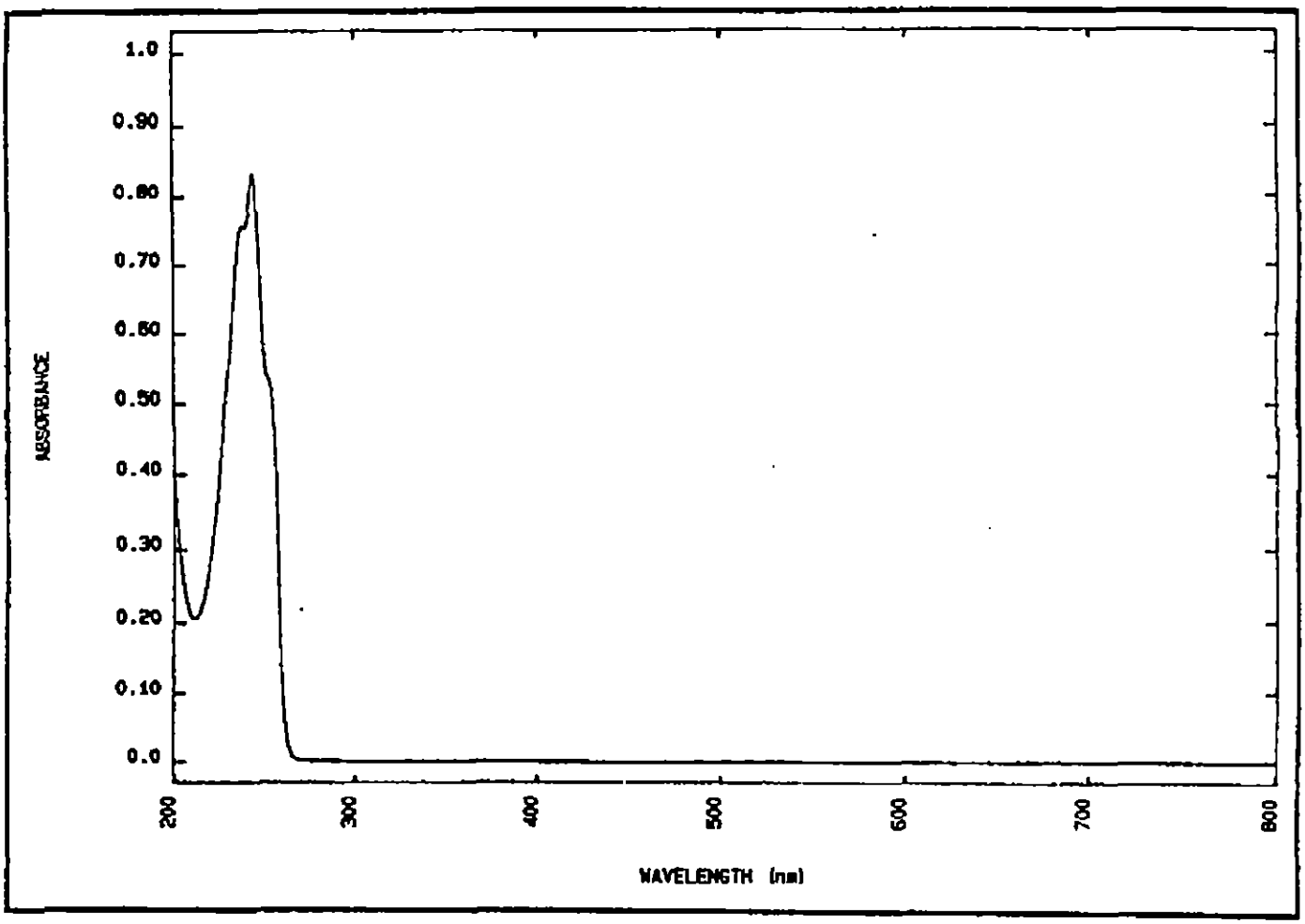
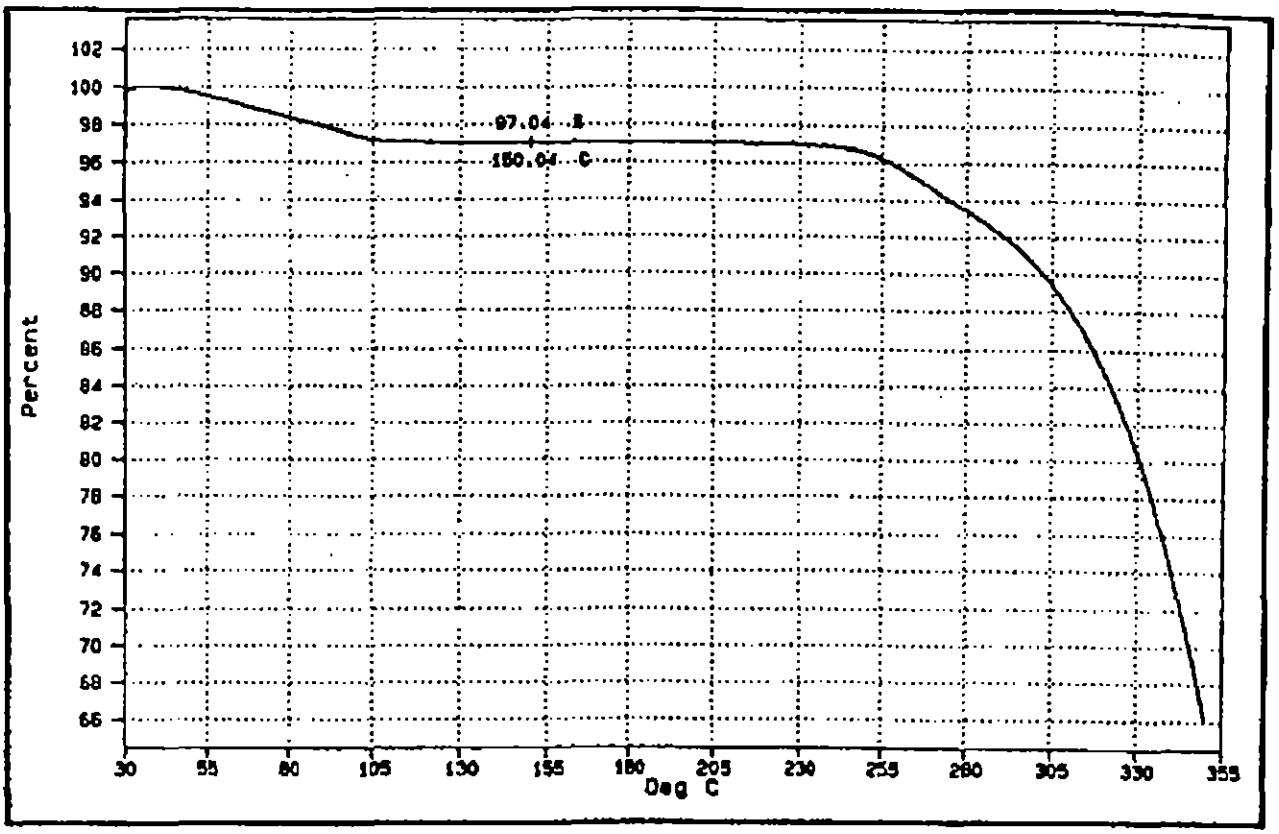


FIGURE 2

TGA PLOT FOR DORAMECTIN



Appendix c-4

The Octanol-Water Partition Coefficient of Doramectin

Report Summary: THE OCTANOL-WATER PARTITION COEFFICIENT OF DORAMECTIN

Study Number: 2438-1088-6132-705

Test System: Two-Phase Solvent System

Summary of Experimental Design: Solutions of radiolabeled doramectin in octanol were prepared in triplicate at approximately 3.7×10^{-4} and 3.7×10^{-5} M concentrations. A 2 ml volume of each solution was shaken gently at 25°C in a capped centrifuge tube, shielded from light, with 200 ml of water for one hour. A preliminary experiment had shown that equilibrium was reached within one hour.

The amount of radiolabeled doramectin present in each phase was then determined by liquid scintillation counting of aliquots. The radiocount for each aliquot was divided by the volume of the aliquot, and the resulting radiocount per unit volume of the phase was divided by the specific radioactivity of the test material to obtain the final concentration of doramectin in the phase.

The partition coefficient (K_{ow}) of doramectin in each of the six two-phase systems was calculated by dividing the final concentration of doramectin in the octanol phase by the final concentration in the aqueous phase. The partition coefficient was converted to its logarithm, log K_{ow}. Mean values of K_{ow} and log K_{ow} were calculated for each set of replicates that contained the same initial concentration of doramectin in octanol.

The radiometric mass balance was checked by multiplying the radiocount per unit volume of each phase by the volume of the phase, summing the resulting total radiocount per phase for the two phases of each system, and dividing the total by the amount of radioactivity originally added to the system in the octanol solution. The result was expressed as a percentage.

The experimentally determined partition coefficient of doramectin was compared to the value estimated from the aqueous solubility of doramectin by means of the following equation:

$\log K_{ow} = 5.00 - 0.67 \log S$, where S is in µg/L equation (1)

It was also used to estimate the bioconcentration factor (BCF) and the soil sorption coefficient (K_{oc}) of doramectin by means of standard equations:

$\log BCF = 0.79 \log K_{ow} - 0.40$ equation (2)
$\log K_{oc} = 0.544 \log k_{ow} + 1.377$ equation (3)

For a variety of reasons, each of these correlations applies to some classes of compounds but not to others. In order to test whether they apply to avermectins like doramectin, the same calculations were carried out with a compound very closely related to doramectin, abamectin (avermectin B_{1a}), and the results were compared with the known, experimentally determined values.

Summary of Results: The table below lists the mean values of the final concentration in the octanol phase, final concentration in the aqueous phase, partition coefficient, logarithm of partition coefficient, and percent radiolabel recovered, for each initial concentration in octanol.

Initial Concentration	Final Concentration mg/ml Octanol	Water	Kow	log Kow	% Recovery
3.7×10^{-5} M	0.0354	1.35×10^{-6}	26,234	4.42	104.5
	0.0340	1.25×10^{-6}	27,226	4.43	100.5
	0.0346	1.25×10^{-6}	27,668	4.44	102.1
Mean			27,043	4.43	102.4
Standard Deviation			735		2.0
3.7×10^{-4} M	0.352	1.48×10^{-5}	23,858	4.38	101.2
	0.346	1.39×10^{-5}	24,821	4.39	100.2
	0.351	1.41×10^{-5}	24,912	4.40	101.6
Mean			24,531	4.39	101.3
Standard Deviation			585		0.9

These results show that doramectin partitions almost exclusively into the octanol phase in preference to the aqueous phase.

The values calculated from equations (1), (2), and (3) are shown below together with known experimentally determined values.

	Doramectin		Abamectin	
Property	Calculated	Found	Calculated	Found
Solubility	--	25 ppb	--	7.8 ppb
Kow	11,571	25,787 (ave.)	25,252	9,900
BCF	1,216	--	571	52
Koc	5,891	--	3,553	4,760

Judging from these data, the correlations of partition coefficient with solubility (equation 1) and of partition coefficient with soil sorption constant (equation 3) apply to these compounds, but the correlation of partition coefficient with bioconcentration factor (equation 2) does not. The lack of correlation with BCF probably stems from the fact that these compounds lack properties required for bioconcentration, such as a sufficiently small molecular size and prolonged persistence.

Appendix c-5

Soil Sorption and Desorption of Doramectin

Report Summary: SOIL SORPTION AND DESORPTION OF DORAMECTIN

Study Number: 2438-1088-6133-710

Test System: Three types of soil in contact with aqueous solutions.

Summary of Experimental Design: The same general procedure was used to conduct a screening test, a soil kinetics test, and an isotherm determination. All tests were conducted in triplicate. Three different types of soil were used: a Mississippi Silty Clay Loam (MSCY), a California Clay Loam (CACY), and a Texas Silty Clay Loam (TXCY). The characteristics of these soils are shown in Table 1.

To study sorption, samples of each soil were shaken in capped centrifuge tubes with solutions of radiolabelled doramectin in 0.01 M aqueous calcium chloride. The ratio of solution to soil was 5:1 in the screening test. In the soil kinetics and isotherm tests the ratios were 1000:1 for Mississippi silty clay loam, 200:1 for California clay loam and 80:1 to Texas silty clay loam. For every combination of soil type and initial concentration, the concentration remaining in the aqueous phase (C_e) was determined by radioassay, and the amount sorbed onto soil (x) was calculated from the difference between the initial and final concentration in the aqueous phases.

To study desorption, soil samples containing sorbed doramectin were equilibrated twice in succession with fresh 0.01 M aqueous calcium chloride, and the concentrations in the aqueous phases were again determined by radioassay. In the screening test, a separate set of sorption and desorption experiments were carried out with deionized water as the aqueous vehicle.

Calculations: The logarithm of the experimentally determined equilibrium concentration, log C_e, was plotted against log (x/m) for each soil, where x/m is the concentration in the soil. The points on the graph were fitted to a logarithmic transformation of the Freundlich isotherm equation:

$$\log (x/m) = \log (K_d) + 1/n \log (C_e)$$

where K_d is the Freundlich sorption coefficient and 1/n, an empirical constant, is the slope of the graph. Log K_d was read off the graph as the intercept. The antilog, K_d, was then calculated and converted to K_{oc}, the sorption constant adjusted for the organic carbon content of the soil, according to the equation:

$$K_{oc} = (K_d \times 100)/\% \text{ organic carbon}$$

The percent of the initially added doramectin that would be sorbed from aqueous solution onto each of the soils at the respective solution to soil ratios (R) was calculated from the K_d values determined for sorption in the isotherm test.

$$\% \text{ Sorbed} = [K_d/(K_d + R)] \times 100$$

Summary of Results: The results of the screening test indicated that doramectin is strongly sorbed to all three soil types, suggesting that it would be advisable to conduct subsequent tests at a high ratio of solution to soil so the low concentrations in the aqueous phases could be determined accurately. It also showed that the presence of calcium chloride, which simulates natural conditions, did not interfere with the sorption of doramectin to soil.

In the soil kinetics test, aqueous concentrations obtained in the equilibrium phase (Table 2) indicated that the time to reach equilibrium varied considerably among the three soil types. Equilibrium was reached after 72 hours for MSCY soil, after 4 hours with CACY soil and after 24 hours with TXCY soil.

The results of the isotherm test confirm that doramectin is strongly sorbed to soil (Table 3 and 4). The value of K_d, the Freundlich sorption coefficient for these three soils ranged from 70.8 to 562 (Table 4). The corresponding ranges of K_{oc} were 7,520 to 86,900 (Table 4). Compounds having a K_{oc} value of 1000 or larger are considered relatively immobile in soils and have a low potential for leaching into the water table or into runoff water. It was calculated that at a solution to soil ratio of 5:1, 99.1% of doramectin will sorb to MSCY, 97.9% will sorb to CACY and 93.4% will sorb to TXCY soil.

Table 1. Characterization of three soils used in the doramectin sorption/desorption coefficient determination.

Source	Mississippi	California	Texas
Texture	Silty Clay Loam	Clay Loam	Silty Clay Loam
% Sand	5.0	32.0	13.0
% Silt	64.0	28.0	59.0
% Clay	31.0	40.0	28.0
% Organic Matter	1.1	3.0	1.6
pH	5.1	7.3	7.8
Cation Exchange Capacity (meq/100g)	7.3	19.4	15.0

Table 2. Soil kinetics test for sorption of doramectin to three soils.

Time interval (Hours)										
0	2	4	8	24	48	72	120	144	168	288
Mississippi Silty Clay Loam										
11.6	8.1	8.3	7.7	8.4	7.5	6.4[a]	4.0	5.8	4.7	5.2
California Clay Loam										
10.6	5.4	3.9[a]	3.0	3.9	3.8					
Texas Silty Clay Loam										
8.2	3.9	4.0	3.4	3.0[a]	3.5					

[a] Equilibrium achieved.

Table 3. Isotherm test: Concentration of doramectin in solution and soil. Mean of three values and standard deviation.

Mean Measured Initial Concen. µg/ml		Mississippi Silty Clay Loam Aqueous µg/ml	Soil µg/g	California Clay Loam Aqueous µg/ml	Soil µg/g	Texas Silty Clay Loam Aqueous µg/ml	Soil µg/g
0.667[a]	Mean	0.00033	0.867	0.00033	0.132	0.00033	0.025
(1.17)[b]	SD	0	0.057	0	0.011	0	0.002
2.23[a]	Mean	0.00096	0.833	0.00066	0.284	0.00096	0.040
(2.27)[b]	SD	0	0.057	0	0.011	0	0.002
6.13[a]	Mean	0.0018	3.300	0.0012	0.780	0.0015	0.155
(4.20)[b]	SD	0	0.173	0	0.029	0	0
10.6[a]	Mean	0.004	6.100	0.004	1.228	0.0035	0.235
(10.7)[b]	SD	0.001	0.656	0	0.039	0.001	0.025

[a] Mean measured initial concentration for MSCY soil type.
[b] Mean measured initial concentration for CACY and TXCY soil types.

Table 4. Linear regression analysis of the sorption data using the Freundlich isotherm $Log_{10}(x/m) = \{log_{10}(K_d) + 1/n\ log_{10}(C_e)\}$ for doramectin with three soil types.

Mean Measured Concentration (µg/L)	Mississippi Silty Clay Loam		California Clay Loam		Texas Silty Clay Loam	
	$log_{10}C_e$	$log_{10}x/m$	$log_{10}C_e$	$log_{10}x/m$	$log_{10}Ce$	$log_{10}x/m$
0.667[a] (1.17)[b]	-3.4771	-0.0621	-3.4771	-0.8787	-3.4771	-1.5886
2.23[a] (2.27)[b]	-3.0147	-0.0792	-3.1761	-0.5470	-3.0147	-1.3965
6.13[a] (4.20)[b]	-2.7447	0.5185	-2.8973	-0.1079	-2.8239	-0.8106
10.6[a] (10.7)[b]	-2.3872	0.7853	-2.3979	0.0891	-2.4518	-0.6295
Correlation:	0.811		0.926		0.871	
Slope (1/n):	0.845		0.914		1.004	
Int ($log_{10}K_d$):	2.75		2.37		1.85	
n:	1.183		1.094		0.996	
K_d:	562		234		70.8	
K_{oc}:[c]	86900		13300		7520	

[a] Mean measured initial concentration for MSCY soil type.
[b] Mean measured initial concentration for CACY and TXCY soil types.
[c] % Organic carbon = % organic matter/1.7

Appendix c-6

Fecal Sorption and Desorption of Doramectin

Report Summary: FECAL SORPTION AND DESORPTION OF DORAMECTIN

Study Number: 2438.1088.6134.711

Test System: Cattle feces in contact with aqueous solutions.

Summary of Experimental Design: The same general procedure was used to conduct a screening test, a kinetics test and an isotherm determination. All tests were conducted in triplicate. Feces were collected from 300 kg steers fed a nonmedicated ration consisting of corn silage plus mineral mix. Fecal organic matter was found to be 13.94% on a wet basis and 77.9% on a dry weight basis. Feces were dried, ground in a blender and sterilized by gamma irradiation before use.

To study sorption, fecal samples were shaken in capped centrifuge tubes with solutions of radiolabeled doramectin in 0.01M aqueous calcium chloride. The ratio of solution to feces was 20:1 in the screening test and 1000:1 in the kinetics and isotherm tests. The concentration of doramectin remaining in the aqueous phase (C_e) was determined by radioassay and the amount sorbed onto feces (x) was calculated from the difference between the initial and final concentration in the aqueous phase.

To study desorption, fecal samples containing sorbed doramectin were equilibrated twice in succession with fresh 0.01 M aqueous calcium chloride, and the concentrations in the aqueous phases were again determined by radioassay. In the screening test, a separate set of sorption and desorption experiments was carried out with deionized water as the aqueous vehicle.

Calculations: The Freundlich sorption constant was determined using the following equation:

$$K_d = \frac{X/m}{C_e}$$

where: X/m = concentration of doramectin in feces as µg/g

C_e = concentration of doramectin in water phase as µg/L

The percent of test article sorbed was calculated as:

$$A = 100 \times \frac{G - (C_e \times V_o)}{G} = 100 \times \frac{X}{G}$$

Note that X was calculated as $G - (C_e \times V_o)$

The percent of test article desorbed was calculated as:

$$D = 100 \times \frac{(C_1 + C_2)\,V - (V_o - V)\,C_e}{X}$$

The sorption coefficient for the screening test was calculated as:

$$K_{ads} = \frac{X/m}{C_e}$$

The sorption coefficient was also calculated as a function of the organic carbon content of the feces as:

$$K_{oc} = K_d \text{ (or } K_{ads}) \times \frac{100}{\% \text{ OC}}$$

Measurable quantities required were:

m	=	dry weight of feces (g)
C_e	=	concentration of test article remaining in solution in the sorption step (µg/L)
C_1	=	concentration of test article in solution in the first wash (µg/L)
C_2	=	concentration of test article in solution in the second wash (µg/L)
V_o	=	original volume of solution (L)
V	=	volume of solution obtained after the desorption step (L)
G	=	quantity of test article recovered from the control lacking feces (µg)
% OC	=	percent organic carbon in the feces

Summary of Results: Total sorption was observed in the screening test and Kd could not be calculated. The solution: feces ratio was adjusted to 1000:1 for subsequent tests.

In the fecal kinetics test, equilibrium of sorbed and dissolved doramectin was observed after 48 hours (Table 1.).

The results of the isotherm test confirm that doramectin is strongly sorbed to feces (Table 2). A value of 15,600 was observed for the Freundlich sorption coefficient (K_d). The corresponding K_{oc} value was 34,100. Compounds having a K_{oc} value of 1000 or higher are considered relatively immobile and have a low potential for leaching into the water table or into runoff water.

Table 1. Aqueous concentration (µg/L) for the feces kinetics test in $CaCl_2$.

	2 Hours	4 Hours	8 Hours	24 Hours	48 Hours	72 Hours
Test Material	5.5	5.6	4.8	3.9	2.8	2.8
Control Bank	0	0	0.35[a]	0	0	0
Control lacking feces	6.5	6.7	6.8	6.3	5.8	5.6

Initial concentration 10 µg/L
[a] Slight contamination occurred

Table 2. Isotherm test: Concentration of doramectin in solution and feces. Mean of three values and standard deviation.

Measured Conc. (µg/L)	Measured Aqueous Conc., C_e (µg/L)	Calculated Feces Conc., x/m (µg/g)		
10 µg/L				
9.84 µg/L (soilless control)				
Mean	2.53×10^{-3}	7.00		
(S.D.)	(1.35×10^{-4})	(0.29)		
			log (C_e)	log (x/m)
6 µg/L				
5.23 µg/L (soilless control)			-2.60	0.845
Mean	1.45×10^{-3}	3.56	-2.84	0.551
(S.D.)	(1.99×10^{-4})	(0.221)	-3.37	0.175
			-3.32	-0.456
2.0 µg/L				
1.98 µg/L (soilless control)				
Mean	4.29×10^{-4}	1.50		
(S.D.)	(1.04×10^{-4})	(5.90×10^{-2})		
			Slope (1/n)	1.29
			Y-intercept (log K_d)	4.19
1.0 µg/L			Correlation Coefficient (R^2)	0.746
0.843 µg/L (soilless control)				
Mean	4.78×10^{-4}	0.350	n	0.774
(S.D.)	(1.00×10^{-6})	(1.27×10^{-2})	K_d	1.56×10^4
			K_{oc}	3.41×10^4

Appendix c-7

Soil Column Leaching of Doramectin

Report Summary: SOIL COLUMN LEACHING OF DORAMECTIN

Study Number: PFZ-520

Test System: ^{14}C doramectin admixed with two soils at a rate equivalent to 0.6 kg/ha (183 ppb) in 30 cm glass columns.

Summary of Experimental Design:

Characteristics of 2 soils employed in the study are as follows:

	Cation Exchange Capacity (meq/100g)	Organic Matter (%)	pH	Bulk density (g/cm^3)
Thoresby Loamy sand	5.0	1.2	7.2	1.38
Alconbury Sandy clay loam	18.7	2.7	7.9	1.04

A leaching study was conducted to estimate the mobility of doramectin in two soils representative of those employed in agriculture. Two glass columns per soil, 5 cm in diameter and 30 cm in height, were packed with dried, sieved soil. Soil containing ^{14}C doramectin at a rate equivalent to 0.6 kg/ha soil (183 ppb) formed the top 20 g of air dried soil in the column. After formation, the columns were saturated with 0.01M calcium chloride solution and the void volume determined by addition of ^{36}Cl sodium chloride. Leachate was then collected in fractions following addition of 1L of water. ^{14}C doramectin and ^{36}Cl sodium chloride content in leachate was quantitated by liquid scintillation counting; radioactivity in soil was determined by combustion analysis after dismantling the column into 5 cm sections.

Summary of Results: No appreciable leaching of doramectin was observed in either of the two soils evaluated. Total mean recoveries of ^{14}C-radioactivity and ^{36}Cl-radioactivity were 92-95% and 96-100%, respectively, of applied amounts (table).

Recovery of radioactivity from soil columns after elution following application of ^{14}C-doramectin

Recovery expressed as % applied ^{36}Cl or ^{14}C

Fraction	Alconbury sandy clay loam		Thoresby loamy sand	
	Column A	Column B	Column A	Column B
^{36}Cl in leachate	92.6	99.4	104.0	95.0
^{14}C in leachate	<0.6	<0.6	<1.2	<0.6
^{14}C in soil extract[a]	95.1	73.4	83.9	88.0
^{14}C in soil residues[a]	2.6	19.8	5.5	5.6
^{14}C Total	97.7	93.2	89.4	93.6

[a]All the radioactivity in soil extracts and residues was recovered in the top 0-5 cm section of the column.

The leachate from both soils contained no detectable ^{14}C-radioactivity (<1.2% applied radioactivity in the total leachate). Most of the applied ^{14}C-radioactivity (89.4-97.7%) was retained in the top 5 cm section of the columns with radioactivity in the lower sections being below the limit of reliable measurement (<3% applied).

Results suggest that amendment of soils with manure or litter from treated animals should not result in contamination of ground water due to the movement of doramectin in soils.

Report Summary: AQUATIC PHOTODEGRADATION OF ^{14}C-DORAMECTIN

Study Number: SC930250

Test System: Exposure of Aqueous Solutions to Simulated Sunlight

Summary of Experimental Design: A sterile aqueous solution containing approximately 1 ppm ^{14}C-doramectin was asceptically transferred to four autoclaved quartz test vessels. Two test vessels were used for irradiated samples and two were wrapped in aluminum foil for dark controls. Air inlet and outlet ports permitted collection of radioactive volatiles and $^{14}CO_2$ from irradiated samples. Actinometer solution, p-nitro acetophenone-pyridine (PNAP/PYR), was added to two additional autoclaved test vessels, one of which was irradiated and the other wrapped in aluminum foil for a dark control. Irradiated vessels were placed in a solid quartz box in an Hereaus Suntest CPS containing a Xenon-Arc lamp with a 290 nm cutoff filter and continuously irradiated directly from above. Lamp intensity was measured directly in watts/m^2. Controls were placed in an environmental chamber in the dark. All vessels were maintained at a temperature of approximately 25°C. Triplicate aliquots from each irradiated and control vessel containing ^{14}C-doramectin and PNAP/PYR were collected at 12 time points over 24 hours and assayed by HPLC for loss of parent ^{14}C-doramectin using a radioactive flow detector or loss of PNAP/PYR using a UV detector set at 280 nm. Triplicate aliquots of trapping solutions were counted for ^{14}C by liquid scintillation. ^{14}C-photodegradate analysis was performed on concentrated samples from the 24 hr sampling of the irradiated ^{14}C-doramectin test solutions using a gradient HPLC system to resolve polar degradates. Degradates were detected using UV detection and fraction collection/^{14}C liquid scintillation counting.

Calculations: The photolytic rate constant, k, was obtained by plotting ln C_o/C_t versus time according to the first order equation:

$$\ln C_o/C_t = kt$$

where C_o = concentration of ^{14}C-doramectin (or actinometer) in the dark controls at time t (in hours)

and C_t = concentration of ^{14}C-doramectin (or actinometer) in the irradiated samples at time t (in hours)

The slope of the regression line, k, was obtained from the graph and used to calculate the half-life, $t_{1/2}$, according to the relation:

$$t_{1/2} = 0.693/k$$

Summary of Results: ^{14}C-doramectin underwent rapid photolysis in dilute aqueous solution upon exposure to simulated sunlight, with a calculated rate constant and half life of 0.16 hours^{-1} and 4.45 hours, respectively in the definitive study (Table 1). The total simulated solar power density (290 to 800 nm) was calculated as 578 watts/m^2 and the UV component (290 to 385 nm) was 49.7 watts/m^2, which compares to a constant sunlight exposure level of a clear day at noon on June 29 in the mid-northern latitudes. Dark controls exhibited no degradation. Only minimal amounts of volatile degradates were detected in irradiated samples (0.2-1.2% of applied radioactivity). Mass balance for irradiated samples ranged from 103.5 to 105.2%.

Appendix c-8

Aquatic Photodegradation of Doramectin

Table 1. Photolytic rate constant and half-life for aqueous solutions of ^{14}C-doramectin and actinometer.

Test Sample	Rate Constant k (hours^{-1})	Linear Regression Coefficient (r^2)	Half-life $t_{1/2}$ (hours)
^{14}C-doramectin	0.16	0.96	4.45
Actinometer	0.07	0.99	10.50

^{14}C-photodegradate analysis of the 24 hour irradiated ^{14}C-doramectin samples revealed that most of the radioactivity (77-78%) eluted on HPLC as a polar peak of materials. Further analysis demonstrated that this polar material consisted of at least 10 minor degradation products, none of which individually accounted for more than 10% of the applied radioactivity. The low UV response of the radiolabeled photodegradates implied these compounds did not contain the macrolide ring chromophore characteristic of doramectin. None of these minor polar degradates was further profiled.

Doramectin does not exhibit absorption in the UV-visible spectrum above 275 nm (Appendix c-3). Yet rapid degradation was observed in this study in the presence of a radiation spectrum from 290-800 nm (simulating the solar spectrum) suggesting an indirect photolytic mechanism. These results are consistent with recent observations by Crouch *et. al.*, 1991 (J. Agric. Food Chem., **39**:1310-1319) who observed rapid degradation of avermectin B_{1a} when applied as thin films to glass under artificial light at wavelengths above 260 nm. The observed photodegradation of avermectin B_{1a} can be rationalized by the abundance of potentially oxygen sensitive sites on the molecule. These same sites are present on doramectin and would be vulnerable to attack by singlet oxygen, thus providing a plausible mechanism for degradation by indirect photolysis.

Appendix c-9

Aerobic Biodegradation of Doramectin in Soil

Report Summary: AEROBIC BIODEGRADATION OF DORAMECTIN IN SOIL

Study Number: SC920011

Test System: ^{14}C doramectin admixed with soils at 12.5 ppm.

Summary of Experimental Design:

Characteristics of 3 soils employed in the study are as follows:

Soil identification (Location)	Cation Exchange Capacity (meq/100g)	Organic Matter (%)	pH	Field Moisture Capacity (%)	Texture (%) Sand	Silt	Clay
Clay Loam (West Jefferson, OH)	15.6	2.0	5.3	23.7	26	46	28
Silt Loam (Illinois)	29.2	4.2	7.9	30.2	22	54	24
Loam (Castleton, ND)	29.3	3.3	7.5	29.5	40	42	18

Three treatments were employed: 1) ^{14}C doramectin at a final concentration of 12.5 ppm in soil (2.518×10^6 DPM activity), 2) glucose (a combination of ^{14}C and unlabeled) at a final concentration of 10 mg C/50 g soil (2.648×10^6 DPM activity), 3) untreated control. Each treatment was evaluated in triplicate for each of the 3 soils. A series of 27 incubation flasks, each containing 50 g of soil, were arranged in a system modified from Marinucci and Bartha (Apparatus for monitoring the mineralization of volatile ^{14}C-labelled compounds. *Appl. Environ. Microbial.* **38**: 1020-1022) for trapping $^{14}CO_2$ and where appropriate, organic volatiles. Flasks were incubated in the dark at 22±3°C. The amount of radiolabeled carbon dioxide in the traps was measured periodically by liquid scintillation counting. All treatments were monitored for 72 days.

The glucose treatment demonstrated rapid mineralization to CO_2 in all three soils with measured time to 50% mineralization of approximately 7, 14 and 35 days, respectively, for Ohio, Illinois and North Dakota soils. Under conditions of the study, mineralization of doramectin to CO_2 did not occur to any appreciable extent (3-4% $^{14}CO_2$ in 72 days). The amount of doramectin transformed to metabolites in 72 days was observed to be 42.2%, 53.5% and 55.6% for Ohio, Illinois and North Dakota soils, respectively. The estimated time to 50% transformation for the same three soils was 79, 62 and 61 days, respectively. The untreated control demonstrated very little $^{14}CO_2$ evolution in any of the soils. Less than 0.005% of organic volatiles were trapped during the test article treatment in any of the three soils.

At the termination of the experiment, material balance achieved for the glucose treatment was 98% (72.6% mineralized to $^{14}CO_2$, 1% extractable, 24.4% bound to soil), 94% (64.1% mineralized to $^{14}CO_2$, 1% extractable, 28.9% bound to soil), and 98% (60.7% mineralized to $^{14}CO_2$, 1.7% extractable, 35.3% bound to soil), respectively, for Ohio, Illinois and North Dakota soils. For the doramectin treatment, it was 98% (3.38% mineralization to $^{14}CO_2$, 79.2% extracted by methanol and acetone:water, 15.4% bound to soil); 98% (4.4% mineralized to $^{14}CO_2$, 73.4% extracted by methanol and acetone:water, and 19.1% bound to soil), and 98% (3.7% mineralized to $^{14}CO_2$, 74.5% extracted by methanol and acetone:water, and 19.3% bound to soil), respectively, for the same three soils.

The study indicates that doramectin is degraded into a series of minor components (15 chromatographic peaks). Only a single degradate in a single soil (Illinois silt loam) constituted more than 10% of the applied dose (range 12.68 - 13.75%). This component was identified as 8α-hydroxydoramectin.

Appendix c-10

Effect of Doramectin on Soil Microbes

Report Summary: EFFECT OF DORAMECTIN ON SOIL MICROBES

Study Number: 2438-1089-6145-790

Test Species: Soil-dwelling microbes

Summary of Experimental Design: The lowest concentrations of doramectin that will inhibit the growth of pure cultures of representative soil bacteria, ascomycetes, fungi, and blue-green algae were determined by the agar plate dilution technique. The following organisms were used.

Clostridium perfringens, a free-living nitrogen-fixing bacterium

Nostoc, a blue-green alga

Pseudomonas aeruginosa, a soil bacterium

Chaetomium globosum, an ascomycete

Aspergillus flavus, a mold

Each of the above organisms was maintained in pure culture under temperature, light and atmosphere conditions appropriate for the species. The following testing procedure was followed separately, in duplicate, for each of the five microbial species. A preliminary range-finding study was conducted at concentrations of 1,000, 100, 10 and 1 ppm. The results were used to select a geometric series of four more closely spaced concentrations. Depending on the species, these ranged from 800 to 20 ppm. Each concentration was obtained by mixing 2 ml of a standard stock solution containing ten times the desired final concentration with 18 ml of molten agar, except that 2 ml of acetonitrile employed to solubilize doramectin was mixed with agar to prepare the negative controls. The agar was then poured into a Petri dish, allowed to cool and solidify, inoculated with the organism, and incubated at an appropriate temperature. When colony growth was well developed on the plates which did not contain any drug, the plates containing doramectin were examined visually for microbial growth. The lowest concentration that completely inhibited growth was recorded as the minimum inhibitory concentration (MIC).

Summary of Results: In the preliminary test, an inhibitory effect was observed at ≥ 100 mg/L for *Clostridium* and *Nostoc*. *Aspergillus*, *Pseudomonas* and *Chaetomium* were observed to be inhibited only at the 1000 mg/L exposure level. Table 1 summarizes the observations made during the preliminary test.

Table 1. Observations recorded during the preliminary exposure of microorganisms to doramectin

Species	Replicate	Solvent Control	Concentration (mg/L) 1.0	10	100	1000
Aspergillus flavus	R1	G	G	G	G	N
	R2	G	G	G	G	N
Chaetomium globosum	R1	G	G	G	G	N
	R2	G	G	G	G	N
Clostridium perfringens	R1	G	G	G	N	N
	R2	G	G	G	N	N
Nostoc	R1	G	G	G	N	N
	R2	G	G	G	N	N
Pseudomonas aeruginosa	R1	G	G	G	G	N
	R2	G	G	G	G	N

G = Growth observed on plate
N = No growth observed (total inhibition)

A definitive test was conducted with the five species and growth inhibition was observed, as follows: *Clostridium perfringens*, ≥ 40 mg/L; *Nostoc*, ≥60 mg/L; *Aspergillus flavus*, ≥ 600 mg/L; and *Pseudomonas aeruginosa* and *Chaetomium globosum*, ≥800 mg/L (Table 2).

Based on these results, the minimum inhibitory concentrations were determined to be: *Clostridium perfringens*, 40 mg/L; *Nostoc*, 60 mg/L; *Aspergillus flavus*, 600 mg/L; and *Pseudomonas aeruginosa* and *Chaetomium globosum*, 800 mg/L.

Table 2. Observations recorded during the definitive exposure of microorganisms to doramectin

Species	Replicate	Solvent Control	Concentration (mg/L) 20	40	60	80
Clostridium perfringens	R1	G	G	N	N	N
	R2	G	G	N	N	N
Nostoc	R1	G	G	G	N	N
	R2	G	G	G	N	N
		Solvent Control	200	400	600	800
Aspergillus flavus	R1	G	G	G	N	N
	R2	G	G	G	N	N
Chaetomium globosum	R1	G	G	G	G	N
	R2	G	G	G	G	N
Pseudomonas aeruginosa	R1	G	G	G	G	N
	R2	G	G	G	G	N

G = Growth observed on plate
N = No growth observed (total inhibition)

Appendix c-11

Effect of Doramectin on Seed Germination and
Root Elongation of Six Plant Species

Report Summary: EFFECT OF DORAMECTIN ON SEED GERMINATION AND ROOT ELONGATION OF SIX PLANT SPECIES

Study Number: 2438-0189-6140-600

Test Species: Six species of plant seeds

Summary of Experimental Design: Seeds of the following species were used:

Monocotyledons: *Lolium perrenne* - perennial ryegrass
Triticum aestivum - wheat
Zea mays - corn

Dicotyledons: *Cucumis sativus* - cucumber
Glycine max - soybean
Lycopersicon esculentum - tomato

Seeds of 3 species of monocotyledons and 3 species of dicotyledons were exposed to varying concentrations of doramectin to determine effects upon germination and root elongation. All species were evaluated initially in a preliminary test at nominal concentrations of 1000, 100, 10 and 1 ppm. A definitive test followed in which cucumber and soybean were exposed to mean measured concentrations of 840 and 990 ppm doramectin, respectively. Tomato and corn were evaluated at measured concentrations of 840, 440, 220, 120 and 57 ppm; perennial ryegrass was evaluated at 6.6, 3.3, 1.6, 0.75 and 0.4 ppm and wheat was evaluated at 57, 27, 14, 6.6 and 3.3 ppm.

In both tests, seeds in petri dishes were exposed to drug by applying doramectin dissolved in a volatile solvent (acetone) to silica sand. Appropriate water and solvent controls were included and each species was evaluated in triplicate. Tests were conducted in the dark and at optimal germination temperatures for each species. Concentrations of drug tested were confirmed analytically prior to initiating the definitive test and on test days 2 and 5. Tests were completed in 5-6 days.

Summary of results: In the preliminary test, monocotyledon species showed effects on root elongation at concentrations between 1000 and 10 ppm, but no effects on seed germination. Germination or root elongation of dicotyledon species was not affected. In the definitive test, no effects upon germination were observed in any species at the concentrations of doramectin that were tested. Significant effects upon root elongation were observed in perennial ryegrass at 6.6 and 3.3 ppm, the two highest levels tested. Wheat roots showed morphological abnormalities at the three highest concentrations but length was statistically equivalent to the solvent controls.

No observable effect concentrations (NOEC) and lowest observable effect concentrations (LOEC) are shown in the table. LOECs for all species except perennial ryegrass appear to be higher than the concentrations tested. Perennial ryegrass was determined to be the most sensitive of the six species exposed to doramectin, with an NOEC of 1.6 mg A.I./kg, and an LOEC of 3.3 mg A.I./kg, based on the effects observed on root elongation.

Species	% Germination[a]		Root Elongation[a]	
	NOEC (mg A.I./kg)	LOEC (mg A.I./kg)	NOEC (mg A.I./kg)	LOEC (mg A.I./kg)
Corn	840	>840	840	>840
Cucumber	840	>840	840	>840
Perennial ryegrass	6.6	>6.6	1.6	3.3
Soybean	990	>990	990	>990
Tomato	840	>840	840	>840
Wheat	57	>57	57	>57

[a] The NOEC and LOEC values were based on statistical analysis of percent germination and root elongation data collected at test termination. Morphological abnormalities were not used to define the NOEC and LOEC values.

Appendix c-12

Effect of Doramectin on Seedling Growth of Six Plant Species

Report Summary: EFFECT OF DORAMECTIN ON SEEDLING GROWTH OF SIX PLANT SPECIES

Study Number: 2438-0189-6141-620

Test Species: Six species of plant seedlings

Summary of Experimental Design: Seedlings of the following species were used:

Monocotyledons: *Lolium perrenne* - perennial ryegrass
Triticum aestivum - wheat
Zea mays - corn

Dicotyledons: *Cucumis sativus* - cucumber
Glycine max - soybean
Lycopersicon esculentum - tomato

Seedlings of 3 species of monocotyledons and 3 species of dicotyledons were exposed to varying concentrations of doramectin to determine morphological abnormalities, survival and effects on shoot length, shoot weight and root weight. In both the preliminary and definitive studies, seedlings were exposed to drug by applying doramectin dissolved in a volatile solvent (acetone) to silica sand. Growth and survival over 21 days were compared to two sets of controls, one which was exposed to the same quantity of acetone and one that was not. Species evaluated included the dicots, cucumber and soybean. Pinto bean was evaluated in the preliminary test and tomato in the definitive test. The monocots, corn, ryegrass and wheat, were evaluated in both the preliminary and definitive tests. In the former test, doramectin was evaluated at the nominal concentrations of 100, 10 and 1 mg/kg. In the latter test, mean measured concentrations were 980, 470, 230, 130, 53 and 33 mg/kg for various species.

Plants were arranged five per pot with 5 replicate pots per treatment and held on trays containing saucers to accommodate watering by subirrigation. Plants were maintained in a growth chamber under conditions that included: 16 hr light per day (mean 2000fc), 73 ± 6.9% mean relative humidity, 23 ± 2.3°C mean temperature and 360 ± 43 ppm mean CO_2. Plants received water containing nutrients daily to keep the sand moist. Due to the hydrophobic nature of the drug, the sand repelled the water for the first three days, and surface watering as well as subirrigation were necessary. Mortality and morphological abnormalities were recorded daily and shoot length was recorded on days 1, 3, 5, 7, 14 and 21. Shoot and root weights were recorded on day 21.

All statistical comparisons of the treatment data were made against the solvent control data. Percentage survival data were analyzed by Fisher's Exact Test. Replicate mean values for shoot length, shoot weight and root weight were used during the statistical analyses, which were calculated from individual observations.

Summary of Results: In the definitive test, the monocotyledons corn and wheat were evaluated at mean measured concentrations of 980, 470, 230, 130 and 53 ppm; perennial ryegrass was evaluated at 470, 230, 130, 53 and 33 ppm. Corn in treated groups showed minor wilting during the first 5 days, and several plants in treated and control groups died during the study.

At day 21, all plants appeared healthy and no morphological abnormalities were observed. There were no statistically significant differences between treated groups and solvent controls in mean shoot length or root weight. Shoot weight was statistically depressed at 980, 230 and 52 mg/kg but not at 470 or 130 mg/kg. The degree of depression was equivalent among the first 3 mentioned groups and did not follow a graded dose response.

Some treated perennial ryegrass became wilted and necrotic after day 5. Mortality occurred in all treated and control groups, but it was statistically significant only in the 130 mg/kg treated group. At 21 days, shoot length was statistically depressed at 230, 130, and 53 mg/kg but not at 470 or 33 mg/kg. Shoot weight was statistically depressed at 230 mg/kg but not at other levels. The degree of reduction did not follow a dose response. No statistically significant reductions were observed in root weight.

No mortality or morphological abnormalities were observed in control or treated wheat plants. At 21 days, mean shoot length and shoot weight were statistically depressed at all levels tested. Root weight was also statistically depressed at all but the lowest dose level. The degree of each depression was about the same at every level and was not dose related.

The dicotyledons soybean and tomato were evaluated at the mean measured concentrations of 980, 470, 230, 130 and 53 ppm and cucumber was evaluated at 470, 230, 130, 53 and 33 ppm. Except for minor wilting in several tomato plants during the first few days of the test, no morphological abnormalities were observed at any time. The NOEC for soybean was 980 ppm and the NOEC for tomato appeared to be between 53-130 ppm. A NOEC for cucumber was not assigned, but reductions in root weights of up to 45% were observed starting at 33 mg/kg, the lowest concentration tested in the definitive test, although the reductions were not statistically significant. The minimum significant differences for cucumber seedling growth were 14.7% for shoot length, 22.3% for shoot weight, and 107.8% for root weight. In contrast to the dicotyledons, statistically significant reductions in various parameters were observed for all 3 monocotyledons (corn, perennial ryegrass, wheat) but not in a dose related fashion and therefore, NOECs were not established. Reductions were likely related to water repellency and the associated dryness of the treatment during the early part of the study rather than any phytotoxic properties of doramectin.

Appendix c-13

Effect of Doramectin on Seedling Growth of Monocot Species, Perennial Ryegrass, Corn and Wheat

Report Summary: EFFECT OF DORAMECTIN ON SEEDLING GROWTH OF MONOCOT SPECIES, PERENNIAL RYEGRASS, CORN AND WHEAT.

Study Number: 2PFF-01

Test Species: Three Species of Plant Seedlings.

Summary of Experimental Design: Seedlings of the following species were used:

Monocotyledons: *Lolium perrenne* - perennial ryegrass
Triticum aestivum - wheat
Zea mays - corn

Seedlings of 3 species of monocotyledons were exposed to varying concentrations of doramectin to determine morphological abnormalities, survival and effects on shoot length, shoot weight and root weight. Only a definitive test was conducted. Seedlings were exposed to graded concentrations of doramectin in acetone added to the aqueous nutrient solution. One additional treatment was evaluated in which drug was applied in a volatile solvent (acetone) to the silica sand support media. Growth and survival of the drug treatments over 21 days was compared to two sets of controls, one in which the silica sand was exposed to the same quantity of acetone and one in which the nutrient solution was diluted with an equivalent amount of diluent (acetone).

Plants were arranged 5 seedlings per container with 5 replicate containers per treatment and held in waterproof trays to permit watering by subirrigation. Plants were maintained in a greenhouse under the following conditions: 16 hr light per day from sunlight and supplemental lights to maintain a minimum 387 μ Einsteins $m^{-2}s^{-1}$ light intensity, 25 ± 5°C mean temperature, >60% relative humidity and 350 ± 50 ppm mean CO_2. Plants received doramectin in the aqueous nutrient solution 3 times a week and nutrient solution without the drug on additional days as needed. Nutrient solution containing nominal drug concentrations of 0.2, 1, 5 and 50 ppb and silica sand containing 50 ppm doramectin were assayed by HPLC and mean measured concentrations of 0, 0.27, 3.79 and 45 ppb in nutrient solution as well as 47 ppm in sand were determined.

All statistical comparisons of the treatment data were made against respective controls by analysis of variance (ANOVA) by use of data collected on the last day of the test. NOECs were established when the ANOVA showed significant effects of treatment. A single degree of freedom F-test was used to compare the treatment means with the appropriate controls.

Summary of Results: On day 21, all plants appeared healthy and no morphological abnormalities were evident.

No significant effects were observed in any of the 3 crops tested in terms of growth (shoot length) or shoot dry weight. Reductions in ryegrass shoot length of 15% at 3.7ppb and 11% at 45 ppb, and reductions in shoot weights of 23% and 29% at the same respective doses in nutrient solutions were observed. These reductions were not statistically significant, but the power of the test was low for ryegrass analysis, with a minimum significant difference of 79% for shoot weight. However, doramectin coated on a sand support medium at 47 ppm did not elicit the same response with ryegrass. Only the root dry weights of corn showed a statistically significant increase when doramectin was applied as a solution at the lowest and highest concentrations but not at the intermediate concentrations. NOECs of 45 ppb for drug in solution and 47 ppm for drug applied to sand were established for each species for each criteria except

for corn root dry weight where a NOEC was not established for drug administered in solution. The latter effects were likely anomolous since the intermediate doses showed no effect and drug applied to sand at 1000 times the solution concentration also showed no effect.

Effects of doramectin on perennial ryegrass, corn and wheat. A single degree of freedom F-test was used to compare the treatment means with the appropriate controls at day 21.

Treatment Mean Measured Doramectin Concentrations		Perennial Ryegrass			Corn			Wheat		
		Shoot Length (cm)	Shoot Weight (mg)	Root Weight (mg)	Shoot Length (cm)	Shoot Weight (mg)	Root Weight (mg)	Shoot Length (cm)	Shoot Weight (mg)	Root Weight (mg)
T1	0 ppb	12.4	36.2	9.2	60.6	1114.6	488.8[a]	26.7	158.0	78.7
T2	0.27 ppb	15.1	43.7	12.7	60.3	1155.5	405.0	27.3	201.0	101.7
T3	3.7 ppb	10.0	23.0	7.6	55.6	1124.2	434.0	26.8	222.3	89.7
T4	45 ppb	10.5	21.1	8.0	64.4	1104.0	483.1[a]	26.8	203.8	102.0
Power (5%)[b]		<0.40	<0.40	<0.40	0.41	0.74	0.87	<0.40	<0.40	<0.40
C2	Control solution	11.8	29.8	8.3	62.4	1144.0	437.7	24.0	158.2	69.0
T5	47 ppm sand	11.7	33.5	9.1	55.3	1009.8	391.6	24.7	150.9	53.8
C1	Control (sand)	8.5	15.8	6.6	56.3	1065.2	429.4	20.4	149.20	56.8

[a] Statistically different from control (C2 for T1-T4, C1 for T5)
b Pearson-Hartley Power statistic values for detection of a 5% difference

Appendix c-14

Subacute Toxicity Studies with Doramectin on Earthworms

Report Summary: SUBACUTE TOXICITY STUDIES WITH DORAMECTIN ON EARTHWORMS

Study Numbers: 92181, 92239

Test Species: *Eisenia foetida*

Summary of Experimental Design: The toxicity of doramectin to the earthworm *Eisenia foetida* was determined by exposing worms (10/repitition, 4 repititions/treatment) in an artificial soil (AS) matrix [70% industrial sand, 20% kaolinite clay, 10% sphagnum peat, 25% moisture and rabbit feces (50 g dry weight/kg AS)] to logarithmically (1000, 100, 10, 1, 0.1 ppm) or geometrically (16, 8, 4, 2, 1 ppm) spaced dose levels for 28 days in two separate tests. Glass jars containing the worms in AS were maintained in a growth chamber at 20±4°C under continuous lighting (400-800 lux). Earthworm mortality and health were assessed after 7, 14, 21 and 28 days. The health assessment consisted of noting any abnormal behavior and appearance, such as lethargy, absence of burrowing, and softness. Worms were weighed on days 0 and 28. Triplicate aliquots of AS from each replicate were assayed for doramectin by soil combustion and liquid scintilliation counting at initiation and termination of the study.

Summary of Results: No mortality was observed in any of the medicated treatments or in the nonmedicated group. Worms exposed to 1000 or 100 ppm doramectin exhibited lethargy and required considerably longer periods of time to burrow beneath the soil surface than did other treatments. Worms exposed to 2, 1 or 0.1 ppm doramectin were normal in appearance and gained weight equivalent to the control group. Worms exposed to 16-4 ppm were normal in appearance but exhibited reduced weight gains compared to nonmedicated controls.

Based on these data, worms would have to be exposed to doramectin well in excess of 1000 ppm to sustain a consequent 50% reduction in survival. Based on weight gain, the most sensitive criteria monitored, the no observed effect concentration (NOEC) was 2 ppm.

Test 1

	Mean Weight (gm)		
Dose (ppm)	Day 0	Day 28	Mean change (gm)
0	0.249 ± 0.004	0.389 ± 0.029	+ 0.140 ± 0.030
0.1	0.248 ± 0.017	0.396 ± 0.027	+ 0.148 ± 0.043
1.0	0.233 ± 0.018	0.412 ± 0.006	+ 0.179 ± 0.021
10.0	0.242 ± 0.015	0.311 ± 0.011	*+ 0.069 ± 0.020
100.0	0.264 ± 0.018	0.183 ± 0.018	*- 0.081 ± 0.019
1000.0	0.235 ± 0.017	0.157 ± 0.007	*- 0.078 ± 0.012

Test 2.

Mean Weight (gm)

Dose (ppm)	Day 0	Day 28	Mean change (gm)
0	0.271 ± 0.035	0.562 ± 0.066	0.291 ± 0.051
1.0	0.289 ± 0.017	0.549 ± 0.035	0.260 ± 0.021
2.0	0.276 ± 0.030	0.512 ± 0.077	0.236 ± 0.061
4.0	0.274 ± 0.029	0.456 ± 0.028	*0.182 ± 0.020
8.0	0.273 ± 0.030	0.441 ± 0.026	*0.168 ± 0.040
16.0	0.272 ± 0.035	0.337 ± 0.026	*0.065 ± 0.041

* means differ signifcantly (p = 0.05) from control

Appendix c-15

Effect of Doramectin *In vitro* Upon Immature Dung Beetles and Hornflies

Report Summary: EFFECT OF DORAMECTIN *IN VITRO* UPON IMMATURE DUNG BEETLES AND HORN FLIES.

Study Numbers: 151-91-13
221-92-13
223-92-13

Test Species: Horn fly (*Haematobia irritans*) and dung beetle (*Onthophagus gazella*)

Summary of Experimental Design: The susceptability of immature stages of the horn fly and dung beetle to doramectin was determined by exposing horn fly eggs and dung beetle brood balls to varying concentrations of doramectin which had been mixed with cattle feces. Freshly oviposited horn fly eggs (100/repitition, 3 repititions/treatment) were seeded on cattle feces containing the drug. Larvae were allowed to develop and pupate and number of adults eclosing from pupae were recorded. Two mating pairs of dung beetles were placed in a container on cattle feces containing varying concentrations of doramectin (3 containers per drug level). Brood balls buried by adults were allowed to develop and progeny were subsequently trapped and counted.

Summary of Results: Initial tests were conducted to evaluate doramectin over logarithmically spaced dose levels (e.g. 250, 25, 2.5 and 0.25 ppb). Subsequent tests evaluated the effects of more closely spaced drug concentrations (Table 1 & 2) in order to calculate LC_{90} values. Against horn flies, concentrations of 2.4 ppb or less doramectin had no effect upon larvae development or emergence of adults from the puparium. At concentrations between 5-15 ppb, pupation was inhibited and development within the puparium was not completed. At concentrations of 20 ppb or higher, pupation was virtually eliminated. The data indicate that doramectin affects actively feeding horn fly larvae, resulting in death before emergence from the puparium. The LC_{90} was calculated to be approximately 3.0 ppb.

Against dung beetles, doramectin at concentrations up to 250 ppb had no effect upon number of brood balls produced indicating that neither mating nor oviposition was affected. The number of viable progeny were reduced compared to nonmedicated controls at concentrations of 25 or 16 ppb and higher in the first and second tests, respectively. In the same test, no progeny were observed at drug concentrations of 250 or 64 ppb, respectively. The LC_{50} and LC_{90} were calculated to be approximately 12.5 ppb and 38.2 ppb, respectively.

Table 1. Effect of doramectin on hornfly pupation and number of emerging adults.

Treatment (ppb)	Rep.	Number of Pupae	Percent Pupation	Numbers Adults Emerged	Percent Control[a] of Pupae	Percent Control[b] of Adults
Control	1	64	60.0	57		
	2	59		59		
	3	57		56		
0.5	1	69	60.7	55	0.0	8.1
	2	44		43		
	3	69		60		
1.0	1	65	61.7	51	0.0	25.6
	2	63		42		
	3	57		35		
5.0	1	16	18.0	0	70.0	100
	2	17		0		
	3	21		0		
10.0	1	9	5.7	0	90.6	100
	2	8		0		
	3	0		0		
15.0	1	2	9.0	0	100	100
	2	11		0		
	3	14		0		
20.0	1	0	0	0	100	100
	2	1		0		
	3	0		0		

[a] $$\% \text{ Control} = \frac{\text{Proportion of Eggs Resulting in Pupae in Controls} - \text{Proportion of Eggs Resulting in Pupae in Treated}}{\text{Proportion of Eggs Resulting in Pupae in Controls}} \times 100$$

[b] $$\% \text{ Control} = \frac{\text{Proportion of Adults Eclosed in Controls} - \text{Proportion of Adults Eclosed in Treated}}{\text{Proportion of Adults Eclosed in Controls}} \times 100$$

Table 2. Effect of doramectin on dung beetle brood ball production and emergence of progeny

Treatment (ppb)	Replicate	Brood balls	Progeny	Progeny emergence (%) from brood balls
Control	1	39	9	
	2	86	64	55.8
	3	40	19	
0.25	1	64	28	
	2	24	25	55.1
	3	48	22	
1.0	1	26	8	
	2	17	29	92.6
	3	52	51	
4.0	1	61	40	
	2	39	33	73.8
	3	45	34	
16.0	1	48	5	
	2	34	3	16.7
	3	26	10	
64.0	1	33	0	
	2	9	0	0.0
	3	42	0	

Appendix c-16

Effect of Doramectin on Disintegration
of Dung Pats in Pasture

Report Summary: EFFECT OF DORAMECTIN ON DISINTEGRATION OF DUNG PATS IN PASTURE.

Study Numbers: 1430C-60-90-003
1430C-60-90-005

Test Species: Grazing cattle

Summary of Experimental Design: A group of 14 pasture grazed steers, 6-12 months of age averaging 257 kg in weight were individually identified and divided into two groups of 7 each. Each steer in one group received 200 µg/kg doramectin 1% injectable solution by subcutaneous injection in the lateral midline of the neck. The other group received a comparable injection of saline. Steers were returned to pasture, and on the 4th, 32nd and 60th day after treatment, the first dung pat deposited from 5 animals from each treatment group on those days was identified and fenced to protect from trampling.

Following pasture deposition, steers were placed in clean outdoor pens by treatment group (7 steers/treatment) and dung was collected for a 28 hr period from each group (4-5, 32-33, 60-61 days post treatment) and homogenized. Bulk dung was formed into 14 circular constructed pats (30 cm diameter by 3 cm thickness) and randomly arranged along a fenced transect on the pasture previously occupied by the steers. Basal areas of deposited and formed pats were determined by monthly measurements during the 1990 (2 July-19 November) and 1991 (8 April-21 October) grazing seasons. Additionally, a portion of the formed pats were monitored for moisture content and residual dry matter as well as the identification and the biomass of invertebrates. Meteorological observations were recorded daily during the two grazing seasons.

Summary of Results: The field study found that treatment of cattle with doramectin had no effect on the rate of degradation of their naturally formed dung pats in the first two years of decomposition (Table 1). Rate of degradation was related to initial pat area; larger pats degraded faster than smaller pats. After adjusting for initial sizes, degradation rates of pats formed 4 and 32 days after doramectin injection were not different from rates of pats from corresponding control steers. In contrast, the rate of degradation of doramectin pats from day 60 was significantly reduced compared to untreated counterparts. The latter result is most likely a type I error, because no effect of the drug was detected among day 4 and 32 pats, ones excreted at times when fecal concentrations of the drug would have been higher.

Table 1 Least squares means[a] and results of corresponding ANOVAs of degradation rates (Δcm^2 per day) of dung pats passed by experimental steers, grouped by excretion day, year of degradation, and treatment group.

Excretion day	Year of degradation	Mean Rate Saline	Mean Rate Doramectin	SE[b]	*df*	*F*	*p*
4	1990	-0.204	-0.286	0.179	6	0.275	0.62
32		-0.600	-0.753	0.148	7	2.418	0.16
60		-0.972	-1.225	0.249	7	2.349	0.17
4	1991	-0.799	-0.648	0.411	7	0.207	0.66
32		-1.041	-0.430	0.383	6	4.941	0.07
60		-0.998	-0.345	0.190	7	26.433	<0.001

[a] Estimated with general linear model of form $Rate = \alpha + \beta T + \gamma A$, where T is categorical code for treatment group, A is initial pat area at time of formation in 1990, or on 6 April, 1991, and α, β and γ are constants estimated separately for each combination of excretion day and year of degradation.

[b] Square root of error mean square after fitting model.

No effects of doramectin were revealed on any aspect of the degradation rate of constructed dung pats during the first two years on pasture (Table 2). Rate of loss in basal area depended on initial pat size and on date of pat formation; larger pats eroded faster than smaller pats, and erosion of pats from dung excreted on days 32-33 was substantially faster than those of pats from dung excreted on days 4-5 and 60-61. The effect of formulation date on erosion is attributable to the hot dry weather experienced during the first day of field exposure.

Destructive sampling of randomly selected pats during the course of the study indicated that pat moisture content, residual dry matter, and all components of the available invertebrate fauna (Table 3) were unaffected by presence of doramectin. However, invertebrates of all kinds were generally sparse among pats formed from dung excreted on days 4-5, a time when treatment effects would likely be greatest. It appears the weather precluded adequate colonization by invertebrates of pats in the first excretion period.

Table 2 Least squares means[a] and results of corresponding ANOVAs of rates of change in basal area (Δ cm^2 per day), arranged by excretion period, year of degradation, and treatment group of pats constructed from dung collected from doramectin and saline treated steers.

Excretion days	Year of degradation	Mean rate (β) Saline	Doramectin	SE[b]	*df*	*F*[c]	*p*
4-5	1990	-0.865	-0.815	0.080	7	0.993	0.35
32-3		-0.951	-0.968	0.096	6	0.050	0.83
60-1		-1.664	-1.503	0.164	7	1.844	0.22
4-5	1991	-0.305	-0.467	0.054	6	17.399	<0.01
32-3		-2.441	-2.049	0.629	7	0.629	0.45
60-1		-0.553	-0.243	0.239	7	3.025	0.13

[a] Estimated with general linear model of form $Rate = \alpha + \beta T + \gamma A$, where T is categorical code for treatment group, A is initial pat area at time of formation in 1990, or on 6 April, 1991, and α, β and γ are constants estimated separately for each combination of period and year of degradation.

[b] Square root of error mean square after fitting model.

[c] F statistic for significance of treatment effect after adjusting for initial pat areas.

Table 3 Mean density and biomass (milligrams dry weight) of all invertebrates in pats constructed from dung collected from doramectin and saline treated steers.

Excretion Period[a]	Excretion Date	Assessment Day[b]	Assessment Date	Mean density ± 2SE Saline	Mean density ± 2SE Doramectin	df	*t*	*p*
5	3 Jul	28	31 Jul	2.7 ± 1.2	10.8 ± 3.5	4	-1.405	0.23
		112	23 Oct	0.0 ± 0.0	0.6 ± 0.4		na[c]	
		279	8 Apr	0.6 ±0.4	2.7 ± 1.7	4	-0.745	0.50
33	31 Jul	28	8 Aug	255.7 ± 53.4	244.9 ± 48.6	4	0.096	0.93
		112	20 Nov	0.7 ± 0.4	0.0 ± 0.0		na	
		280	7 May	4.8 ± 1.6	6.6 ± 1.8	4	-0.472	0.66
61	28 Aug	28	25 Sep	97.6 ± 9.4	188.8 ± 27.4	4	-2.029	0.11
		112	18 Dec	0.0 ± 0.0	0.7 ± 0.4		na	
		280	4 Jun	2.7 ± 0.4	0.0 ± 0.0		na	
				Mean biomass ± 2SE				
5	3 Jul	28	31 Jul	trace	trace		na	
		112	23 Oct	0.0 ± 0.0	trace		na	
		279	8 Apr	12.7 ± 8.1	117.8 ± 75.8	4	-0.887	0.43
33	31 Jul	28	8 Aug	909.2 ± 241.1	942.9 ± 311.0	4	-0.055	0.96
		112	20 Nov	3.2 ± 2.1	0.0 ± 0.0		na	
		280	7 May	14.2 ± 4.6	19.1 ± 5.7	4	-0.434	0.69
61	28 Aug	28	25 Sep	438.5 ± 75.5	597.5 ± 75.4	4	-0.958	0.39
		112	18 Dec	0.0 ± 0.0	1.4 ± 0.9		na	
		280	4 Jun	395.9 ± 135.6	0.0 ± 0.0		na	

[a] Days after steers injected on 28 June, 1990.
[b] Days dung exposed in field.
[c] *t*-test not applicable when values of one group or both are all 0.0.

Appendix c-17

Effect of Doramectin on Invertebrate Colonization and Disintegration of Dung Pats in Pasture

Report Summary: EFFECT OF DORAMECTIN ON INVERTEBRATE COLONIZATION AND DISINTEGRATION OF DUNG PATS IN PASTURE.

Study Numbers: GR25643

Test Species: Grazing cattle

Summary of Experimental Design: A group of 15 pasture grazed steers, 6-12 months of age averaging 263 kg in weight were individually identified and divided into two groups of 7 or 8 each. Each steer in one group received 200 µg/kg doramectin 1% injectable solution by subcutaneous injection in the lateral midline of the neck. The other group received a comparable injection of saline. Steers were returned to pasture on the 5th day after treatment, and the first dung pat deposited from each animal was identified and fenced to protect from trampling.

Following pasture deposition, steers were placed in clean outdoor pens by treatment groups, and dung was collected for a 28 hour period from each group and homogenized. Bulk dung was formed into 29 circular constructed pats (20 cm diameter by 3 cm thickness) and randomly arranged along a fenced transect on the pasture previously occupied by the steers. Basal areas of deposited and formed pats were determined by monthly measurements during the 1992 (23 June-15 October) and 1993 (2 April-15 October) grazing seasons. Additionally, a portion of the formed pats were monitored for moisture content and residual dry matter as well as the identification and the biomass of invertebrates. Meteorological observations were recorded daily during the two grazing seasons.

Summary of Results: Rates of degradation of natural pats within each year are summarized in Table 1. In 1992, the doramectin pats eroded at a rate of 0.96 cm^2/day, 0.13 cm^2/day less than the untreated pats. In contrast, the doramectin pats in 1993 eroded at the same rate as the untreated pats. Overall, the differences between treatment groups were well within the realm of chance ($F=0.25$ with 1,27 df; $p=0.61$). In contrast, effects of year were significant; the pats in both treatment groups eroded faster in their first year than in their second year ($F=5.25$ with 1,27 df; $p=0.030$). It is likely that basal areas of all pats diminished faster in the first year because of dehydration.

Tables 1: Summary statistics for daily rate of change in basal areas (cm^2 per day) of natural pats passed by experimental steers 5 days after receiving injections, grouped by year of observation and treatment.

Year	Treatment	Mean rate[a]	SE	n[b]	Difference[c]
1992	Vehicle	-1.09	0.15	8	
	Doramectin	-0.96	0.11	7	0.13
1993	Vehicle	-0.72	0.15	8	
	Doramectin	-0.72	0.11	7	0.00
1992	Treatments combined	-1.03	0.13	15	
1993	Treatments combined	-0.72	0.13	15	0.31

[a] Estimated with regression of measured area vs. day of year observed, excluding winter months.
[b] Number of pats observed in each year.
[c] Doramectin - Vehicle, or Year 1993 - 1992.

Rates of degradation of the constructed pats in each year are summarized in Table 2. The doramectin pats degraded at a rate of -0.79 cm^2/day, 0.16 cm^2/day less than the untreated pats. In contrast, the doramectin pats in 1993 degraded at a rate of -1.37 cm^2/day, 0.36 cm^2/day faster than the untreated pats. Overall, the differences between treatment groups ($F=0.09$ with 1,17 df; $p=0.76$) and between years ($F=0.97$ with 1,17 df; $p=0.34$) were well within the realm of chance. The rates of loss in basal areas were not affected by treatment with doramectin.

Table 2 Summary statistics for daily rate of change in basal areas (cm^2 per day) of constructed pats formed from dung passed by experimental steers 5 days after receiving injections, grouped by year of observation and treatment.

Year	Treatment	Mean rate[a]	SE	n[b]	Difference[c]
1992	Vehicle	-0.95	0.17	5	
	Doramectin	-0.79	0.09	5	0.16
1993	Vehicle	-1.01	0.19	5	
	Doramectin	-1.37	0.61	5	-0.36
1992	Treatments combined	-0.87	0.13	10	
1993	Treatments combined	-1.19	0.40	10	-0.32

[a] Estimated with regression of measured area vs. day of year observed, excluding winter months.
[b] Number of pats observed in each year.
[c] Doramectin - Vehicle, or Year 1993 - 1992.

Effects of doramectin on dung invertebrates varied among the component functional-taxonomic groups, and among pat ages after formation. In the 5-day old pats (Table 3), large and small dung feeding flies were the dominant components of the fauna. Doramectin reduced substantially the density and biomass of these flies. Density of predators (which presumably eat flies) was also lower in the treated pats, but their biomass was not affected. In contrast, there were no apparent effects of doramectin on the density or biomass of adult dung feeding beetles, or on the density or biomass of earthworms burrowing at the bases of the pats.

In the 28-day old pats (Table 4), retrieved after the flies had vacated, larvae of dung feeding scarab beetles (Aphodiinae) were the major element of the fauna. In the pats of this age, doramectin had no measurable effects on the density or biomass of the dung feeding beetles, residual flies, predatory beetles, or earthworms.

In the 310-day old pats (Table 5), retrieved after spring thaw, several species of earthworms were the dominant elements of the fauna. Doramectin had no measurable effects on their density or biomass.

Table 3: Density (specimens per pat) and biomass (milligrams dry weight per pat) of four functional groups of invertebrates in 5-day old constructed pats.

Group	Mean Density ± 2SE Vehicle	Doramectin	Functional Diff[a]	df	t	p
Dung feeding flies	1277.6 ± 313.8	21.0 ± 19.4	-1256.6	14	-8.65[b]	<.01
Dung feeding beetles	43.8 ± 15.4	49.4 ± 7.3	5.6	14	1.07[b]	0.31
Predatory beetles	58.9 ± 27.5	16.2 ± 7.1	-42.7	14	-4.11[b]	<.01
Earthworms	0.3 ± 0.5	1.0 ± 1.5	0.7	14	0.80[b]	0.44
	Mean Biomass ± 2SE					
Dung feeding flies	1.0 ± 0.3	0.1 ± 0.1	-0.9	14	-5.57	<.01
Dung feeding beetles	0.2 ± 0.1	0.4 ± 0.1	0.2	14	2.66	0.02
Predatory beetles	0.04 ± 0.02	0.04 ± 0.03	0.0	14	0.09	0.93
Earthworms	0.002 ± 0.003	0.081 ± 0.118	<0.1	14	1.36	0.22

[a] Difference in means, Doramectin - Vehicle
[b] Tested after transformation (Ln[x+1]) to counteract unequal variances and non-normality.

Table 4: Mean density (specimens per pat) and biomass (milligrams dry weight per pat) of four functional groups of invertebrates in 28-day old constructed pats.

Group	Mean Density ± 2SE Vehicle	Doramectin	Functional Diff[a]	df	t	p
Dung feeding flies	0.0	2.5 ± 4.9	2.5		na[b]	
Dung feeding beetles	43.0 ± 19.3	34.3 ± 17.2	-8.7	14	-0.68	0.51
Predatory beetles	21.4 ± 14.3	27.3 ± 13.1	5.9	14	0.72	0.49
Earthworms	4.8 ± 5.0	9.5 ± 8.9	4.7	14	0.92	0.38
	Mean Biomass ± 2SE					
Dung feeding flies	0.0	trace	trace		na[b]	
Dung feeding beetles	0.4 ± 0.2	0.2 ± 0.1	-0.2	14	-1.40	0.18
Predatory beetles	0.01 ± 0.01	0.01 ± 0.01	0.0	14	-0.98	0.34
Earthworms	0.093 ± 0.111	0.151 ± 0.103	<0.1	14	0.76	0.46

[a] Difference in means, Doramectin - Vehicle
[b] Test not applicable when all values in one or both treatment groups are 0.

Table 5: Mean density (specimens per pat) and biomass (milligrams dry weight per pat) of four functional groups of invertebrates in 310-day old constructed pats.

Group	Mean Density ± 2SE Vehicle	Doramectin	Functional Diff[a]	df	t	p
Dung feeding flies	0.0	0.0			na[b]	
Dung feeding beetles	0.4 ± 0.5	0.0	-0.4		na[b]	
Predatory beetles	0.1 ± 0.3	0.4 ± 0.5	0.3	14	0.79	0.44
Earthworms	13.3 ± 4.6	15.3 ± 5.0	2.0	14	0.59	0.57
	Mean Biomass ± 2SE					
Dung feeding flies	0.00	0.00			na[b]	
Dung feeding beetles	<0.01 ± <0.01	0.00			na[b]	
Predatory beetles	trace	<0.01 ± <0.01	<0.01		na[b]	
Earthworms	0.59 ± 0.20	0.68 ± 0.25	0.09	14	0.57	0.58

[a] Difference in means, Doramectin - Vehicle
[b] Test not applicable when all values in one or both treatment groups are 0.

Appendix c-18

Effect of Doramectin on Freshwater Algae

Report Summary:THE EFFECT OF DORAMECTIN ON FRESHWATER ALGAE

Study Number: 2438-0189-6138-430

Test Species: *Selenastrum capricornutum*, a freshwater green alga

Summary of Experimental Design: The effect of doramectin was determined on the growth rate and cell density of the freshwater algae *Selenastrum capricornutum*. Preliminary and definitive assays were conducted, each in 125 ml flasks containing 50 ml of Algal Assay Procedure (AAP) medium. Nominal concentrations of 1.0, 0.10, 0.01, and 0.001 ppm doramectin were evaluated in singlet in the preliminary test. Mean measured concentrations of 26 and 14 ppb, encompassing the maximum water solubility (25 ppb) of doramectin, were evaluated in triplicate in a definitive test. Both tests included media and solvent (acetone) controls. Each flask was inoculated with about 10^4 algal cells per ml and placed on a gyrotory shaking table in an environmental chamber. Light and temperature favorable to algal growth were maintained. At 24 hours and at each subsequent 48 hour interval, triplicate cell counts were conducted on each flask using a hemocytometer and a compound microscope. The test was continued until day 13 when cell density in all flasks increased by less than 5%.

Test endpoints were 1) cell density and 2) growth rate (μ)

1) Cell density = (Number of Cells X Number of Microscope Fields) ÷ Field Volume

Field Volume = Volume of hemocytometer grid (0.1 x 0.1 x 0.01 cm)

2) growth rate (μ) was calculated using the formula:

$$\mu = \frac{\ln (X_2/X_1)}{t_2-t_1}$$

where ln = natural logarithm, X_1 and X_2 are cell density measured at times t_1 and t_2 and μ is expressed in units of days^{-1}. The maximum growth rate (μ max) for each culture vessel is the highest value of μ calculated for any 24 hour interval during the test.

From the observed values for maximum culture density and the calculated values for maximum growth rate, the highest test concentration that caused no significant growth inhibition or stimulation (No Observed Effect Limit, NOEL) and the lowest test concentration that caused significant inhibition (Minimum Inhibitory Concentration, MIC) were determined using one-way analysis of variance (Sokal and Rohl, 1981) and Dunnett's Procedure (Dunnett 1955, 1964).

Summary of Results: Ninety-six hours after initiation of the preliminary tests, cell densities in treated flasks were 314, 299, 313 and 339 x 10^4 cells/ml compared to 147 and 353 x 10^4 cells/ml for control and solvent control flasks, indicating that doramectin was not acutely toxic to *Selenastrum capricornutum* over the range of concentrations tested. Therefore, the test was terminated without enumerating growth rates.

In the 14 day definitive test, cell densities were observed to increase over time in all replicates of each treatment level and control. Maximum cell densities of controls and solvent control differed statistically, the latter was statistically equivalent to both concentrations of doramectin that were tested. Growth rates of both controls were statistically equivalent. Therefore, data were pooled and compared against both doramectin treatment levels and found to be equivalent.

HPLC assays conducted at initiation of the definitive test indicated concentrations of 26 and 14 ppb for nominal levels of 40 and 20 ppb. Drug concentrations had declined below the level of assay sensitivity (<5.8 ppb) on day 14. Possible reasons for the decline include the known photoinstability of doramectin (Appendix c-8) sorption to particulate matter (Appendices c-5, c-6) and a propensity for sorption to glass. Loss of doramectin from solution indicates that the test organisms were not exposed to doramectin throughout the test period and actual exposure concentrations are not known. Although NOEL and MIC values cannot be assigned, doramectin does not appear to be acutely toxic to *S. capricornutum*, even at nominal initial concentrations up to 1.0 ppm.

Appendix c-19

Acute Toxicity Study with Doramectin on Daphnia

Report Summary: ACUTE TOXICITY STUDY WITH DORAMECTIN ON *DAPHNIA*

Study Number: 2438-1088-6138-110

Test Species: Water Flea (*Daphnia magna*)

Summary of Experimental Design: The acute toxicity of doramectin was determined against the water flea, *Daphnia magna* under static test conditions. A 48 hr preliminary test was conducted in 1 L glass beakers each containing 1 L of test solution and 10 daphnids. Nominal concentrations of 94, 9.4, 0.94 and 0.094 ppb doramectin were evaluated in singlet. The 48 hr definitive test employed 250 ml glass beakers containing 225 mL test solution and 5 daphnids. Mean measured concentrations of 0.32, 0.21, 0.11, 0.066 and 0.025 ppb doramectin were evaluated in quadruplicate. Both preliminary and definitive tests included water and solvent (acetone) controls.

Summary of Results: In the preliminary test, 100% of *Daphnia* exposed to the upper 3 levels (94-0.94 ppb) were immobilized after 48 hr; none were immobilized at the lowest level tested, 0.094 ppb. In the definitive test, 100% immobilization was observed after 48 hr at the highest measured concentration (0.32 ppb). Immobilization over the next three test concentrations (0.21, 0.11, 0.066 ppb) ranged from 75-30%. Immobilization was not statistically significant (<10%) in the lowest measured test level. Replicated values are shown in the table. EC_{50} value were established for 24 and 48 hr as follows:

Observation Period	EC_{50} (µg/L)	Confidence Interval Lower (µg/L)	Confidence Interval Upper (µg/L)
24-hour[a]	> 0.32	---	---
48-hour[b]	0.10	0.080	0.12

[a] EC_{50} value empirically estimated as greater than the highest concentration tested.
[b] EC_{50} value and 95% confidence interval calculated by moving average angle analysis.

The NOEC established for this study was 0.025 ppb. It is the highest concentration of test material that had no statistically significant adverse effect on exposed organisms as compared to controls.

Concentrations tested, corresponding cumulative percent of immobilized organisms and observations made during the 48-hour static exposure of daphnids (*Daphnia magna*) to doramectin (N=20)

Mean Measured Concentration (µg/L)	Cumulative Percent of Immobilized Organisms 24-Hour					48-Hour				
	A	B	C	D	Mean	A	B	C	D	Mean
0.32	0	20	0	20	10[ab]	100	100	100	100	100
0.21	40	20	0	0	15[c]	100	40	80	80	75[a]
0.11	20	20	20	20	20[bd]	40	40	40	80	50[af]
0.066	0	0	40	0	10[e]	0	20	40	60	30[af]
0.025	0	0	0	0	0[e]	0	20	0	0	5[b]
Solvent Control	0	0	0	0	0	0	0	0	0	0[e]
Control	20	20	0	0	10	20	20	0	0	10[b]

[a] Several of the surviving daphnids were lethargic.
[b] Two of the surviving daphnids were caught on particulate matter.
[c] One of the surviving daphnids was lethargic.
[d] One of the surviving daphnids was on the surface of the test solution.
[e] One of the surviving daphnids was caught on particulate matter.
[f] Several of the surviving daphnids were caught on particulate matter.

Appendix c-20

Acute Toxicity Study with
[5-^{3}H]-3"-0-desmethyldoramectin on Daphnia

Report Summary: ACUTE TOXICITY STUDY WITH [5-^{3}H]-3"-0-DESMETHYLDORAMECTIN ON *DAPHNIA*

Study Number: 260A-105

Test Species: Water Flea (*Daphnia magna*)

Summary of Experimental Design: The acute toxicity of [5-^{3}H]-3"-0-desmethyldoramectin was determined against the water flea *Daphnia magna* under static test conditions. Preliminary and definitive tests of 48 hour duration each employed four replicates of five daphnids per test concentration plus water and solvent (acetone) controls. Test vessels were 250 ml glass beakers containing 200 ml water. Nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 ppb were evaluated in the preliminary test. Drug concentrations in the definitive test were determined by liquid scintillation analysis. Organisms were observed at 3, 6, 10, 24 and 48 hours to determine the number of mortalities, immobilities and number of individuals exhibiting clinical signs of toxicity or abnormal behavior.

Summary of Results: Daphnids in the negative and solvent control groups were healthy and appeared normal. The average values of drug concentrations determined at 0 and 48 hour were as follows: less than the limit of quantitation (0.0831 ppb), 0.16, 0.27, 0.59 and 1.2 ppb. As shown in the Table, no daphnids at the two lowest test concentrations exhibited signs of toxicity nor did any become immobile. At the highest concentration, immobility/mortality was 100% by 24 hour. No immobilization was observed in the two middle concentrations but lethargy ranged from 5-40%. EC_{50} values of 0.84 ppb were established at 24 and 48 hours and a 48 hour no observed effect concentration was calculated to be approximately 0.16 ppb.

Concentrations tested, corresponding cumulative percent of immobilized organisms and observations made during the 48-hour static exposure of daphnids (*Daphnia magna*) to [5-^{3}H]-3"-0-desmethyldoramectin (N=20)

Mean Measured Concentration (µg/L)	Cumulative Percent of Immobilized or Dead Organisms 24-Hour					48-Hour				
	A	B	C	D	Mean	A	B	C	D	Mean
1.2	100	100	100	100	100	NA	NA	NA	NA	NA
0.59	0	0	0	0	0	0	0	0	0	0[a]
0.27	0	0	0	0	0	0	0	0	0	0[b]
0.16	0	0	0	0	0	0	0	0	0	0
<LOQ[c]	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

[a] Eight surviving daphnids were lethargic.
[b] One surviving daphnid was lethargic.
[c] LOQ = Limit of quantitation = 0.0831 µg 3"-0-desmethyldoramectin equivalents/L.

Appendix c-21

Acute Toxicity Study with
[^{3}H]-8-αhydroxydoramectin on Daphnia

Report Summary: Acute Toxicity Study with [^{3}H]-8-αhydroxydoramectin on *Daphnia*

Study Number: 260A-106

Test Species: Water Flea (*Daphnia magna*)

Summary of Experimental Design: The acute toxicity of [^{3}H]-8-αhydroxydoramectin was determined against the water flea *Daphnia magna* under static test conditions. Preliminary and definitive tests of 48 hour duration each employed 4 replicates of 5 daphnids per test concentration plus water and solvent (acetone) controls. Test vessels were 250 mL glass beakers containing 200 mL water. Nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 ppb were evaluated in the preliminary test. Drug concentrations in the definitive test were determined by liquid scintillation analysis. Organisms were observed at 2, 6, 10, 24 and 48 hour to determine the number of mortalities, immobilities and number of individuals exhibiting clinical signs of toxicity or abnormal behavior.

Summary of Results: Daphnids in the negative and solvent control groups were healthy and appeared normal. The average values of drug concentrations determined at 0 and 48 hour were as follows: 0.075, 0.13, 0.39, 0.69 and 1.2 ppb. Daphnids in the three lowest test concentrations appeared normal and exhibited no signs of toxicity. At the two highest concentrations, mortality/immobility was 10 and 60%, respectively, at 48 hour. EC_{50} values of >1.2 and 1.1 ppb were established at 24 and 48 hour and a 48 hour no observed effect concentration was calculated to be 0.39 ppb.

Concentrations tested, corresponding cumulative percent of immobilized organism and observations made during the 48-hour static exposure of daphnids (*Daphnia magna*) to [^{3}H]-8-αhydroxydoramectin (N=20).

Mean Measured Concentration (µg/L)	Cummulative Percent of Immobilized or Dead Organisms									
	24 hour					48 hour				
	A	B	C	D	Mean	A	B	C	D	Mean
1.2	20	0	0	0	5	40	80	60	60	60
0.69	0	0	0	0	0	0	0	20	20	10[a]
0.39	0	0	0	0	0	0	0	0	0	0
0.13	0	0	0	0	0	0	0	0	0	0
0.075	0	0	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

[a] One surviving daphnid was lethargic

Appendix c-22

Acute Toxicity Study with Doramectin
on Bluegill Sunfish

Report Summary: ACUTE TOXICITY STUDY WITH DORAMECTIN ON BLUEGILL SUNFISH

Study Number: 2438-1088-6138-100

Test Species: Bluegill sunfish (*Lepomis macrochirus*)

Summary of Experimental Design: The acute toxicity of doramectin was determined against bluegill sunfish under static test conditions. A 96 hr preliminary test was conducted in 18.9 L glass aquaria each containing 15L of test solution and 10 fish. Nominal concentrations of 940, 94, and 9.4 ppb doramectin were evaluated in singlet. The 96 hr definitive test employed similar aquaria and volume sizes and the same number of fish per treatment. Mean measured concentrations of 47, 25, 12, 7.1 and 2.3 ppb doramectin were evaluated in duplicate. Both preliminary and definitive tests included water and solvent (acetone) controls.

Summary of Results: In the preliminary test 100% of the bluegill sunfish exposed to 940 and 94 ppb doramectin died within 96 hr. No mortality was observed at 9.4 ppb, the lowest level tested. In the definitive test, 100% mortality was observed in the two highest measured concentrations (47 and 25 ppb). During the same period, 55% and 5% mortality was observed at 12 and 7.1 ppb. Sublethal effects (e.g. loss of equilibrium) were also observed at the 12 ppb concentration. No mortality or sublethal effects were observed at the lowest measured concentration (2.3 ppb). Data are summarized in the table.

Observation Period	LC_{50} (µg/L)	Confidence Interval Lower (µg/L)	Confidence Interval Upper (µg/L)
24-Hour[a]	>47	---	---
48-Hour[b]	34	25	47
72-Hour[c]	13	11	15
96-Hour[c]	11	10	14

[a] The LC_{50} value was empirically estimated to be greater than the highest mean measured concentration tested.
[b] LC_{50} value estimated by non-linear interpolation; 95% confidence interval calculated by binomial probability.
[c] LC_{50} value and 95% confidence interval was calculated by probit analysis.

The NOEC through 96 hr was unequivocally established at 2.3 ppb, the lowest measured concentration.

HPLC and radiometric assays conducted at the initiation and termination of the definitive test indicated mean concentrations of 47, 25, 12, 7.1 and 2.3 ppb for nominal levels of 60, 37, 22, 13 and 8 ppb. The concentrations generally decreased by about 40% between 0 and 96 hours, likely because of sorption to particulates (Appendices c-5, c-6) and a propensity for sorption to glass.

Mean measured concentrations tested, corresponding cumulative percent mortalities and observations made of bluegill sunfish (*Lepomis macrochirus*) exposed to doramectin during a 96-hour static acute exposure (N=20).

Mean Measured Concentration (µg/L)	Cumulative Mortality (%)											
	24-Hour			48-Hour			72-Hour			96-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
47	50	40	45[a]	100	100	100	100	100	100	100	100	100
25	0	0	0	0	0	0[b]	80	100	90[c]	100	100	100
12	0	0	0	20	0	10	40	70	55[b]	40	70	55[d]
7.1	0	0	0	0	0	0	10	0	5	10	0	5
2.3	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0

[a] Several of the surviving fish exhibited a partial loss of equilibrium.
[b] Several of the surviving fish exhibited a complete loss of equilibrium.
[c] All of the surviving fish exhibited a complete loss of equilibrium.
[d] One of the surviving fish exhibited a complete loss of equilibrium.

Appendix c-23

Acute Toxicity Study with Doramectin on Rainbow Trout

Report Summary: ACUTE TOXICITY STUDY WITH DORAMECTIN ON RAINBOW TROUT

Study Number: 2438-1088-6138-103

Test Species: Rainbow trout (*Onchorhynchus mykiss*)

Summary of Experimental Design: The acute toxicity of doramectin was determined against rainbow trout under static test conditions. A 96 hr preliminary test was conducted in 18.9 L glass aquaria, each containing 15L of test solution and 10 fish. Nominal concentrations of 10, 1 and 0.1 ppb doramectin were evaluated in singlet. The 96 hr definitive test employed similar aquaria and volume sizes and the same number of fish per treatment. Mean measured concentrations of 26, 13, 7.6, 2.5 and 1.9 ppb doramectin were evaluated in duplicate. Both preliminary and definitive tests included water and solvent (acetone) controls.

Summary of Results: In the preliminary test 100% of the rainbow trout exposed to 10 ppb doramectin died within 96 hr. No mortality or abnormal behavior was observed at 1 and 0.1 ppb, the two lowest levels tested. In the definitive test, mortality in the 3 highest treatment levels ranged from 100-85% (26-7.6 ppb); no mortality was observed in the 2 lower levels over the same time period. Data are summarized in the table.

LC_{50} values were calculated for 24-96 hour as follows:

Observation Period	LC_{50} (µg A.I./L)	Confidence Interval Lower (µg A.I./L)	Confidence Interval Upper (µg A.I./L)
24-Hour[a]	21	18	25
48-Hour[a]	9.9	8.7	11
72-Hour[b]	6.6	2.5	13
96-Hour[b]	5.1	2.5	7.6

[a] The LC_{50} value and 95% confidence interval was calculated by probit analysis.
[b] LC_{50} value estimated by non-linear interpolation; 95% confidence interval calculated by binomial probability.

The NOEC through 96 hr was established at 2.5 ppb.

HPLC and radiometric assays conducted at the initiation and termination of the definitive test indicated mean concentrations of 26, 13, 7.6, 2.5 and 1.9 ppb for nominal levels of 47, 27, 17, 10 and 6 ppb. The concentrations generally decreased by about 40% between 0 and 96 hours, likely because of sorption to particulates (Appendices c-5, c-6) and a propensity for sorption to glass.

Concentrations tested, corresponding cumulative percent mortalities and observations of rainbow trout (*Oncorhynchus mykiss*) exposed to doramectin during a 96-hour static acute exposure (N=20).

Mean Measured Concentration (µg/L)	Cumulative Mortality (%)											
	24-Hour			48-Hour			72-Hour			96-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
26	50	100	75[bc]	100	100	100	100	100	100	100	100	100
13	10	0	5[a]	90	80	85[f]	100	100	100	100	100	100
7.6	0	0	0	30	0	15[de]	70	50	60[bh]	90	80	85[jk]
2.5	0	0	0	0	0	0	0	0	0[g]	0	0	0
1.9	0	0	0	0	0	0	0	0	0	0	0	0[i]
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0	0	0	0	0

[a] One of the surviving fish exhibited a complete loss of equilibrium and was observed to be at the bottom of the exposure vessel.
[b] Two of the surviving fish exhibited a partial loss of equilibrium.
[c] Several of the surviving fish exhibited a complete loss of equilibrium and were observed to be at the bottom of the exposure vessel.
[d] One of the surviving fish was observed on the bottom of the exposure vessel and exhibited rapid respiration.
[e] Several surviving fish exhibited partial loss of equilibrium.
[f] Several surviving fish were observed on the bottom of the exposure vessel and exhibited rapid respiration.
[g] Two of the surviving fish exhibited darkened pigmentation.
[h] Several surviving fish exhibited a complete loss of equilibrium.
[i] One of the surviving fish exhibited darkened pigmentation and a partial loss of equilibrium.
[j] One of the surviving fish exhibited a partial loss of equilibrium.
[k] Two of the surviving fish exhibited a complete loss of equilibrium.

Appendix c-24

Acute Dermal and Ocular Irritation Studies with
Doramectin in Albino Rabbits

Report Summary: ACUTE DERMAL AND OCULAR IRRITATION STUDIES WITH DORAMECTIN IN ALBINO RABBITS

Study Number: 91-657-22

Test Species: Albino rabbit (New Zealand White)

Summary of Experimental Design:

1) Dermal irritation: Two males and one female rabbit were used. Their bodyweights ranged from 2.7-3.1 kg. A dose of 0.5 gram of doramectin was applied to one intact and one abraded site on the back of each rabbit and was held in continuous contact with the skin under an occlusive patch for 24 hours. Each test site measured approximately two inches square. During the dosing procedure, both the compound and the skin were thoroughly wetted with distilled water until an aqueous paste of the compound was formed. The total dose of 1 gram applied to each animal was equivalent to a dose of 322-369 mg/kg of doramectin. All rabbits were observed for 3 days after dosing.

2) Ocular irritation: Two males and one female rabbit were used. Their bodyweights ranged from 2.8-3.0 kg. A dose of 18.8 mg doramectin, equivalent to the 0.1 ml volume of solid specified in the procedure, was introduced into the conjunctival sac of the left eye. The treated eye of each rabbit was not rinsed after dosing. The animals were observed to 3 days. On the day of dosing (day 0), the eyes were evaluated with minimal manipulation and without the use of fluorescein.

   Skin reactions and ocular changes were evaluated visually according to the standard Draize scoring system, in which a score of zero denotes no effect and higher scores denote increasingly severe reactions. A Primary Irritation Score for skin was calculated as the sum of the mean erythema scores at 24 and 72 hours, divided by 4.

Summary of Results:

Skin irritation: Following a 24-hour exposure to the compound, very slight (score = 1), non-confluent erythema was apparent at both the intact and the abraded site of one animal and at the abraded site of a second rabbit. No erythema was evident at either site in the third rabbit, and there was no edema at any of the application sites. There was no obvious change at any of the sites at 48 hours post dose. However, by 72 hours post dose, the erythema had subsided completely, and the skin at each intact and abraded site appeared essentially normal:

Condition of Skin	Time after Application (hr)	Mean Value of Score	
		Erythema	Edema
Intact	24	0.33	0
	72	0	0
Abraded	24	0.67	0
	72	0	0

Clinical Observations: Throughout the 72-hour observation period, all animals remained alert and active, but the food consumption of one rabbit was reduced. The final body weight of each rabbit was essentially comparable to the animal's pre-test weight.

Results of this test indicate that doramectin is not a primary skin irritant.

Ocular Irritation: Immediately after dosing, each rabbit blinked and rubbed the treated eye; however, none of the rabbits exhibited signs of obvious pain or discomfort. Within 1 hour of dosing, slight circumcorneal reddening was apparent in the treatment eye of each rabbit. Slight conjunctival reddening and chemosis were also evident in two of the rabbits, and iritis was apparent in the treated eye of one of these animals. By 6 hours post dose, the ocular changes were subsiding, and at 24 hours, the only changes noted were slight circumcorneal reddening in one rabbit and slight reddening of the conjunctive in another animal. By 48 hours post dose, the treated eye of each rabbit appeared normal.

Time After Application (hr)	Cornea Opacity	Iritis	Conjunctive Redness	Conjunctive Chemosis
1-6	0/3	1/3	0/3	0/3
24-72	0/3	0/3	0/3	0/3

Clinical Observations: All rabbits were asymptomatic throughout the 72-hour test period, and they all gained weight.

Results of this test indicate that doramectin is not an ocular irritant.