Environmental Assessment

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

Pfizer Inc

March 1996

ENVIRONMENTAL ASSESSMENT

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1. DATE: March 11, 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 swine 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 swine producing regions of the United States and doramectin 1% injectable will fulfill a need for treatment and control of parasitic diseases caused by various infectious agents.

Doramectin 1% injectable solution would be administered to swine by intramuscular injection at the recommended dose level of 300 µg doramectin per kilogram of body weight. Each ml of doramectin 1% injectable solution contains 10 mg doramectin, sufficient to treat 75 lb (34 kg) of body weight. Doramectin 1% injectable solution will be used wherever swine are raised in the U.S., but particularly in Iowa, Illinois, Missouri, Indiana, Minnesota, Nebraska and North Carolina.

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

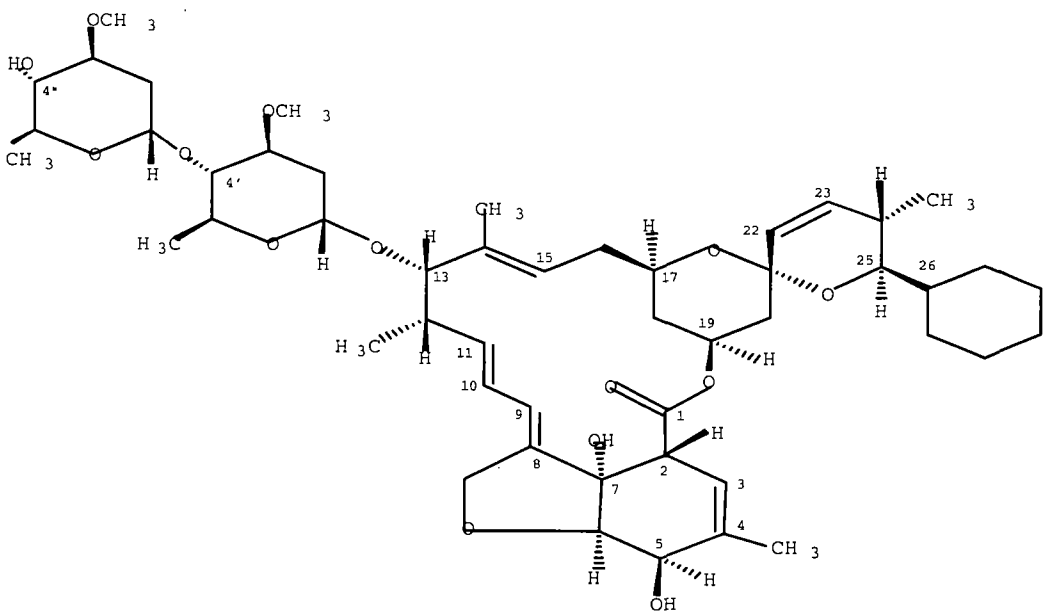
CAS Registry Number: 117704-25-3

Pfizer Code Number: UK-67,994

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

Molecular Weight: 899.13

Structural Formula:



Physical Description: White solid, m.p. 160.5-162.2°C

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 occupational safety and 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 medium consisting of carbohydrates, organic nitrogen sources, fats, fatty acids, oils, mineral salts, miscellaneous inorganic and organic compounds and antifoaming agents 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-21).

Instillation of 18.8 mg doramectin to the conjunctival sac caused slight reddening of the conjunctivae, 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-21).

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. The attached statements (Appendix a-2 and a-3) certify that the manufacturing sites are in compliance or will be in compliance with all applicable occupational safety requirements.

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
Choline 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
Ethyl 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-methionine	63-68-3	NL	NL
L-tyrosine	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 Butyric 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 propionate	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 occupational safety and emissions 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 Swine

Doramectin will be administered once to feeder pigs and once or twice to breeder pigs. Assuming average body weights at treatment of 30 kg and 125 kg for feeder and breeder pigs, respectively, and a dose of 0.3 mg/kg body weight, animals will receive 9.0 mg or 37.5 mg of doramectin per treatment, respectively:

30 kg x 0.3 mg/kg = 9.0 mg
125 kg x 0.3 mg/kg = 37.5 mg

2. Metabolism and Excretion of Doramectin by Swine

Doramectin would be introduced into the environment intermittently and in low concentrations through the feces and urine of medicated swine. Following intramuscular administration of tritiated doramectin at 300 µg/kg to two feeder swine of each sex averaging 40 kg in weight, urine and feces samples were collected daily for 7 and 21 days, respectively, and assayed for radiolabeled residues. Less than 1% of the administered dose was recovered in urine. In feces, a mean total of 61% of the dose was recovered over the 21-day period, with a mean of 17% of the dose (28% of the excreted residues) present as unchanged drug (Appendix c-1). The maximum mean daily concentration of total drug residues in feces was 1214 ppb, representing 6.6% of the dose, which occurred on day 4 after treatment, with a corresponding maximum mean concentration of unchanged drug in feces of 301 ppb, accounting for 1.6% of the administered dose. Since feces accounted for only about one-third of the total raw waste (feces and urine), peak residue concentrations in combined raw waste would be about one-third of these values, or about 400 ppb total residues and 100 ppb unchanged parent. A single major metabolite of doramectin was observed in swine feces collected at days 3, 7, 14, and 21, accounting for a mean of 31% of the total radiolabeled residues in feces. This metabolite was identified as 3"-O-desmethyldoramectin.

3. Concentration of Doramectin in Excreted Swine Wastes

The concentration of doramectin-related residues in excreted swine wastes can be estimated from the dose administered/head and the average amount of excreta produced/head. For this example, we will consider a farrow-to-finish unit producing approximately 1200 market pigs annually. The design of this unit is modeled after an all-in, all-out production facility as described by Jones et. al. (Reference 1) for maximizing facilities utilization and optimizing sanitation. This unit will have a one-room, 20 crate farrowing house housing 75 sows and will operate with 3 sow groups having a 51-day interval between successive farrowings. There will be 8 pigs/litter and a 180-day finishing time. Therefore, at any given time there will be four different, separately housed groups of 160 market-bound pigs, differing in age by approximately 7 weeks, as follows:

	Age Range (weeks)	Weight (kg)
Farrowing	0 - 7	1.4 - 18
Nursery	7 - 14	18 - 45
Growing	14 - 21	45 - 70
Finishing	21 - 28	70 - 100

The unit will also house 12 boars. Manure production by the various groups of animals and by the whole facility can be estimated from values provided in Reference 2 (Table 2-1) and are presented in the table below:

Group	Number	Average Raw Manure (kg/day) per head	per group
Boars	12	4.1	49
Gestating sows	55	4.1	226
Sows + Litters	20	10.2	204
Nursery	160	1.9	304
Growing	160	3.7	592
Finishing	160	5.4	864
Total			2239

The peak concentration of total drug residues in manure will occur on day 4 post treatment, when 6.6% of the administered dose is excreted (Appendix c-1). Assuming treatment of feeder pigs at 30 kg (i.e., nursery group), the maximum drug residue concentration in raw manure from these pigs on day 4 would be 0.31 ppm:

(30 kg)(0.3 mg/kg)(0.066)/1.9 kg manure = 0.31 mg/kg or ppm

Since unchanged drug represents only about 30% of the excreted residues (Appendix c-1), the concentration of doramectin in these wastes would be only 0.09 ppm.

Estimates of residue concentrations in manure from treated breeders can also be made, taking into account the different volumes of raw waste produced by the different groups and assuming all animals are treated simultaneously. A 125 kg breeder would receive 37.5 mg of doramectin (Section 6.C.1). Assuming peak daily excretion of 6.6% of the dose (2.5 mg) as drug residues, the 75 sows and 12 boars would excrete 217.5 mg. Since the total daily manure production for these groups is 479 kg (226 + 204 + 49, from table above), the maximum total residue concentration in raw waste from the breeder unit would be 0.45 ppm:

(217.5 mg)/479 kg = 0.45 mg/kg or ppm

The maximum concentration of unchanged doramectin would be 30% of this or 0.14 ppm.

The estimates presented above represent maximum residue concentrations, assuming no dilution with manure containing lower or no residues. This might occur under a manure management regime where manure is scraped or removed daily and not mixed with other manure, with wash water, or diluted into a lagoon. These would also represent maximum concentrations in manure excreted into open lots or pasture. This worst case and intermittent situation would occur only once during every treatment cycle. In the production example described, there would be a total of 8 groups of feeder pigs/year, or 8 treatment periods, and one or two treatments administered to breeders. Therefore, there would only be 8 days/year when residues in wastes from feeders could be at the maximum estimated level of 0.31 ppm and only 2 days/year when residues in wastes from the breeder unit would reach the maximum level of 0.45 ppm.

In a facility where pigs are maintained on slotted floors and manure is collected into pits, residues would be diluted to varying degrees, depending upon storage times and conditions. For example, assume underfloor storage pits are pumped out every 30 days and further assume 100% of the administered dose is excreted over this interval. In the nursery unit housing 160 pigs (see above), 1440 mg of drug residues would be excreted (9 mg/pig x 160 pigs) in a total of 9120 kg of raw wastes (304 kg/day x 30 days), giving maximum concentrations of 0.16 mg/kg (ppm) total residues or 0.05 ppm unchanged doramectin in the waste from this unit. A similar estimate can be made for waste from the breeder unit. In this case, total excreted residues would be 3263 mg ([75 sows + 12 boars] x 37.5 mg) in 14370 kg raw wastes (479 kg/day x 30 days), resulting in maximum total residues of 0.23 mg/kg (ppm) and maximum doramectin residues of 0.068 ppm. Combining wastes for the entire facility for this period would dilute total residue concentrations to 0.07 ppm:

(1440 mg + 3263 mg)/(2239 kg waste/day x 30 days) =
0.07 mg/kg or 0.07 ppm total residues

The maximum concentration of doramectin in these combined wastes would be only 0.02 ppm. Transferring wastes to a common waste lagoon would dilute residues even more.

Estimates can be made for the maximum residue concentrations for various manure storage periods (e.g., in a lagoon), assuming wastes from the entire facility are combined for storage, no degradation of the doramectin residues occurs during storage and not accounting for dilution by wash water or rainfall/runoff. Values presented in the following table assume treatment of breeders on day 0 and treatment of individual groups (160 pigs/group) of 30 kg feeder pigs on days 0, 51, 102 and 153 with excretion of 100% of the administered dose within approximately 30 days:

Storage Period (Days)	Number of treatments (cumulative)	Total Residues* (mg)	Raw Wastes (kg)	Total Residue Concentration (mg/kg)	Doramectin Concentration (mg/kg)
30	1 feeder + 1 breeder	1440 + 3263	6.7×10^4	0.070	0.021
90	2 feeder + 1 breeder	2880 + 3263	2.0×10^5	0.030	0.009
180	4 feeder + 1 breeder	5760 + 3263	4.0×10^5	0.022	0.007

* Feeder + Breeder; assumes 9 mg/feeder, 37.5 mg/breeder

4. Potential Concentration of Doramectin in Waste-Amended Soil

Use of swine waste containing doramectin residues as fertilizer would result in incorporation of the residues into the soil. The expected concentration of residues in soil can be estimated from the concentration of residues in raw waste and the rate of application of waste to soil.

The quantity of swine manure applied to agricultural soils is determined by the nitrogen (N) and phosphorous (P) content of the soil and the manure as well as on various crop needs and desired yield. Typically, manure is applied at a rate sufficient to provide 100-400 lbs of nitrogen/acre/year; an average application rate for common crops is 200 lbs N/acre/year (Reference 2, Table 10-3). Since swine manure contains 10-14 lbs N/ton of raw waste (References 2, 3, 4), application of the equivalent of 25 tons (22.7 metric tons) raw waste/acre/year would provide for 250-350 lbs added N/acre/year, sufficient for most crop needs. Assuming annual application of 22.7 metric tons of raw waste per acre with incorporation into the top 15 cm of soil (9.09×10^5 kg soil/acre, Reference 5) and waste management scenarios presented in section 6.C.3 above, maximum residue concentrations can be estimated. In the worst case, undiluted waste from the breeder unit removed on day 4 post treatment, containing 0.45 ppm total residues, applied directly to the field would yield maximum soil concentrations of 11 ppb total residues or 3.3 ppb unchanged parent:

$(22.7 \times 10^3$ kg waste/acre)(0.45 mg residues/kg waste) =
1.02×10^4 mg/acre
$(1.02 \times 10^4$ mg/acre)/$(9.09 \times 10^5$ kg soil/acre) =
11.2×10^{-3} mg/kg or 11 ppb total residues x 0.3 = 3.3 ppb doramectin

It should be noted that the one-day collection of manure from this unit would be dispersed to only 0.02 acres (479 kg manure/22.7×10^3 kg waste/acre).

A more realistic scenario for estimating residue concentrations in soils assumes collecting manure from the facility at 30 day intervals for field application. In such a practice, where residues in manure are 0.07 ppm (Section 6.C.3), maximum total residue levels in soil would be 1.75 ppb and maximum unchanged drug would be 0.53 ppb:

$(22.7 \times 10^3$ kg waste/acre)(0.07 mg residues/kg waste) =
1.59×10^3 mg/acre
$(1.59 \times 10^3$ mg/acre)/$(9.09 \times 10^5$ kg soil/acre) =
1.75×10^{-3} mg/kg or 1.75 ppb total residues x 0.3 = 0.53 ppb doramectin

The above estimates assume no degradation of residues in the stored waste prior to incorporation into soil and application of the entire annual manure (N) allotment at one time. Various manure management practices may impact on the specific details of manure application to soil, affecting localized drug residue concentrations. For example, in some regions manure is typically applied to soil semi-annually, in the spring before crops are planted and in the fall following harvest. The total amount applied should not exceed that needed to provide the desired annual N allotment, so the amount of drug residues introduced annually should not exceed those projected above. However, some degradation of the applied residues will have occurred in the interim between applications (see Section 7.B.2), so maximum residue levels in such soil would be below those estimated above.

5. Amount of Drug Used and Introduced into the Environment

It is estimated that use of doramectin for the therapy of parasitic infections of swine could result in up to approximately 0.36 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 Swine Statistics Division of the USDA has estimated the 1992 U. S. swine crop at 99×10^6 and a breeder inventory of about 7.1×10^6. If as many as 25% of these animals were treated with doramectin in a given year, about 356 kg of the drug would be used:

$$(0.25)(99 \times 10^6 \text{ feeders})(9 \text{ mg/feeder}) = 223 \text{ kg}$$
$$(0.25)(7.1 \times 10^6 \text{ breeders})(37.5 \text{ mg/treatment})(2 \text{ treatments/yr}) = 133 \text{ kg}$$

Total for 25% of feeders + breeders = 223 + 133 kg = 356 kg or 0.36 metric tons

6. Number of Acres Affected

The figures cited in scenarios presented above can be used to estimate the amount of swine manure containing doramectin that would be produced annually and the number of acres that could be fertilized with this manure.

In Section 6.C.3, it was estimated that manure combined from all units of a farrow-to-finish facility that was collected at 30-day intervals for broadcast to fields would contain a maximum of 0.07 mg/kg total doramectin-related residues, assuming excretion of 100% of the dose. Thus, if in a given year 356 kg of doramectin is administered to swine (Section 6.C.5), 5.1×10^9 kg or 5.1×10^6 metric tons of manure would contain residues at the estimated level:

$$356 \times 10^6 \text{ mg doramectin}/0.07 \text{ mg per kg manure} = 5.1 \times 10^9 \text{ kg manure}$$

At a field application rate of 22.7 metric tons/acre, approximately 2.25×10^5 acres would be required:

$$5.1 \times 10^6 \text{ tons manure}/22.7 \text{ tons per acre} = 2.25 \times 10^5 \text{ acres}$$

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 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 8α-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 swine are treated, 2) sites where swine 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 Swine Feedlot Waste to Rainfall Runoff

Only insignificant amounts of doramectin are expected to partition into surface waters in runoff from open lots or pasture due to the strong sorption of drug to feces (Appendix c-6). Furthermore, runoff from open lots must be controlled following local guidelines, generally by collection and direction to settling and

storage basins. Doramectin residues would be expected to partition almost exclusively into the solids phase of the settling basins, where they would ultimately be disposed of by application to soil as described in Section 6.C.4. Nevertheless, one can estimate a distribution of residues into surface runoff to illustrate the minimal concentrations that would be found in the aqueous phase. It was estimated above (Section 6.C.3) that raw wastes collected in underfloor pits for 30 day intervals will contain a maximum of 0.16 mg/kg total doramectin-related residues from feeder pigs and 0.23 mg/kg total residues from breeders. These same concentrations would be found in raw wastes for pigs raised in open feedlots. However, in order to consider partitioning of the doramectin residues between the solid and aqueous phases, the estimated concentrations of residue in raw waste must be adjusted for the solids content of the manure, which is about 10% of the total raw waste (Reference 2, Table 2-1). Therefore, the maximum expected concentration of total drug residues associated with manure solids would be 10-fold higher than the above estimates, or 1.6 mg/kg for feeders and 2.3 mg/kg for breeders; corresponding concentrations of unchanged doramectin would be 0.5 mg/kg and 0.7 mg/kg, respectively. Assume a rainfall event occurs on day 30 post-treatment. The concentration of unchanged doramectin in surface water equilibrated with the manure, C_w, can be calculated using the feces/water partition coefficient according to 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 of 15,600, determined using cattle feces (Appendix c-6), will be used to estimate partitioning of doramectin into the aqueous phase. The maximum concentration of doramectin in undiluted surface runoff is 32 ppt from the feeder unit and 45 ppt from the breeder unit:

$$(0.5 \text{ mg/kg})/15{,}600 = 3.2 \times 10^{-5} \text{ mg/kg or } 32 \text{ ppt}$$
$$(0.7 \text{ mg/kg})/15{,}600 = 4.5 \times 10^{-5} \text{ mg/kg or } 45 \text{ ppt}$$

Runoff from rainfall events occurring at later times after drug administration will contain even less, as the concentration of residues in manure will have decreased by further dilution and equilibration with fresh manure. Residues in such runoff would also be diminished by sorption to soil during the runoff event, dilution into a receiving basin or holding pond, and sorption to suspended solids and settled sludge in the holding pond.

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 (Reference 6) 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

Considering the worst case situation, if the initial concentration of unchanged doramectin in manure-amended soil is 3.3 ppb (Section 6.C.4) and assuming a time to 50% transformation of 79 days, the most conservative value obtained from soil biodegradation studies, 0.134 ppb will remain in the soil 365 days after application (log C = log 3.3 -[0.00877 x 365/2.3] = -0.873; C = 0.134 ppb). The table below indicates that the maximum concentration of approximately 3.4 ppb doramectin in soil is reached after two successive annual applications of manure:

Number of Successive Reapplications	Concentration (ppb) of Doramectin Residues in Soil
0	3.3
1	0.134 + 3.3 = 3.434
2	0.139 + 3.3 = 3.439
3	0.140 + 3.3 = 3.440

Thus, annual field application of swine manure containing doramectin-related residues would not be predicted to lead to accumulation of increasing concentrations of doramectin in soil. The maximum estimated soil level would be found only in fields where manure from day 4 post-dose breeder units was broadcast, representing a minimal percentage of the total annual manure deposition area (see section 6.C.4). In a manure management scenario where combined facility waste is collected at 30-day intervals and the maximum

initial doramectin concentration in soil is 0.53 ppb (Section 6.C.4), levels will not exceed 0.55 ppb upon annual reapplication:

Number of successive reapplications	Concentration (ppb) of doramectin in soil
0	0.53
1	0.0215 + 0.53 = 0.5515
2	0.0224 + 0.53 = 0.5524
3	0.0224 + 0.53 = 0.5524

If the annual manure allotment is applied in two semi-annual applications, introducing only half the drug-related residues at each application, concentrations would be even less.

In summary, depending upon management and manure application practices, maximum concentrations of doramectin in agricultural soils fertilized with manure from doramectin-treated swine would not exceed 0.55 to 3.4 ppb. These maximum levels would be found only at the times of manure application to the fields.

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 (References 7 and 8) 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 swine 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 concentrations. The maximum doramectin

concentration in soil amended annually with swine waste from a worst case, 4-day post dose disposal is 3.4 ppb (Section 7.B.2). Therefore, maximum concentrations of doramectin in undiluted surface runoff from acreage containing these levels would be 48 ppt:

$$C_w = (3.4 \times 10^{-3} \text{ mg/kg})/70.8 = 4.8 \times 10^{-5} \text{ mg/kg or 48 ppt}$$

Dilution of such runoff even as little as 10-fold into a receiving water body would reduce aqueous concentrations of doramectin to only 5 ppt . It should again be noted that only a portion of a producer's manure disposal area would receive manure containing the maximum level of drug residue (see Section 6.C.4); adjacent acreage would receive lower levels or no drug residues and runoff in a watershed encompassing the entire manure application area would mix and dilute out the residues. Therefore, a more reasonable estimate of maximum residue concentrations in surface runoff from manure-amended soils can be made using the estimated maximum soil concentration of 0.55 ppb (Section 7.B.2). In this case, the maximum doramectin concentration in undiluted runoff would be only 7.8 ppt:

$$C_w = (5.5 \times 10^{-4} \text{ mg/kg})/70.8 = 7.8 \times 10^{-6} \text{ mg/kg or 7.8 ppt}$$

Dilution of the runoff into a receiving water body would immediately reduce doramectin levels. Using a general scenario of a 10 hectare watershed draining into a 1 hectare pond of 2 m depth (Reference 9) and assuming a rainfall event produces 1 inch (2.5 cm) of runoff across the entire watershed, the total volume of runoff would be 2.5×10^6 L:

$$0.025 \text{ m} \times 10 \text{ ha} \times (1 \times 10^4 \text{ m}^2/\text{ha}) = 2.5 \times 10^3 \text{ m}^3 = 2.5 \times 10^6 \text{ L}$$

The volume of the receiving pond is 2×10^7 L (1×10^4 m^2/ha x 2 m), so the incoming runoff would be diluted 9-fold, reducing the concentration of doramectin residues from 7.8 ppt to 0.9 ppt. These residues will partition between the aqueous phase and the organic matter in the receiving pond, reducing aqueous concentrations even further. An estimate of this redistribution of residues can be made using the partition coefficient, K_d, and the following equation:

$$K_d = \frac{C_s}{C_w} = \frac{A_s}{m} \div \frac{A_w}{V} = \frac{A_s \times V}{m \times A_w}$$

where C_s = concentration of residue in sediment
C_w = concentration of residue in the water column
A_s = amount of residue partitioned into the sediment
A_w = amount of residue in the water column
m = mass of sediment
V = volume of water = 2×10^7 L + 2.5×10^6 L runoff = 2.25×10^7 L

Assumptions used:

K_d = 70.8 (lowest measured value for soil)
Depth of sediment sorbing residue = 5 cm with density = 1.5 x 10^3 kg/m^3, therefore
m = [0.05 m x 1 ha x (1 x 10^4 m^2/ha)] x (1.5 x 10^3 kg/m^3) = 7.5 x 10^5 kg

The total amount of doramectin entering the pond = 7.8 x 10^{-6} mg/L x (2.5 x 10^6 L) = 19.5 mg
Therefore, $A_w = 19.5 - A_s$

These values are substituted into the above equation to solve for A_s:

$$70.8 = \frac{A_s \times (2.25 \times 10^7)}{(7.5 \times 10^5) \times (19.5 - A_s)} = \frac{(2.25 \times 10^7)A_s}{(1.5 \times 10^7) - (7.5 \times 10^5)A_s}$$

$$(1.06 \times 10^9) - (5.3 \times 10^7)A_s = (2.25 \times 10^7)A_s$$

$$(7.55 \times 10^7)A_s = 1.06 \times 10^9$$

$$A_s = 14.04 \text{ mg}$$

$$A_w = 19.5 - 14.04 = 5.46 \text{ mg}$$

The concentration of doramectin remaining in the water column is therefore only 0.24 ppt:

$$C_w = A_w/V = 5.46 \text{ mg}/(2.25 \times 10^7 \text{L}) = 2.4 \times 10^{-7} \text{ mg/L or 0.24 ppt}$$

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

As noted above, the strong sorption of doramectin to soils and to 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 (References 10, 11 and 12):

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

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 manure (Section 7.B.2), the low concentration in surface water equilibrated with waste-amended soils (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. Summary of Fate of Doramectin Residues in Environmental Compartments

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

Compartment	Maximum Expected Concentration		EA Section
	Total Residues	Doramectin	
Raw waste, breeders, day 4 post-dose	0.45 ppm	0.14 ppm	6.C.3
Raw waste, combined, 30-day	0.07 ppm	0.02 ppm	6.C.3
Waste-amended soil, day 4 wastes	11 ppb	3.4 ppb	6.C.4/7.B.2
Waste-amended soil, 30 day wastes	1.75 ppb	0.55 ppb	6.C.4/7.B.2
Undiluted surface runoff, feedlot	---	45 ppt	7.B.1
Undiluted surface runoff, soil, day 4 wastes	---	48 ppt	7.B.3
Undiluted surface runoff, soil, 30-day wastes	---	7.8 ppt	7.B.3
Surface water body	---	0.24 ppt	7.B.3
Ground water	Insignificant	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:

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.

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.

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 *Daphnia 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-15.

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-16, c-17 and c-18.

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 summary is presented in Appendix c-19.

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-20.

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

1. Terrestrial Species

As discussed above under Sections 6.C.4 and 7.B.2, the maximum estimated concentration (MEC) of drug residues in soil is 11 ppb, with doramectin present at concentrations not exceeding 3.4 ppb. Since doramectin is the most significant bioactive component of the excreted residues, with the only other major component (3"-O-desmethyl doramectin) 8-fold less toxic against *Daphnia magna*, maximum expected concentrations of doramectin of 3.4 ppb will be used for evaluation of possible effects of drug usage on non-target organisms. This concentration could only occur when swine manure from 4-day post dose animals containing doramectin residues had just been mixed into soil, assuming no degradation of doramectin had taken place in the manure prior to application; levels from 30 day accumulated wastes will be 6-fold less or 0.55 ppb (Section 7.B.2). However, even this worst case

maximum estimated concentration in soil is not expected to have an adverse effect on non-target terrestrial species. Minimum inhibitory concentrations of doramectin were 40 ppm or above for soil microorganisms tested, 1.2×10^4 times the soil doramectin MEC. The NOEC for earthworms was 2 ppm, a level that exceeds the soil MEC by 6×10^2 times; no lethal effects were observed for earthworms at concentrations up to 1000 ppm, 2.9×10^5 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, nearly 1.0×10^3 times the soil MEC. Seedling growth of the dicotyledons tomato and soybean was not affected at concentrations of 53-980 ppm, between 1.6×10^4 and 2.9×10^5 above the 3.4 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 9.7×10^3 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, 1.4×10^4 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, 13 times the soil MEC and 9.4×10^2 times the 48 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 or other non-target terrestrial organisms.

2. Aquatic Species

The potential exposure of aquatic organisms to doramectin is expected to be intermittent, since it depends upon rain runoff from feedlot wastes or soil fertilized with swine 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 swine feedlot is 45 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. Nevertheless, dilution into the retention basin would immediately reduce levels to < 10 ppt. Maximum concentrations in runoff from waste-amended soil could range from 8 to 48 ppt (Section 7.B.3), with 8 ppt likely a more representative estimate. Even these maximum estimated levels would be transient due to the susceptibility of doramectin residues to microbial degradation. Runoff from waste-amended soil may enter ponds or streams,

where it would also be diluted into the receiving water body. As little as a one-to-ten dilution of the runoff into the receiving water body would immediately reduce maximum doramectin levels to the 1 to 5 ppt range, even in areas impacted by localized concentrated residue application. 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. Following equilibration, maximum levels of 0.24 ppt could be found in surface water bodies. These maximum levels 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 more than 100-fold greater than the 0.24 ppt maximum concentration that might be found in a surface water body. The 8-α- hydroxy and desmethyl 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-17 and c-18). Finally, the doramectin NOECs for bluegill sunfish and rainbow trout of 2.3 and 2.5 ppb, respectively, are about 1×10^4 times higher than the 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 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, Pfizer Inc, Groton, Connecticut:

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M.A. in Organic Chemistry
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Principal Research Investigator, Environmental Safety
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The following individuals are members of the production staff of Pfizer's U.S. Animal Health Operations at Lee's Summit, MO; and International Manufacturing Division, Nagoya, Japan.

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
13 years experience in quality control

N. Nishimura

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

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.

3,13,96

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

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Appendix a-1

Material Safety Data Sheets

Pfizer Inc
Central Research
Eastern Point Road
Groton, Connecticut 06340
Emergency Telephone: 203 441-410(

Central Research Experimental Substance Material Safety Data Sheet

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/ft^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

DATE: April 9, 1993

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 emissions requirments and is expected to remain in compliance when doramectin is produced at the site.

H. Kikuchi

H. Kikuchi
Vice President, Manufacturing
Pfizer Pharmaceuticals Inc.
Nagoya Plant

Appendix a-3

Certification of Compliance - Injectable Product Manufacturing Site

March 15, 1993

TO WHOM IT MAY CONCERN:

This is to certify that, to the best of our knowledge, Pfizer Inc's manufacturing plant at Lee's Summit, Missouri is in compliance with all applicable federal, state and local emissions' requirements and is expected to remain in compliance when the Doramectin 1% injectable solution is produced.

Richard H. Bartel
Manager, Environmental Compliance
North American Animal Health Division

Appendix b

Data Summary Charts

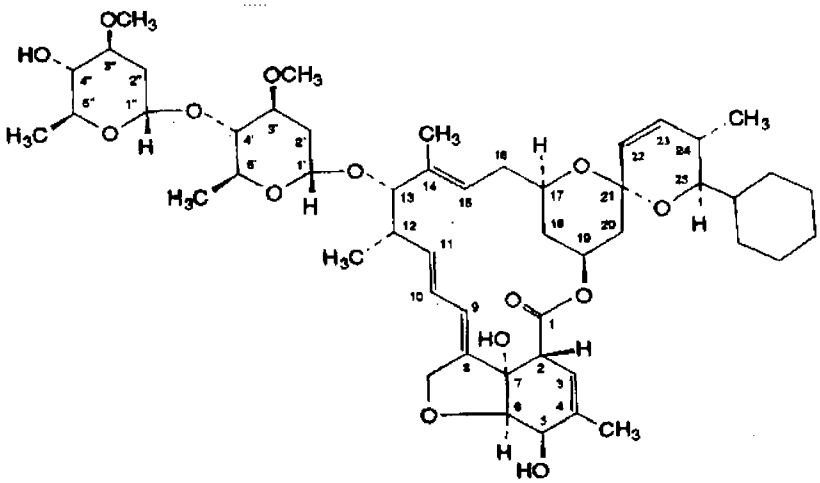
APPENDIX b

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

AQUATIC ORGANISMS

ORGANISM	ENDPOINT		
	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 Swine

Report Summary: EXCRETION OF DORAMECTIN BY MEDICATED SWINE

Study Number: 1525N-60-90-012

Test System: Excreta obtained from medicated swine

Summary of Experimental Design: A mixed-sex group of swine, two male castrates and two female swine, with a mean weight of 40 kg each received a single intramuscular injection of [^{3}H] doramectin in a formulation of 75:25 sesame oil:ethyl oleate at 300 μg/kg. Individual collections of urine and feces were made over 24 hour periods for 7 days (urine) and 21 days (feces) after dosing to assess the percentage of the dose excreted and the concentration of unchanged drug in excreta. Fecal samples collected on days 3, 7, 14, and 21, pooled by sex, were examined for an assessment of the metabolic profile of radioactivity.

For the determination of total radioactivity, urine samples were assayed in replicate by liquid scintillation counting. Fecal samples were combusted in replicate 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 derivatized solid phase extracts of the drug. The profile of drug and metabolites in feces was characterized by liquid scintillation counting of fractions eluted from a liquid chromatographic gradient system.

Summary of Results: By day 7, less than 1% of the dose was recovered in urine. The portion of the dose recovered in feces by day 21 showed considerable variability among the four swine, ranging from 38% to 87%, with a mean of 61% (Table 1). A mean total of 17% of the dose (28% of the excreted residues) was present as unchanged drug in feces (Table 1). The concentration of total residues in feces peaked at day 4 with a mean of 1214 ppb (6.6% of the dose), and declined thereafter to 235 ppb (1.2% of the dose) on day 21 (Table 2). Doramectin represented a mean of 22 - 39% of the total daily residues in feces (Table 2). A single major metabolite of doramectin was observed in feces collected at days 3, 7, 14, and 21, accounting for a mean of 31 ± 5% of the total radiolabeled residues excreted in feces. This metabolite was identified as 3"-O-desmethyldoramectin.

Table 1

Excretion of [^{3}H] Doramectin and Total Radiolabeled Residues in Feces of Swine Following IM Administration of [^{3}H]Doramectin 300 µg/kg, SID x 1

Experiment No. 1525N-60-90-012

	Animal No.	Days -1[1]	0[1]	1	2	3	4	5	6	7	8	9	10
		Percent of Dose											
Unchanged Drug													
	2552	<0.1	<0.1	1.3	2.1	2.6	2.9	2.0	2.0	1.3	1.6	1.5	1.3
	2562	<0.1	<0.1	0.9	0.6	1.3	1.3	1.9	1.9	1.3	1.1	0.7	1.3
	2570	<0.1	<0.1	0.3	0.4	1.0	1.1	0.5	0.8	0.6	0.6	0.6	0.6
	2580	<0.1	<0.1	0.7	0.7	1.0	1.2	0.8	1.4	1.0	0.9	0.9	0.9
	Mean	<0.1	<0.1	0.8	1.0	1.5	1.6	1.3	1.5	1.1	1.1	0.9	1.0
	± SD	---	---	0.4	0.8	0.8	0.9	0.8	0.6	0.3	0.4	0.4	0.3
Total Residues													
	2552	<0.1	0.1	3.0	6.2	10.1	11.2	8.4	8.1	4.9	4.6	5.3	3.9
	2562	<0.1	<0.1	2.0	1.5	3.2	4.6	7.7	6.9	4.4	2.5	2.1	4.0
	2570	<0.1	0.2	1.2	1.5	4.2	6.0	2.2	4.1	2.4	1.4	2.5	1.7
	2580	<0.1	0.3	2.1	1.8	3.1	4.5	3.0	5.4	3.2	2.5	3.1	2.9
	Mean	<0.1	<0.2	2.1	2.8	5.2	6.6	5.3	6.1	3.7	2.8	3.3	3.1
	± SD	---	---	0.7	2.3	3.3	3.2	3.2	1.7	1.1	1.3	1.4	1.1

Table 1 (continued)

Excretion of [^{3}H] Doramectin and Total Radiolabeled Residues
in Feces of Swine Following IM Administration of [^{3}H]Doramectin
300 µg/kg, SID x 1

Experiment No. 1525N-60-90-012

	Animal No.	Days 11	12	13	14	15	16	17	18	19	20	21	Total
		Percent of Dose											
Unchanged Drug													
	2552	0.8	0.5	0.7	0.4	0.5	0.4	0.3	0.5	0.4	0.3	0.5	23.9
	2562	0.8	0.7	0.5	0.6	0.8	0.3	0.6	0.4	0.3	0.3	0.4	18.2
	2570	0.4	0.5	0.4	0.3	0.3	0.2	0.3	0.1	0.2	0.1	0.3	9.8
	2580	0.6	0.9	1.0	0.5	0.7	0.6	0.3	0.6	0.6	0.5	0.4	16.4
	Mean	0.7	0.7	0.7	0.5	0.6	0.4	0.4	0.4	0.4	0.3	0.4	17.1
	± SD	0.2	0.2	0.3	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	5.8
Total Residues													
	2552	2.6	1.9	3.1	1.8	2.8	2.1	1.2	2.0	1.3	1.2	1.3	87.1
	2562	2.3	2.2	2.3	2.8	3.6	1.5	2.5	2.1	1.4	1.2	1.1	61.8
	2570	1.4	1.4	1.3	1.1	1.1	0.9	0.9	0.4	0.7	0.5	0.7	37.8
	2580	2.0	2.7	3.6	2.0	3.1	3.0	1.5	2.5	2.6	1.6	1.5	58.0
	Mean	2.1	2.1	2.6	1.9	2.7	1.9	1.5	1.8	1.5	1.1	1.2	61.2
	± SD	0.5	0.5	1.0	0.7	1.1	0.9	0.7	0.9	0.8	0.5	0.3	20.2

Note:

[1]Considered to be the value presented for the calculation of the mean ± SD values.

Table 2

Concentration of [^{3}H] Doramectin and Total Radiolabeled Residues in Feces of Swine Following IM Administration of [^{3}H] Doramectin 300 µg/kg, SID x 1

Experiment No. 1525N-60-90-012

	Animal No.	Days -1	0	1	2	3	4	5	6	7	8	9	10
		Equivalents of [^{3}H] UK-67,994 (ng/g)											
Unchanged Drug													
	2552	<25	<25	296	436	445	451	371	346	302	313	231	201
	2562	<25	<25	213	247	310	287	299	269	219	319	274	240
	2570	<25	<25	182	231	297	273	242	264	284	263	250	191
	2580	<25	<25	111	155	155	191	201	208	193	225	172	151
	Mean	<25	<25	201	267	302	301	278	272	250	280	232	196
	± SD	---	---	77	119	119	109	74	57	52	44	44	37
Total Residues													
	2552	<0.8	24	682	1274	1715	1738	1556	1417	1146	913	809	617
	2562	<0.8	15	483	612	761	995	1186	959	719	762	796	717
	2570	<0.8	48	642	788	1297	1427	1067	1297	1054	564	989	501
	2580	<0.8	59	352	408	497	695	808	817	658	646	586	461
	Mean	<0.8	37	540	771	1068	1214	1154	1123	894	721	795	574
	± SD	---	20	152	370	545	461	311	281	242	151	165	116
Mean Percent Drug to Total Residues		---	<68	37	35	28	25	24	24	28	39	29	34

Table 2 (continued)

Concentration of [^{3}H] Doramectin and Total Radiolabeled Residues in Feces of Swine Following IM Administration of [^{3}H] Doramectin 300 µg/kg, SID x 1

Experiment No. 1525N-60-90-012

	Animal No.	Days 11	12	13	14	15	16	17	18	19	20	21
		Equivalents of [^{3}H] UK-67,994 (ng/g)										
Unchanged Drug												
	2552	171	158	115	113	81	68	69	69	68	50	56
	2562	255	209	163	163	114	132	93	74	78	99	93
	2570	240	139	129	128	95	87	92	86	83	79	58
	2580	176	177	147	159	116	108	123	101	91	105	93
	Mean	211	171	139	141	102	99	94	83	80	83	75
	± SD	43	30	21	24	17	28	22	14	10	25	21
Total Residues												
	2552	545	562	497	488	448	326	277	278	237	221	165
	2562	754	707	695	743	500	562	413	351	314	433	264
	2570	813	408	451	450	378	329	278	301	291	282	154
	2580	562	561	546	573	513	500	543	409	413	368	356
	Mean	669	560	547	564	460	429	378	335	314	325	235
	± SD	135	122	106	130	61	120	127	58	74	93	95
Mean Percent Drug to Total Residues		32	31	25	25	22	23	25	25	25	26	32

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

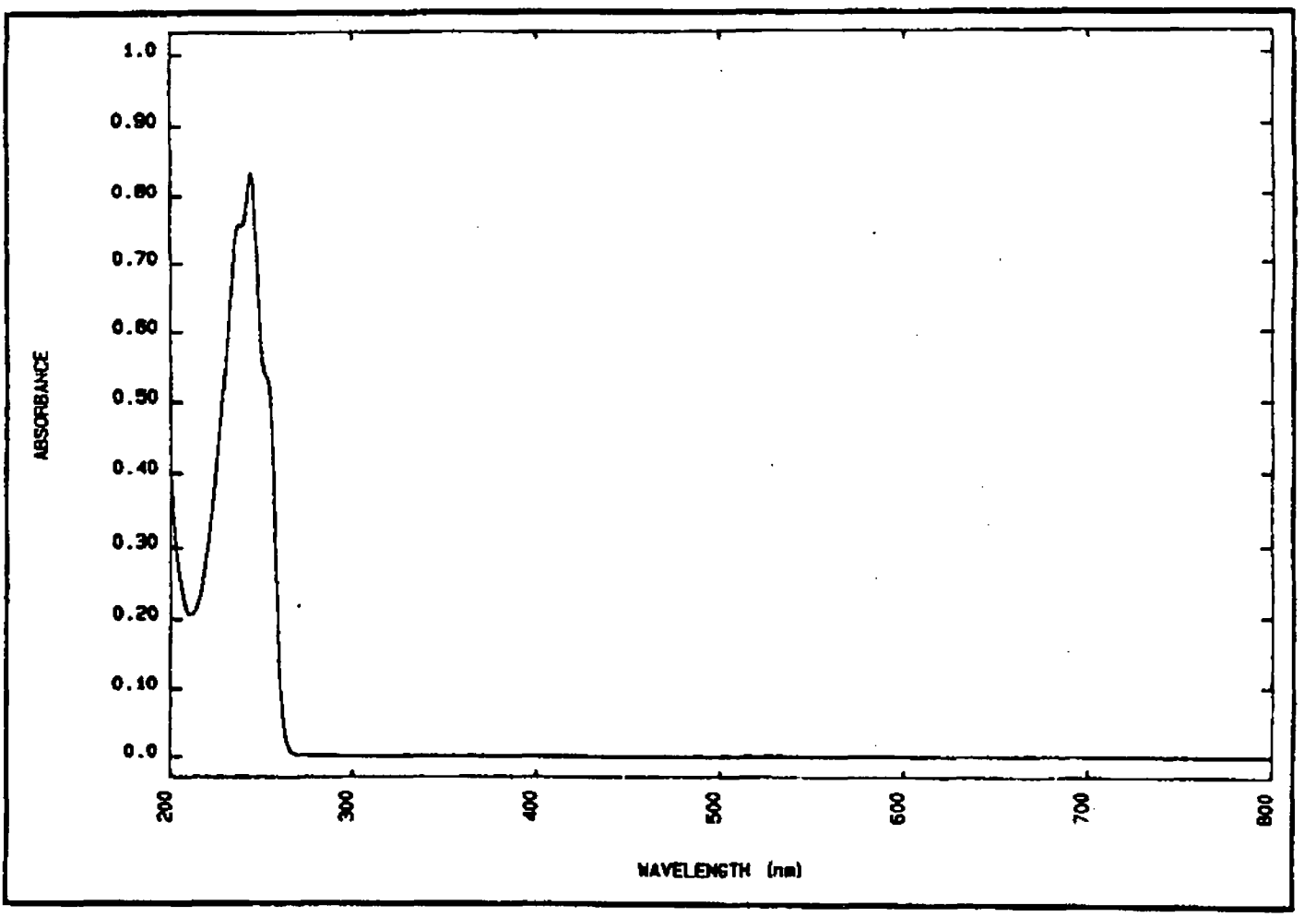
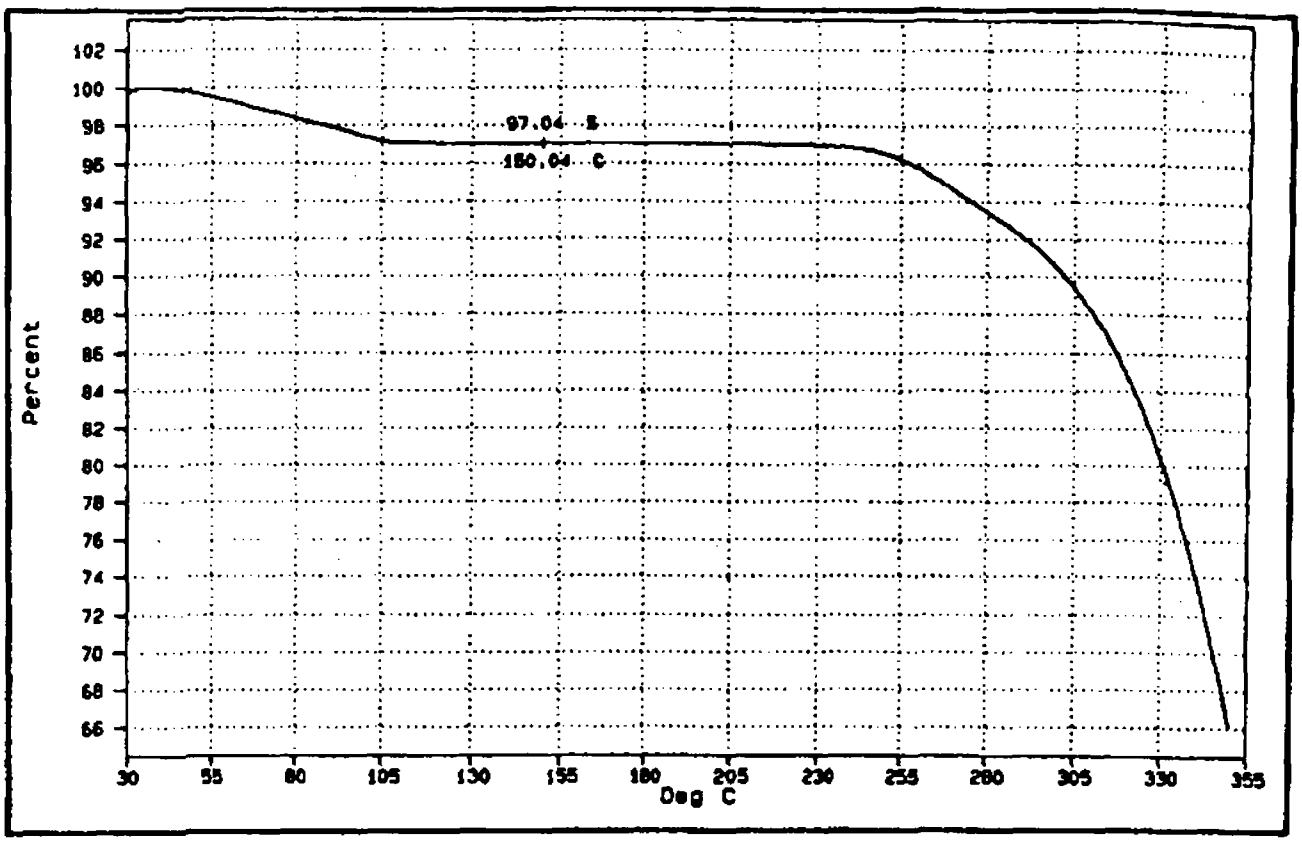


FIGURE 2

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.

Appendix c-8

Aquatic Photodegradation of Doramectin

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%.

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		Perennial Ryegrass			Corn			Wheat		
Mean Measured Doramectin Concentrations		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

Dose (ppm)	Mean Weight (gm) 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 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-16

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-17

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-18

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-19

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-20

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-21

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.