

Environmental Assessment

Doramectin 0.5% pour-on solution
for the treatment of parasitic
infections in cattle

Pfizer Inc

Original August 1996
Revision June 2002 (NADA 141-095)

ENVIRONMENTAL ASSESSMENT

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ENVIRONMENTAL ASSESSMENT

Doramectin 0.5% pour-on solution for the treatment of parasitic infections in cattle

1. DATE: August 2, 1996 (Revised, June 2002 NADA 141-095)
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 0.5% pour-on solution in beef and non-lactating dairy cattle for the treatment and control of a variety of internal and external parasitic infections. Parasitism continues to be a primary cause of production losses in all cattle producing regions of the United States and doramectin 0.5% pour-on solution will fulfill an unmet need for treatment and control of parasitic diseases caused by various infectious agents.

Doramectin 0.5% pour-on solution would be applied topically along the dorsal midline of the back between the withers and tail head at the recommended dose level of 500µg doramectin per kilogram of body weight. Each mL of doramectin 0.5% pour-on solution contains 5 mg doramectin, sufficient to treat 22 lb (10 kg) of body weight. Medication would not be given within 75 days of slaughter. Doramectin 0.5% pour-on solution will be used wherever cattle are raised in the U.S., but particularly in Texas, Nebraska, Kansas, Oklahoma, Missouri, South Dakota, Montana, Kentucky, Tennessee and Florida.

B. Locations Where Bulk Drug or Pour-on 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 pour-on product, a 0.5% solution, will be manufactured at Pfizer's Lee's Summit, Missouri plant.

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*, 15*S*, 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- α -L-arabino-hexopyranosyl)-3-*O*-methyl- α -L-arabino-hexopyranoside

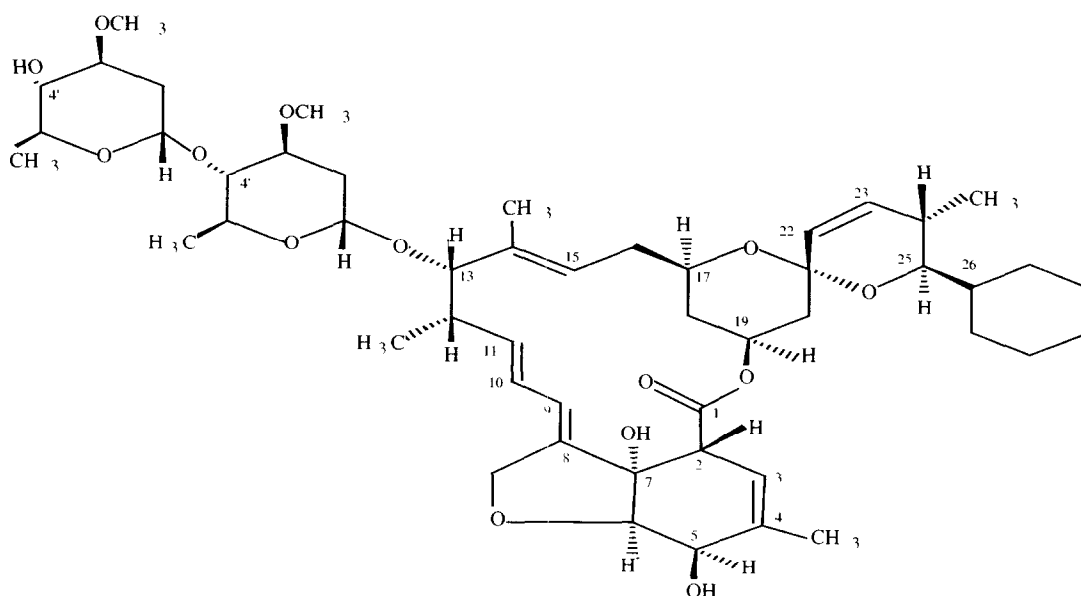
CAS Registry Number: 117704-25-3

Pfizer Code Number: UK-67,994

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

Molecular Weight: 899.13

Structural Formula:



B. Other Pour-on Solution Ingredients:

In addition to doramectin, DECTOMAX 0.5% pour-on solution contains 63.143% isopropyl alcohol, 16% cetearyl octanoate, 0.0063% purified water, 0.05% trolamine and 0.0007% FD & C blue dye #1, cert.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

A. From the Site where Bulk Drug is Produced:

The manufacture of doramectin will be carried out in purpose built fermentation and recovery facilities designed with doramectin containment in mind and to be in compliance with all applicable emissions requirements. The plant is located in Nagoya, Japan and will operate in accordance with local environmental regulations. A description of occupational safety, disposal procedures and statement of compliance are found in the doramectin injectable EA (NADA 141-061). Substances which could be emitted and/or discharged from Nagoya, Japan along with the respective exposure limits (when available) are listed in the doramectin injectable EA (NADA 141-061).

B. From the Site where Pour-on Solution will be Produced:

Dectomax (Doramectin) 0.5% Pour-On will be compounded and mixed into a 0.5% topical solution then 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 emissions and occupational safety requirements (Appendix A-2).

The Dectomax Pour-On 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 solution are added to a mixing tank in prescribed order and mixed. After the necessary quality assurance tests are complete, they are transferred through a clarifying filter to bottles via a filling machine. The production of this solution will not normally generate hazardous waste 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 wastes, generated during the manufacturing process and contaminated with doramectin, will be destroyed by incineration. These wastes may include empty metal drums, polyethylene drum liners, empty glass bottles, closures, filters and disposable protective apparel. Under Missouri law, these materials will be classified and managed as special waste. The incineration process is covered under Federal Regulations 40 CFR 264 or 40 CFR 60 and by Missouri Solid Waste Rules 10 CSR 80-5.

Liquid Wastes

The manufacturing process generates two liquid waste streams. One stream is isopropyl alcohol based, and one is aqueous based. The alcohol based stream will consist of residual pour-on solution 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 Dectomax Pour-On solution.

The alcohol based stream will be collected and destroyed by incineration as a hazardous waste. The incineration process is regulated under 40 CFR 264 or by Missouri Solid Waste Rules 10 CSR 25-7. The aqueous waste streams will be collected and destroyed by incineration as a non-hazardous special waste, as per Missouri law. The incineration process is regulated under 40 CFR 264 or 40 CFR 60 and by Missouri Solid Waste Rules 10 CSR 80-5.

Air Emissions

Of all the ingredients in the formulation of topical products, the only volatile compound of concern is isopropyl alcohol. Isopropyl alcohol is controlled at all times except when it is being added to the product bottles. Isopropyl alcohol emissions from production of Dectomax® 0.5% Pour-On products are very minor.

Emissions of particulate matter during the transfer of the topical products' active ingredient to the mixing tank are controlled by local ventilation and dust collection equipment. Total dust emissions from the production of the topical product are de minimis.

Air emissions are subject to the Clean Air Act and its 1990 Amendments codified in 40 CFR Parts 50, 52, and 60 as well as Missouri Air Pollution Control Regulations 10 CSR 10-2. The attached statement (Appendix A-2) certifies compliance with all Federal, State and local emissions requirements.

1. Manufacturing and Occupational Safety

a. Material Safety Data Sheets

Each 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 doramectin 0.5% pour-on solution will contain the information shown in the attached examples (Appendix A-1).

b. Hazard Evaluation Studies

Results of acute dermal and ocular irritation studies conducted with albino rabbits indicate that 1) doramectin bulk is neither a primary skin irritant nor an ocular irritant, 2) doramectin pour-on solution produced only minimal skin changes. Ocular irritation studies were not conducted with the pour-on solution since it contains isopropyl alcohol which is a known eye 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 bulk. No edema was observed and all six sites appeared normal by 72 hours post dose. 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 (See doramectin injectable EA-NADA 141-061).

Minimal skin changes were produced on intact skin sites of four rabbits exposed to 0.5 mL doses of the 0.5% pour-on solution and placebo solution. In most cases, erythema subsided within 1-3 days of dosing (Appendix C-5).

c. Occupational Safety

The Dectomax Pour-On product will be manufactured in a semi-automated plant located in Lee's Summit, Missouri, which has been specifically designed to minimize employee exposure to dust. Exposure to dust from the active ingredient (doramectin) and the vapor from isopropyl alcohol are minimized by the use of the engineered air handling systems, administrative controls and by personal protective equipment. Dermal contact to active ingredients or isopropyl alcohol is prevented by the use of engineering controls such as air handling systems, and personal protective equipment. During routine manufacturing operations, occupational exposure to doramectin bulk powder will be well below the 8-hr work occupational exposure limit (OEL) set by Pfizer of 0.2 mg/m³ (see MSDS p. 57)

C. Introduction of Substances as a Result of Use

1. Doramectin Administration to Cattle

Doramectin will be administered to both pastured and feedlot cattle. Since the latter represent a denser population, they will be used to estimate upper limits for the amount and concentration of doramectin introduced into the environment. The average amount of drug administered to a single animal can be estimated as follows. Feedlot cattle will most commonly be treated shortly after arrival at the feed lot. Assuming the average body weight of 300 kg upon arrival and a dose level of 0.50 mg/kg, a typically treated animal will receive 150 mg of doramectin:

$$300 \text{ kg} \times 0.50 \text{ mg/kg} = 150 \text{ mg}$$

2. Metabolism and Excretion of Doramectin by Cattle

Doramectin would be introduced into the environment intermittently and in low concentrations through the feces and urine of medicated cattle following administration of the drug percutaneously as a single dose at 500 µg/kg body weight. Over a 14 day period following topical administration of tritiated doramectin at 500 µg/kg to two male and female cattle averaging 183 kg in weight, daily assay of feces and urine accounted for 3.8% and < 0.04%, respectively, of the dose (Appendix C-1). The maximum concentration of total residues in feces during this 14 day period was 52.6 ppb in pooled feces from females (day 14) and 68.8 ppb in pooled feces from males (day 4). Subsequently, feces were collected weekly at 21, 35, 42 and 56 days. At 21 days post dose, the residues peaked at values of 156 and 270 ppb for female and male cattle, respectively, depleting to ≤ 7.4 ppb by 56 days post dose. The total dose excreted over 56 days, estimated by the area under the curve from zero to infinity of rate versus time post dose, was 39% for male and 36% for female cattle, for an average excretion of 38% of the administered dose. Radiotracer profiles of fecal extracts on day 21 post dose indicated that approximately 80% of the residue was doramectin. Only one metabolite, an O-desmethyldoramectin derivative, accounting for about 10% of the radiotracer, was observed.

3. Wash-off of Topically Applied Doramectin

Doramectin could enter the environment by wash-off of a portion of the topically-applied dose during a rainfall. Although not a likely event, such wash-off could introduce additional doramectin into feedlot manure or, for pastured cattle, directly into soil or surface waters. A study designed to determine the percentage of the dose that washed off treated cattle shortly after application showed that an average of 8.5% of the applied dose could be detected in the wash water (Appendix C-2). Assuming an average doramectin dose of 150 mg/300 kg animal, the maximum amount that would wash off is approximately 13 mg/animal. Therefore, the combined maximum amount of doramectin residues that could enter the environment as excreted residues in manure or washed off an individual animal is 57 mg + 13 mg = 70 mg.

4. Concentration of Doramectin in Excreted Cattle Wastes

A feedlot animal typically produces about 27 kg of wet waste per day and over the course of a typical 130 day stay in the feedlot would produce a total of 3510 kg wet waste:

$$27 \text{ kg wet waste/day} \times 130 \text{ days} = 3510 \text{ kg wet waste}$$

A worst case estimate assumes that each animal will be treated once and residues include both excreted and washed off doramectin. Therefore, the average maximum concentration of drug residues in the excreted wet waste would be 20 ppb:

$$\frac{70 \text{ mg drug}}{3510 \text{ kg waste}} = \frac{0.0199 \text{ mg}}{\text{kg}} = 20 \text{ ppb}$$

5. Concentration of Doramectin in Aged Feedlot Wastes

Fresh cattle excreta contains about 80% water by weight (Ensminger, 1976), whereas after aging on the feedlot, moisture content is reduced to about 25-40% (Environmental Protection Agency, 1974; Sweeten and Withers, 1990). Assuming an average moisture content of 30% in aged feedlot waste and no degradation of doramectin residues in the manure, the concentration of doramectin residues would be increased by a factor of 2.7 (0.80/0.30) over that expected in wet waste, giving maximum expected concentrations in aged feedlot waste of approximately 0.054 mg/kg or 54 ppb (0.020 mg/kg x 2.7).

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

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

Manure is incorporated into the top 15 cm of soil at a rate of 5-20 tons aged waste/acre/year (Ensminger, 1976; Sweeten and Withers, 1990). At a density of $1.5 \times 10^3 \text{ kg/m}^3$, 15 cm of soil weighs about $9.1 \times 10^5 \text{ kg/acre}$; therefore, using an average rate of incorporation of 15 tons (13.6 metric tons) manure/acre/year, use of aged manure containing 54 ppb doramectin residues would result in a maximum concentration in soil of only 0.81 ppb drug residue:

$$(0.054 \text{ mg/kg})(13.6 \times 10^3 \text{ kg/acre}) = 7.34 \times 10^2 \text{ mg/acre}$$

$$(7.34 \times 10^2 \text{ mg/acre}) \div (9.1 \times 10^5 \text{ kg/acre}) = 8.1 \times 10^{-4} \text{ mg/kg or } 0.81 \text{ ppb}$$

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

7. Amount of Drug Used and Introduced into the Environment

a. Quantity

Use tracking survey information (Doane 1999-2001) indicate that across the US, the following number of cattle received doramectin pour-on treatment: 1) Approximately 5.5 million cows and 5.5 million calves; 2) approximately 4.5 million stockers; 3) approximately 7.2 million feed lot cattle. A dose level of 0.5 mg/kg is assumed as well as average body weights of 545 and 136 kg respectively for cows and calves, 340 kg for stockers and 300 kg for feed lot cattle. Therefore, approximately 23 million cattle in the segments listed were treated and 1386 kg doramectin was introduced into the environment:

$0.5 \text{ mg/kg} \times 545 \text{ kg} \times 5.5 \times 10^6 \text{ beef cows} = 1.498 \times 10^9 \text{ mg or } 1500 \text{ kg}$

$0.5 \text{ mg/kg} \times 136 \text{ kg} \times 5.5 \times 10^6 \text{ beef calves} = 3.74 \times 10^8 \text{ mg or } 374 \text{ kg}$

$0.5 \text{ mg/kg} \times 340 \text{ kg} \times 4.5 \times 10^6 \text{ stockers} = 7.65 \times 10^8 \text{ mg or } 765 \text{ kg}$

$0.5 \text{ mg/kg} \times 300 \text{ kg} \times 7.2 \times 10^6 \text{ feed lot cattle} = 1.08 \times 10^9 \text{ mg or } 1080 \text{ kg}$

Total = 3719 kg

Since only about 38% of the administered doramectin dose is excreted, treatment of the above number of cattle would result in excretion of approximately 1386 kg of doramectin:

$3719 \text{ kg} \times 0.38 = 1413 \text{ kg or approximately } 1.4 \text{ metric tons}$

b. Pattern of Use

The doramectin injectable EA (NADA 141-061) presented detailed information acquired through surveys that examined cattle pasturing patterns and ivermectin usage in order to better understand the introduction of residues into the pasture environment as a result of use. Surveys focused on the Southwest and Southeast U.S. where non-native (exotic) dung beetles have been introduced and established and where significant numbers of cattle are kept on pastures. Drug usage focused on ivermectin because it was the only avermectin approved for use in the U.S. Survey conclusions follow.

1) Regional Survey: (see NADA 141-061): Across the Southeastern and Southwestern U.S., on the basis of the total number of pasture cattle treated with ivermectin during 1992-1994, peaks occurred in the second and fourth quarters of the year (March - May and September - November). However, percentage treated per quarter tended to remain below 20% of the total cattle population, even during peak times.

Use tracking survey information (Doane, 1999-2001) in Chart 1 indicates that peak endectocide doses across the US including the Southern states occurs in the 2nd and 4th quarters of the year (March-May and September-November). Times of peak usage are similar to what was observed for ivermectin usage during 1992-1994 across Southeastern and Southwestern states (NADA 141-061).

Doane (2002) surveyed veterinary clinics in Texas and Florida that manage cow-calf operations. Local operators purchasing endectocides from these clinics tend to use the total amount purchased all at one time in treating up to 200-250 cattle per day. Daily sales (and presumably use) of endectocides including doramectin from one clinic in Texas and two clinics in Florida are shown in Charts 2-4. During March-May, 2001, local operators purchased sufficient endectocide from a clinic in Matagorda County Texas over 90 days to treat about 8% of the cattle in this county. During March-May 2001, local operators purchased sufficient endectocide from clinic no. 1 in Hardee county Florida to

treat about 2% of the beef cattle in this county. During the same time frame, local operators purchased sufficient endectocide from clinic no. 2 in Hardee county Florida to treat an additional 32% of the beef cattle population of this county.

The data in Charts 2-4 show that drug purchase (and presumably use) occurs throughout the three-month observation period rather than being compressed into a shorter time period. This suggests that operators are unlikely to be treating many herds in adjacent pastures simultaneously. Therefore, over the spring treatment period in any regional area, the total number of pastures containing residues from recently treated animals would be a small percentage of the total pastures.

Chart 1. Endectocide Doses in Total Market & Southern US

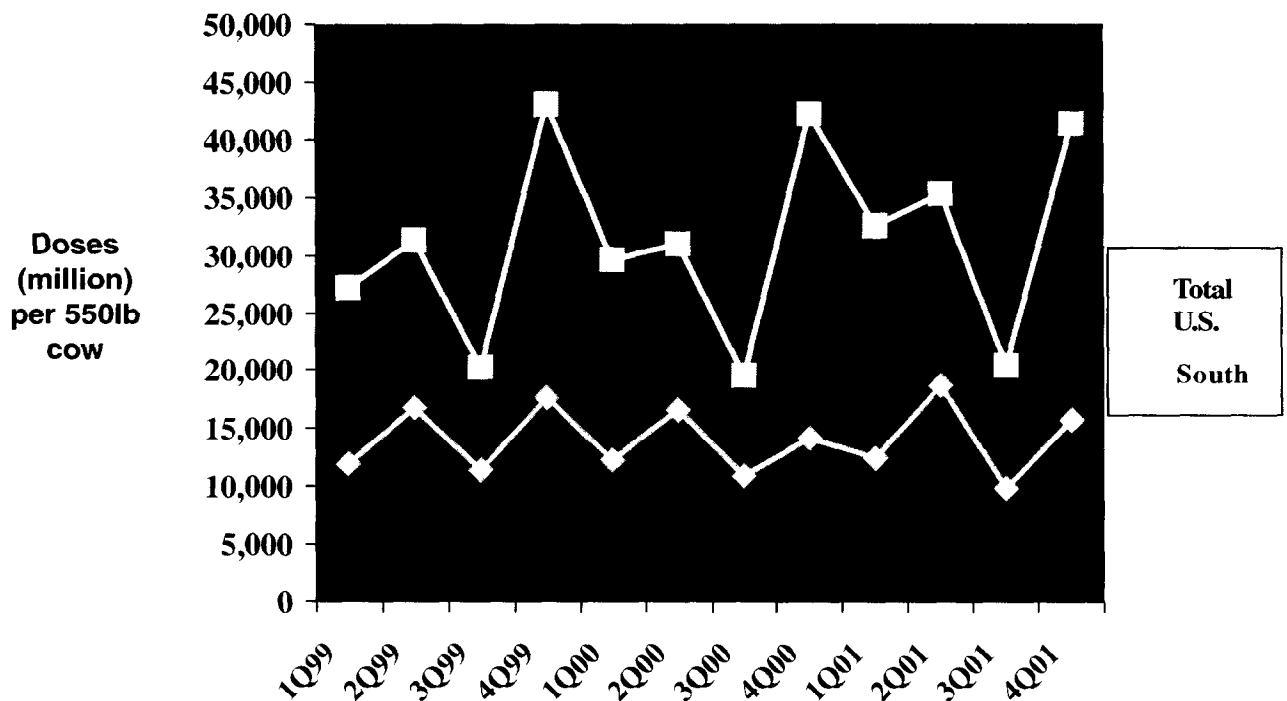
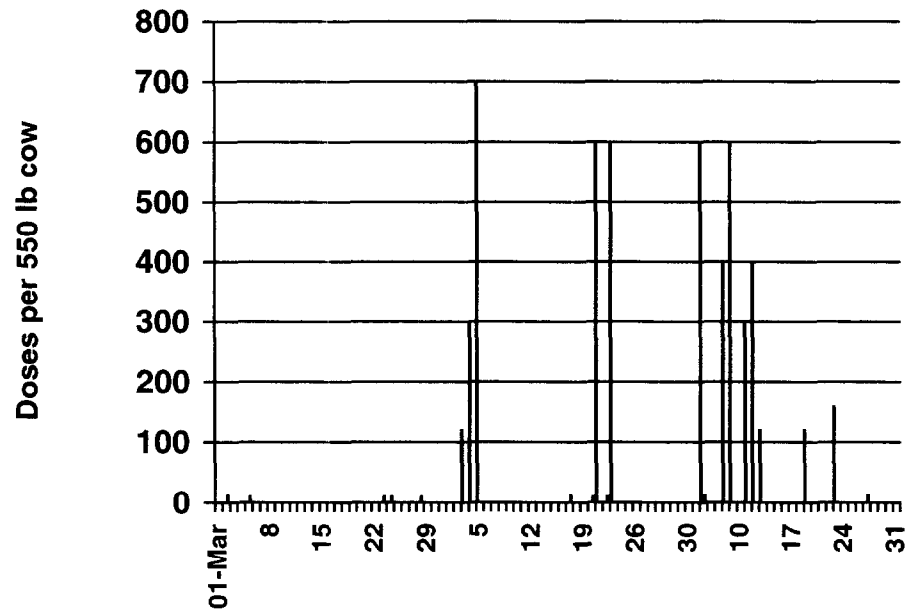
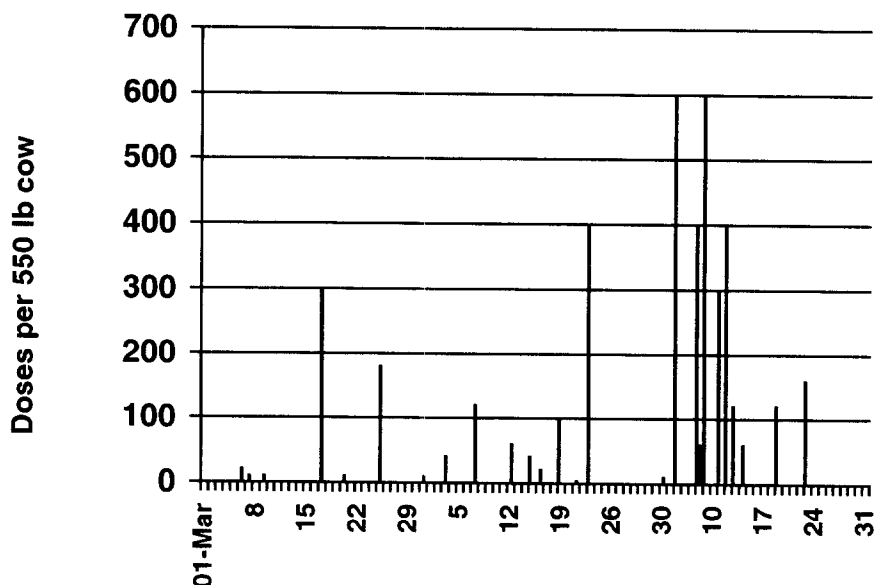


Chart 2. Daily sales of avermectins purchased through a large veterinary practice in Matagorda county Texas from March-May, 2001.



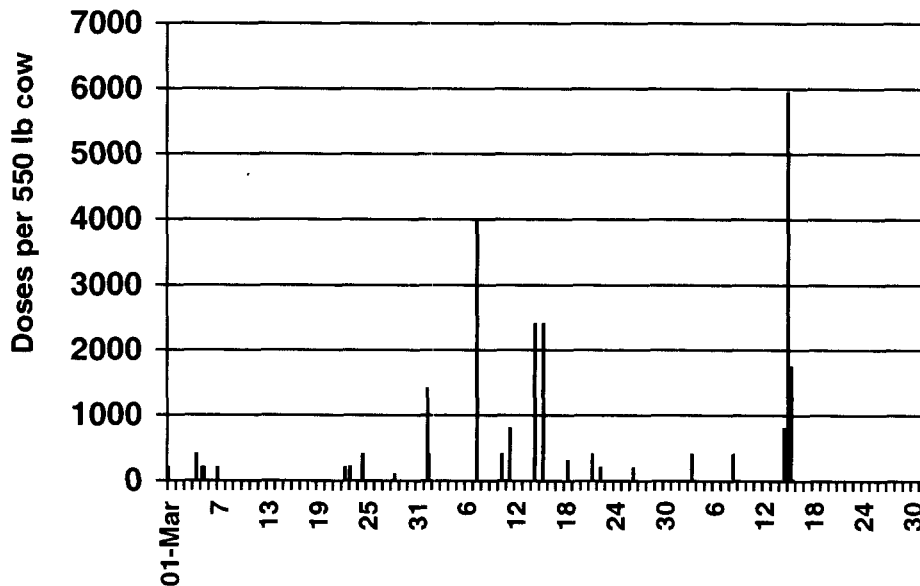
Over the 3 months, daily sales totaled enough to dose 5,128 cows or 8% of Matagorda county's beef cow population (1997 Ag census).

Chart 3. Daily sales of avermectins purchased through a large veterinary practice (clinic no. 1) in Hardee county Florida from March-May, 2001.



Over the 3 months, daily sales totaled enough to dose 1,454 cows or 2% of Matagorda county's beef cow population (1997 Ag census).

Chart 4. Daily sales of avermectins purchased through a large veterinary practice (clinic no. 2) in Hardee county Florida from March-May, 2001



Over the 3 months, daily sales totaled enough to dose 27,210 cows or about 32.5% of Hardee county's beef cow population (1997 Ag census).

8. Number of Acres Affected

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

Each feedlot animal would produce about 3510 kg (3.5 tons) of wet waste or 1300kg (1.3 tons) of aged waste during a 130 day fattening period. Medication of 7.2 million feedlot cattle annually with pour-on (Section 6.C.7.a) would produce 9.4 million tons of aged waste containing residues:

$$1.3 \text{ tons/animal/year} \times 7.2 \text{ million animals} = 9.4 \text{ million tons/year}$$

At an application rate of 13.6 metric tons of aged manure per acre, this manure would be dispersed over about 6.9×10^5 acres:

$$9.4 \text{ million tons} \div 13.6 \text{ tons/acre} = 6.9 \times 10^5 \text{ acres}$$

Medication of 5.5 million beef cows, 5.5 million calves and 4.5 million stockers on pasture annually with pour-on (Section 6.C.7.a) would expose a total of 70 million pasture acres to residues in dung pats, assuming a stocking density of 9 acres/cow-calf pair or 4.5 acres per stocker (finding of local survey reported in the doramectin injectable EA, NADA 141-061):

$$15.5 \text{ million pasture cattle} \times 4.5 \text{ acres per animal} = 70 \times 10^6 \text{ acres}$$

Pasture acres would actually receive only minimal exposure to doramectin residues in dung pats due to the physical and chemical properties of the drug and its degradation by biotic and abiotic mechanisms (Section 7.B.6).

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT:

A. Summaries of Doramectin Environmental Fate Studies

(Full report summaries are found in the doramectin cattle injectable EA, NADA 141-061)

1. Aqueous Solubility

The solubility of doramectin in water is 25 ppb at $25 \pm 0.01^\circ\text{C}$.

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.

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.

3. Octanol-Water Partition Coefficient

The octanol-water partition coefficient, K_{ow} , for doramectin is 25,787; $\log K_{ow}$ is 4.41.

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.

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.

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).

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 -photodegradeate analysis revealed at least 10 minor polar degradation products, none of which individually accounted for more than 10% of the applied radioactivity.

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% $^{14}\text{CO}_2$ in 72 days). Analysis of soils for unchanged doramectin and metabolites by extraction and HPLC analysis at termination of the study (day 72) revealed that doramectin had been transformed to metabolites in all three soils. The amounts transformed were 42.2%, 53.5% and 55.6% for the Ohio, Illinois, and North Dakota soils, respectively. The estimated time to 50% biotransformation for these soils was 79, 62, and 61 days, respectively. One breakdown product accounted for more than

10% of the total applied radioactivity in a single soil, Illinois silt loam (range 12.7-13.8%) and was identified as the 8- α -hydroxy analog of doramectin.

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

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

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

Only insignificant amounts of doramectin are expected to partition into surface waters in runoff from a feedlot due to the strong sorption of drug to cattle feces. 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.6. Nevertheless, one can estimate a distribution of residues into surface runoff to illustrate the very low concentrations that would be found in the aqueous phase. For example, assume that all residues from both wash-off (13 mg) and excretion (57 mg) are present in feedlot manure excreted over 56 days.

The amount of manure excreted over this period would be 1512 kg (27 kg/day x 56 days), so the residue concentration would be 46 ppb:

$$(13 \text{ mg} + 57 \text{ mg})/1512 \text{ kg} = 0.046 \text{ mg/kg}$$

The concentration of doramectin in surface water equilibrated with the doramectin-containing manure, C_w , can be calculated using the relationship

$$C_w = C_m/K_d$$

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

The feces/water partition coefficient for doramectin is 15,600. The maximum concentration of doramectin in equilibrated surface runoff is therefore 3 ppt ($[0.046 \text{ mg/kg}]/15,600 = 3.0 \times 10^{-6} \text{ mg/kg}$ or 3.0 ppt). Runoff from rainfall events occurring at later times after drug administration will contain even less, as the concentration of doramectin residues in manure will have decreased by further dilution with fresh manure. Residues in any runoff would be further diminished by sorption to soil during the runoff event and dilution into the receiving pond or lake.

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

$$\begin{aligned}\text{Amount removed} &= (\text{volume of runoff per acre})(\text{concentration in runoff}) \\ &= (1/12 \text{ acre-ft})(1.233 \times 10^6 \text{ L/acre-ft})(3.0 \times 10^{-6} \text{ mg/L}) = 0.31 \text{ mg}\end{aligned}$$

In a feedlot with a stocking density of 200 head/acre and assuming all of the animals were treated with doramectin, this would represent only 0.002% of the total drug residues:

$$[0.31 \text{ mg} \div (70 \text{ mg/head} \times 200 \text{ head})] \times 100 = 0.002\%$$

Therefore, in this worst case example, 0.31 mg doramectin/acre would be carried in surface runoff at a concentration of 3.0 ppt, representing only 0.002% of the residues expected in fresh feedlot manure.

2. Fate of Doramectin in Waste-Amended Soil

The innate biodegradability of doramectin in soil has clearly been shown by demonstration that the drug undergoes biotransformation to approximately 14 quantifiable metabolites which collectively account for as much as 56% of residues extracted from soil at 72 days. The estimated time for transformation of 50% of doramectin to metabolites in three different soils was 61, 62 and 79 days. Although the kinetics of doramectin degradation in soils cannot be predicted from the studies conducted and are likely to be complex, first order kinetics have been found applicable for describing degradation of a variety of chemicals present at very low (e.g., ppm) concentrations (Alexander and Scow, 1989) and will be used to describe the degradation of doramectin in soil.

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

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

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

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

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

<u>Time to 50% Biotransformation (days)</u>	<u>k (Days⁻¹)</u>
61	0.01136
62	0.01117
79	0.00877

If the initial concentration of doramectin in manure-amended soil is 0.81 ppb (Section 6.C.6) and assuming a time to 50% transformation of 79 days, the most conservative value obtained from soil biodegradation studies, 0.033 ppb will remain in the soil 365 days after application ($\log C = \log 0.81 - [0.00877 \times 365/2.3] = -1.48$; $C = 0.0329$ ppb). The table below indicates that a maximum concentration of approximately 0.84 ppb doramectin residues in soil is reached after application of manure to the soil two times with a 365 day interval:

<u>Number of successive reapplications</u>	<u>Concentration (ppb) of doramectin residues in soil</u>
0	0.81
1	$0.0329 + 0.81 = 0.8429$
2	$0.0342 + 0.81 = 0.8442$
3	$0.0343 + 0.81 = 0.8443$

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

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

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

Estimates of the amount of doramectin that might enter surface waters after feedlot waste is applied to agricultural soils can be made from the doramectin soil/water partition coefficients determined in the soil sorption/desorption study. 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. Using the mean K_d value for the three soils tested, 289, and the maximum doramectin concentration in

soil amended with aged manure, 0.84 ppb or 8.4×10^{-4} mg/kg (Section 7.B.2), $C_w = (8.4 \times 10^{-4} \text{ mg/kg})/289 = 2.9 \times 10^{-6} \text{ mg/kg}$ or 2.9 ppt. This is the maximum concentration that would be found in surface water that has equilibrated with the doramectin-amended soil; this would be diluted as the surface water mixed with water in a receiving pond, lake or stream and would decline further as the doramectin is rapidly degraded by sunlight.

The amount of doramectin that could be transported to the aquatic environment during a rainfall event can be estimated by assuming that 1% of the total drug residue per acre (Wauchope, 1978) applied to a 10-acre watershed moves into a 1 acre pond which is 2 m deep. The pond volume is 8.1×10^6 liters (1 acre x 2 m x $4047 \text{ m}^2/\text{acre} = 8094 \text{ m}^3 \times 1000 \text{ L/m}^3 = 8.1 \times 10^6 \text{ L}$). At a maximum application rate of 734 mg/acre (Section 6.C.6), the maximum amount entering the pond would be 73.4 mg:

$$734 \text{ mg/acre} \times 0.01 \times 10 \text{ acres} = 73.4 \text{ mg}$$

If this entire amount were present in the aqueous phase of the receiving pond, the concentration would be 9 ppt:

$$\frac{73.4 \text{ mg}}{8.1 \times 10^6 \text{ L}} = 9.06 \times 10^{-6} \text{ mg/L} = 9 \text{ ppt}$$

However, these residues will partition between the aqueous phase and the organic matter in the receiving pond, significantly reducing aqueous concentrations. 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 = $8.1 \times 10^6 \text{ L}$

Assumptions used:

K_d adjusted for a sediment organic matter content of 5%, or approximately 2.9% organic carbon, estimated from the mean K_{oc} of 35,900 for 3 soils:

$$K_d = 0.029 \times K_{oc} = 0.029 \times 35,900 = 1041$$

Depth of sediment sorbing residue = 5 cm with density = $1.5 \times 10^3 \text{ kg/m}^3$, therefore:
 $m = [0.05 \text{ m} \times 1 \text{ acre} \times (4047 \text{ m}^2/\text{acre})] \times (1.5 \times 10^3 \text{ kg/m}^3) = 3 \times 10^5 \text{ kg}$

The total amount of doramectin entering the pond = 73.4 mg; therefore:

$$A_w = 73.4 - A_s$$

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

$$1041 = \frac{A_s \times (8.1 \times 10^6)}{(3 \times 10^5) \times (73.4 - A_s)} = \frac{(8.1 \times 10^6)A_s}{(2.2 \times 10^7) - (3 \times 10^5)A_s}$$

$$(2.29 \times 10^{10}) - (3.12 \times 10^8)A_s = (8.1 \times 10^6)A_s$$

$$(3.2 \times 10^8)A_s = 2.29 \times 10^{10}$$

$$A_s = 71.47 \text{ mg}$$

$$A_w = 73.4 - 71.47 = 1.93 \text{ mg}$$

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

$$C_w = A_w/V = 1.93 \text{ mg}/(8.1 \times 10^6 \text{ L}) = 2.4 \times 10^{-7} \text{ mg/L or } 0.24 \text{ ppt}$$

Note that the percentage of the introduced drug residue partitioning into the aquatic compartment using this representative pond configuration is only 2.6% ($1.93 \text{ mg}/73.4 \text{ mg} \times 100$).

4. Potential Concentration of Drug in Surface Water Body after Wash-off

Although doramectin pour-on formulation is not to be used to treat cattle outdoors during rainy weather, a chance rain shower shortly after application could wash off as much as 13 mg of the dose applied to a 300 kg animal (Section 6.C.3). Assuming 10 cattle are standing in a pond of the configuration described above during a rainstorm and all the washed off doramectin remained in the aquatic compartment, the concentration would be 16 ppt:

$$([13 \text{ mg/animal}] \times 10 \text{ animals})/(8.1 \times 10^6 \text{ L}) = 1.6 \times 10^{-5} \text{ mg/L} = 16 \text{ ppt}$$

However, as demonstrated above, most of the doramectin will partition into the sediments, with only 2.6% remaining in the aquatic compartment. Therefore, the concentration of doramectin in the aqueous phase after wash-off will be only 0.42 ppt:

$$(0.026) \times 16 \text{ ppt} = 0.416 \text{ ppt}$$

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

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

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

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

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

d_s = density of soil solids

θ = pore fraction of the soil

Using the lowest K_{oc} value measured for doramectin in the soil sorption and desorption study (7,520), $\theta = 0.5$ and additional soil properties corresponding to the two soils that were used in the soil column leaching study, 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 ($50 \text{ cm} \times R_f$), 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 ($0.5 \times 25.4 \text{ cm} \times R_f$); even 10 times this amount of rainfall

(i.e., 100 inches) would not lead to significant movement of doramectin through the soil.

Given the low concentration of doramectin in soil following repeated application of cattle feedlot manure (0.84 ppb; Section 7.B.2), the low concentration in undiluted surface water equilibrated with waste-amended soils (2.9 ppt; Section 7.B.3), the very high K_{oc} values, and the susceptibility of doramectin to biotransformation in soil, doramectin is not expected to leach into ground water to any significant extent.

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

Doramectin present in dung pats of pastured cattle would be tightly sorbed to the excreta and would not be expected to leach from the dung pats into the soil or into surface run-off. As noted in Section 6.C.2, the maximum concentration of drug residue in fresh manure excreted by treated cattle was 270 ppb, occurring in feces collected on day 21 post-dose; manure collected at other times had lower levels of residue. The feces/water partition coefficient (K_d) of 15,600 will limit concentrations in equilibrated surface water to ≤ 17 ppt:

$$C_w = C_m/K_d = 270/15,600 = 0.017 \text{ ppb or } 17 \text{ ppt}$$

This water can permeate into soil around or beneath the dung pats or flow over the soil surface; in either case, any drug residues will partition from the water to the soil, depleting the waterstream of residues. Once in the soil, doramectin will be subject to biotransformation to minor metabolites (Section 7.B.2) and will be gradually depleted from the soil environment. Likewise, the susceptibility of doramectin to biodegradation and photodegradation will reduce levels of residues in the dung pats. Rates of degradation will likely depend upon various climatic and environmental parameters, as has been reported for ivermectin (Halley et al., 1989). Disruption of dung pats by weather, i.e. freeze-thaw cycles and rainfall, as well as the activity of vertebrates, i.e. trampling by livestock and foraging by mammals and birds, will tend to disperse the dung and any associated residues into the soil, where biodegradation will continue.

7. Summary of Fate of Doramectin Residues in Environmental Compartments

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

<u>Compartment</u>	<u>Maximum Expected Concentration</u>	<u>EA Section</u>
Wet feedlot wastes (130 days, 80% moisture)	20 ppb	6.C.4
Aged feedlot wastes (130 days, 30% moisture)	54 ppb	6.C.5
Surface runoff from feedlot wastes	0.003 ppb	7.B.1
Waste-amended soil, first application	0.81 ppb	6.C.6
Waste-amended soil, reapplication	0.84 ppb	7.B.2
Surface runoff, waste-amended soil	0.0029 ppb	7.B.3
Receiving pond, 10 acre watershed	0.00024 ppb	7.B.3
Surface water body, wash-off	0.00042 ppb	7.B.4
Ground water	Insignificant	7.B.5

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

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

(Full report summaries are found in the doramectin cattle injectable EA NADA 140-061 except where noted)

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.

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.

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, 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. 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. *Eisenia fetida*, acute study

No mortality was observed in the earthworm *Eisenia fetida* 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.

5. *Eisenia fetida* sublethal effects and reproductive output

Adult worms were exposed to doramectin for 56 days and showed no effects except for worms exposed to the highest concentration (17mg/kg) requiring longer time to burrow. Juvenile production was reduced in worms exposed to a doramectin concentration of 1.6 mg/kg or higher. The no observed effect concentration based on fecundity data was 0.89 mg/kg doramectin. A full report summary is presented in Appendix C-6.

6. *Enchytraeus albidus* sublethal effects and reproductive output

Adult worms were exposed to doramectin for 42 days and no effects were observed at any concentration. Juvenile production was reduced in worms exposed to a doramectin concentration of 24 mg/kg or higher. The no observed effect concentration based on fecundity data was 13 mg/kg doramectin. A full report summary is presented in Appendix C-7.

7. Immature Dung Beetles and Horn Flies

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

8. Effects of doramectin pour-on on three species of dung inhabiting insects

No effects were observed on either viability or mating of 2 species of dung burying Scarabaeidae, *Euoniticellus intermedius* and *Onthophagus gazella* and 1 species of predaceous Staphylinidae, *Philonthus flavolimbatus* adults following exposure to dung collected weekly from cattle treated with doramectin pour-on. Numbers of progeny recovered from dung collected from doramectin treated cattle were reduced compared with saline treated cattle for 7-14 days post dose, indicating that residues excreted in dung during this time period were present at concentrations that impacted beetle development. A full report summary is presented in Appendix C-3.

9. Invertebrate Colonization and Disintegration of Dung Pats in Pasture

Dung pats deposited by pastured cattle or constructed of bulked dung collected 4, 32 or 64 days after doramectin injectable treatment degraded at rates equivalent to nontreated controls. Numbers of larval and adult dung beetles (*Aphodius* spp. and *Sphaeridium* spp.) were equivalent in pats from control and treated animals. Larvae of dung feeding flies, mainly *Ravinia* spp., *Neomyia cornicina* and *Musca autumnalis* were reduced in pats from treated cattle. Predatory beetles, primarily larval *Sphaeridium* spp. and adult *Staphylinidae* were also reduced at 4 days but not at 28 days.

10. Acute Oral Toxicity (LD_{50}) of Doramectin in Bobwhite Quail

The acute oral (single dose) LD_{50} of doramectin for Bobwhite quail lies in excess of 2000 mg/kg. Following doses of 500, 1000 or 2000 mg/kg, clinical signs of toxicity were mild and only infrequently observed; those receiving 2000 mg/kg were necropsied 14 days post dose and no abnormalities were observed. A full report summary is presented in Appendix C-4.

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*.

2. *Daphnia magna*

Acute toxicity of doramectin, 3"-O-desmethyldoramectin and 8- α -hydroxy-doramectin for the water flea *Daphnia magna* was measured under static conditions. The 48 hour EC₅₀ concentrations and NOECs are as follows:

	<u>EC₅₀</u>	<u>NOEC</u>
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

3. Bluegill Sunfish

Acute toxicity of doramectin for bluegill sunfish (*Lepomis macrochirus*) was measured under static conditions. The 96 hour LC₅₀ is 11 ppb and the NOEC is 2.3 ppb.

4. Rainbow Trout

Acute toxicity of doramectin for rainbow trout (*Onchorhynchus mykiss*) was measured under static conditions. The 96 hour LC₅₀ is 5.1 ppb and the NOEC is 2.5 ppb.

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

1. Terrestrial Species

a. Terrestrial Plants

As discussed above under Sections 6.C.6 and 7.B.2, the maximum predicted environmental concentration (PEC) of doramectin residues in soil is 0.84 ppb. This concentration could only occur when cattle manure containing doramectin residues had just been mixed into soil, assuming no degradation of doramectin had taken place in the manure, and accounting for the very small residual amount of drug that may remain from previous annual fertilizations. Seed germination or root elongation for six different species of agricultural crop seeds were affected only at concentrations of 3.3 ppm or greater, 3.9×10^3 times the soil PEC. Seedling growth of the dicotyledons tomato and soybean was not affected at concentrations of 53 - 980 ppm, between 6.3×10^4 and 1.2×10^6 above the 0.84 ppb maximum predicted 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 3.9×10^4 times the soil PEC. 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, 5.6×10^4 times the PEC 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, 54 times the soil PEC and 1.6×10^4 times the 2.9 ppt PEC for doramectin in undiluted soil surface runoff (Section 7.B.3), which would correspond to maximum interstitial water concentrations to which seedlings would be exposed. Importantly, the tight binding of doramectin to soil and its extremely low water solubility will limit doramectin availability to plants to such an extent that residues are not expected to affect plant growth. Moreover, the susceptibility of doramectin residues to degradation prior to and following land application will result in exposure of terrestrial species to drug residues at concentrations likely to be significantly below the maximum estimated soil concentration. Such exposures will be transient as doramectin residues further degrade in the soil environment. Therefore, doramectin residues in soils are not expected to affect plant growth.

b. Soil Dwelling Microbial and Invertebrate Species:

The maximum predicted concentration of doramectin in soil is not expected to have an adverse effect on non-target, soil dwelling terrestrial species. Minimum inhibitory concentrations of doramectin were 40 ppm or above for soil microorganisms tested, nearly 5×10^4 times the soil PEC. The NOEC for the earthworm *E. fetida* in the acute study was 2 mg/kg, a level that exceeds the soil PEC by 2.4×10^3 times; no lethal effects were observed for this species at concentrations up to 1000 ppm, 1.2×10^6 times the soil PEC. In chronic studies, the NOEC for *E. fetida* and *E. albidus* were 0.89 mg/kg and 13 mg/kg, levels that exceed the soil PEC by 1×10^3 and 1.5×10^4 times respectively. No lethal

effects were observed for earthworms at concentrations up to 9.3 mg/kg and 140 mg/kg, 1×10^4 and 1.6×10^5 times the soil PEC respectively.

c. Dung Dwelling Species:

Dung-dwelling arthropods are sensitive to doramectin. Laboratory studies in which immature stages of the horn fly *Haematobia irritans* and dung beetle *Onthophagus gazella* were exposed to fresh cattle dung spiked with doramectin, indicated that actively feeding larvae were affected by the doramectin-containing dung. In a laboratory environment, the LC_{90} value for hornfly larvae in cattle feces is approximately 3 ppb; the NOEC for larvae development or emergence of adults from the puparium is 2.4 ppb. The LC_{50} and LC_{90} of doramectin for immature dung beetles are approximately 12.5 ppb and 38.2 ppb, respectively; concentrations up to 250 ppb had no effect upon number of brood balls produced by mating pairs. Bioassays conducted in the laboratory showed that *Euoniticellus intermedius* and *Onthophagus gazella* produced significantly fewer progeny when exposed to feces collected from cattle 7 and 14 days after treatment with doramectin pour-on compared with exposure to feces collected from saline treated cattle. *Philonthus flavolimbatus* progeny development was reduced only on day 7. No effects on progeny development were observed at later time points and no effects were observed at any time post dose on viability of adults, mating or brood ball production (Appendix c-4). A study conducted with pastured cattle showed that in dung pats deposited or constructed of bulked dung collected 4, 32 or 64 days after doramectin injectable treatment, numbers of larval and adult dung beetles (*Aphodius* spp. and *Sphaeridium* spp.) were equivalent in pats from control and treated animals. Larvae of dung feeding flies, mainly *Ravinia* spp., *Neomyia cornicina* and *Musca autumnalis* were reduced in pats from treated cattle. Predatory beetles, primarily larval *Sphaeridium* spp. and adult *Staphylinidae* were also reduced at 4 days but not at 28 days, probably due to the absence of flies upon which they feed at the early time point rather than any drug effect.

Ecology of Dung Beetles in the U.S.: Concern has been expressed that use of avermectins in pasture cattle in the U.S. may adversely affect dung dependent arthropods (Schmidt, 1983) and dung beetles have been identified specifically as insects that may be threatened (Ridsdill-Smith, 1993). The doramectin injectable EA (NADA 141-061) provides a literature review on the ecology of dung beetles, e.g. geographic and temporal distribution, mobility, dung preference and breeding period. Literature appearing since 1996 is summarized as follows: Recent reports indicate that exotic dung beetle species introduced mainly from Africa continue to rapidly expand their habitat. Flanders et al (2000) trapped beetles in three regions of Alabama from May through August and found *O.gazella* to be abundant across all three regions at all times. Montes de Oca and Halffter (1999) describe *O.gazella* and *E.intermedius* from 27 new capture sites in Mexico and cite evidence that populations had spread from their original introduction sites across the Southern US. Smith (1997) reports *O.taurus* as far north as Indiana while Hunter & Fincher (1996) trapped *O.depressus* in mid Florida, 550 Km from its original Georgia collection site. Hoebeke and Beucke (1997) report spread of *O.gazella* as far west as Kansas and *O.taurus* as far north as Ohio, Pennsylvania and New York and as far west

as Missouri. This information has permitted species to be identified whose breeding populations could be threatened by exposure to doramectin residues in dung pats (Section 6.C.7.b, doramectin pattern of use survey).

Potential Effects of Avermectins including Doramectin Treatment on Soil and Dung Dwelling Organisms, Higher Trophic Species and Impact on Dung Degradation: Concern has been raised, i.e. Strong, 1992, that treatment of cattle with avermectins (such as doramectin) might delay the degradation of dung pats on pasture due to the insecticidal activity of residues excreted in dung. Studies conducted with doramectin injectable on pastured cattle failed to demonstrate any effect on rate of dung pat degradation (see doramectin EA, NADA 141-061); however, it may not be possible to extrapolate results from the site of these studies to other parts of the country or to more extended pasture areas. To provide a broader perspective, literature describing effects of avermectins on dung fauna and dung degradation was reviewed and presented in the doramectin injectable EA (NADA 141-061). This literature plus papers published since 1996 concerning impact of avermectins including doramectin on soil and dung dwelling organisms, higher trophic species and dung pat degradation is presented below. Findings from this literature review will be considered in relationship to doramectin exposure resulting from pour-on administration in a hazard assessment that follows the literature review.

Avermectins appear to have a broad range of activities against nematodes and arthropods. Their action either by glutamate-gated chloride channels (nematodes and arthropods) or GABA-gated chloride channels (arthropods) will inevitably extend beyond the targeted parasitic organisms when the residues of the drug reach the environment. Overall, the conditions of drug use, the type of species and their developmental stage (larval or adult), and the presence of other environmental factors including the diversity of fauna will influence the impact

McKellar (1997) discussed potential ecotoxicological impacts of anthelmintic residues. Impact was related to the specific deleterious effect on organisms in the locus of the excreta, the quantity of active residue excreted, the temporal nature of the excretion and the stability of residues in the environment. Studies measuring the insecticidal activity of avermectin residues in dung and rate of dung pat degradation have appeared since 1983 (Schmidt). However, a study on the impact of ivermectin administered in a sustained release device (Wall and Strong, 1987) has elicited the most attention and follow up. The last mentioned workers reported that the dung from calves administered an experimental ivermectin slow release bolus would not support the development of some dung breeding arthropods and degraded at a much slower rate than pats formed from the dung of non-medicated calves. Pats formed from bulk dung collected every 10-20 days after placement of the boluses showed major differences in numbers of Coleoptera and Diptera larvae and adults compared to controls through 100 days. By this time, control pats had largely disintegrated, but pats from treated cattle were largely intact, based on relative differences in wet weight of the pats. The same authors published a later article (Strong and Wall, 1988) describing an additional segment of the above study. Pats formed from bulk dung containing ivermectin added at 0.5, 0.25 and 0.125

ppm were placed on pasture and subsequently examined for dung inhabiting arthropods. After 33 days on pasture, equal numbers of Scarabaeidae larvae were collected from non-medicated pats and those containing 0.125 ppm ivermectin; no larvae were found in pats containing 0.25 or 0.5 ppm drug. After 70 and 121 days on pasture, all pats including non-medicated controls were almost devoid of insects except for dipteran pupae.

Strong and Wall (1994a) and Strong *et al.* (1996) studied the effect of the Ivomec® SR bolus, introduced in the UK in 1993 and releasing 50% more ivermectin than the earlier tested device. Dung was recovered from the calves 21 days after introduction of the bolus, formed into pats and placed in a field for 7-42 days. Upon examination, pats from bolus treated calves were devoid of larval Cyclorrhapha Diptera and had significantly fewer larval Scarabaeidae but there were no differences in numbers of adult beetles between treated and control groups. Fenbendazole and moxidectin were also evaluated and neither drug showed these effects.

Barth *et al.* (1993) studying the same bolus in Germany evaluated natural pats on pasture deposited 21, 70 and 119 days after dosing. A decrease in numbers of Coleoptera and Diptera larvae were observed at all time points in pats from ivermectin treated cattle. No differences in numbers of adult Coleoptera species were noted between treated and controls. Comparing surface areas of treated and control dung pats, a delay in degradation rate of treated pats was observed but differences were not statistically significant.

In Australia, Wardaugh *et al.* (2001) conducted bioassays with the bush fly *Musca vetustissima* and dung beetles *Onthophagus taurus* and *Euoniticellus fulvus* to assess the insecticidal duration of ivermectin when administered to sheep in controlled release capsules. Newly emerged fly larvae failed to pupate when placed on feces from sheep 6-49 days after they received the capsules. Beetle development was inhibited for 39 days after dosing. *O. taurus* adults emerging at later times showed some reduction in fecundity but reproduced normally within a week of being transferred to feces from untreated sheep. Using a model that simulated local dung beetle populations, the authors concluded that use of the device at the flock level in the spring or early summer could deplete beetle populations and cause losses in diversity but such losses would only be temporary.

Many studies have been conducted over the last 15 years to assess the impact of treating cattle and sheep with ivermectin injectable or pour-on formulations on rate of dung degradation and survival of dung dependant insects. In Denmark, the insect colonization and/or disintegration of formed dung pats was investigated (Madsen *et al.*, 1988 and 1990). Pats were formed from bulk dung collected from cattle following subcutaneous administration of ivermectin at 200 µg/kg. Larvae of aphodid beetles were inhibited by dung collected one day after treatment while pupae and larvae of dipteran nematocera and cyclorrhapha were inhibited for 1-10 days and 30 days, respectively, after treatment. In both studies, pats formed from dung collected one day after injection and placed on composted soil in flower pots or on pasture degraded more slowly than controls, based on visual observations or decreases in percent

organic matter. Pats formed 20 days after injection also degraded more slowly based on the latter criteria but not pats collected 30 days post dose.

Similar results were obtained in another Danish study in which cattle received ivermectin by subcutaneous injection at 200 µg/kg or via the pour-on formulation at 500 µg/kg (Sommer et al., 1992). As before, larvae of aphodid beetles were inhibited by dung collected 1-2 days after treatment with either formulation. Larval development of nematoceran Diptera were not inhibited at any time point, but cyclorrhaphan Diptera were inhibited in dung collected for 13-14 days after administration of the pour-on and for 28-29 days after subcutaneous injection. Rate of degradation of pats formed from dung collected for one to two days after ivermectin administration was reduced relative to controls after 45 days on pasture, on the basis of organic matter remaining in the pats. Similar studies were conducted in Spain with formed pats to determine the effect of intramuscularly or subcutaneously administered ivermectin treatment at 200 µg/kg on insect development (Wardhaugh and Rodriguez-Menendez, 1988; Lumaret et al., 1993). In the first mentioned study, feeding larvae of the dipteran fly *Orthelia cornicina* were inhibited in dung collected for 32 days post treatment. Ninety percent of larvae *Copris hispanus* were inhibited in development in dung collected three days post dose and 20% were inhibited in dung collected after 16 days. Sublethal effects were noted as a result of adult *C. hispanus*, *Bubas bubalus* and *Onitis belial* beetles feeding on dung from recently treated cattle. Effects included suppressed feeding activity, reduced ovipositing rates and egg viability. In the second study, larval development of the dipteran fly, *Neomyia cornicina* was prevented when exposed to dung collected 10 days post dose. Development of larval *E. fulvus* beetles was prevented by exposure to dung collected 1 but not 10 days post dose; however, larvae exposed to dung collected 10 days post dose took longer to develop than beetles exposed to control dung. Strong and Wall (1994b) conducted a similar study comparing ivermectin and moxidectin injectable products. *Aphodius* spp larvae and cyclorrhaphan Diptera larvae were absent from dung of ivermectin treated cattle for 7 and 14 days post dosing respectively. Dung from control and moxidectin treated cattle showed comparable numbers of larvae.

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

Studies with formed and natural dung pats were conducted in Germany (Schaper and Liebsich, 1991; Barth et al., 1994) to evaluate the impact of ivermectin injectable treatment. In the first mentioned study, development of various larval Diptera (muscid, sepsid, sphaerocerid) were reduced in pats formed from dung collected from cattle for several weeks after treatment. Dung was collected weekly beginning after the second of two injections given at 5

week intervals. Scarabaeidae (species not differentiated) development was not inhibited, nor was there any difference in rate of degradation of dung pats between treated and control groups. The second study monitored insect invasion and rate of degradation of natural pats that were voided on pasture during and after treatment at 3, 8 and 13 weeks after turn out. Populations of Diptera larvae in pats voided up to 28 days after treatment were reduced as were populations of some dung specific nematodes. Based on measurement of surface area and organic matter content (as a percentage of dry weight), pats from control and treated cattle degraded at statistically equivalent rates.

In south-central Australia (Wardhaugh and Mahon, 1991) higher numbers of adult *O. australis* and *O. pexatus* were found in dung from cattle treated three days and 25 days previously with abamectin (subcutaneously at 200 µg/kg) compared to dung from untreated cattle. An examination of pats from treated cattle revealed more dung beetle tunneling, suggesting a greater degree of dung burial. This suggests that beetles from treated pats were spending more time in the dung. Moreover, pats formed three days after treatment and recovered after six weeks of field exposure had significantly less residual dry weight than untreated pats. Pats from treated cattle had nearly disintegrated compared to pats from untreated cattle.

In western Australia, Dadour and Cook (1999) and Dadour *et al.* (1999) compared the activity of the introduced dung beetle species, *O. taurus* during the period of maximum beetle activity on pats formed from feces of nonmedicated cattle and those receiving ivermectin injectable at 0.2mg/kg. Pats formed from feces collected 7 and 10 days after treatment and placed on the ground for 24hrs had significantly fewer beetles than control pats and dung was significantly less dispersed. Pats formed from dung collected 3 and 15 days after treatment were not different from controls in terms of *O. taurus* adult populations or degree of dung dispersion. In a recent abstract, Dadour (2001) indicated that ivermectin significantly impacted survival of *O. binodis* in dung from treated cows, irrespective of the diet (grain or pasture). Data were not presented in the abstract. The report of Dadour and Cook (1999) and a more recent paper (Dadour *et al.*, 2000) also described a laboratory experiment measuring the impact of doramectin residues excreted in cattle dung on development of *O. binodis*, an introduced species now abundant in Western Australia. The latter report also describes the impact of abamectin on this species and describes doramectin excretion kinetics. Newly emerged adults exposed to abamectin residues 3 and 6 days post dose and doramectin residues 9 days post dose were reduced by 35% and 20% respectively compared to controls. Authors indicate that newly emerged adults are susceptible as a consequence of their voracious feeding prior to reaching sexual maturity. The authors observe that once sexually mature, adults were no longer impacted by either drug. Brood mass production of females exposed to feces collected for 42 days after abamectin treatment was significantly lower than controls for each week. For doramectin, brood mass production was lower by weekly averages but significantly reduced only at 3, 6 and 42 days; at 18, 24 and 34 days, production was significantly higher than controls. Abamectin residues impacted the ovarian condition of females exposed to feces collected at all time points post dose compared to 3 and 6 days post injection for

doramectin as indicated by a decline in mean number of developing oocytes and F_1 emergence. In this study, doramectin residues attained maximal concentrations of 101 µg/kg 3 days after injection followed by a linear decline with an elimination half-life estimation of 15 days (Dadour et al., 2000). Authors suggest that doramectin residues of <60 µg/kg have minimal impact on mortality and reproductive potential.

Mahon et al. (1993) observed that the sheep blowfly *Lucilia cuprina* exhibited reduced survival, delayed ovarian development and reduced egg production when fed dung from sheep treated 14hr previously with ivermectin oral drench. Feces collected 2 or more days after treatment had little effect. Wardaugh and Mahon (1998) evaluated the sheep drench product in cattle along with ivermectin and abamectin injectable formulations in a bioassay to determine impact against larvae survival of the bush fly, *M. vetustissima*. All formulations suppressed or reduced larval development through 16 days post treatment. Injectable formulations numerically but not statistically impacted survival at 32 days.

In Canada, Floate (1998, 2001) evaluated ivermectin and abamectin impact on insect activity and degradation of pats formed from feces of cattle collected up to 16 weeks after injectable or pour-on treatment. Reductions in insect populations were observed across taxonomic groups including coprophagous flies, parasitic wasps and both predacious and coprophagous beetles, some out to 12 weeks after drug administration. The data, however, are difficult to interpret because insect populations in pats from treated cattle were compared to insects enumerated from pretreatment control pats rather than control pats that were sampled weekly. Reduced insect activity was associated with slower dung pat degradation; however, the author stated that insect activity was observed to be only one factor affecting rate of dung pat degradation.

Several studies have been reported in which authors monitored dung pat degradation at the pasture level. Two studies conducted in 1987, one near London (Jacobs et al., 1988) and the other near Glasgow (McKeand et al., 1988) and a third study conducted over two grazing seasons (1988, 1989) near Southampton (Wratten et al., 1993) measured the disintegration of natural fecal pats in continuously grazed paddocks. Groups of cattle received either no medication or ivermectin pour-on at 500 µg/kg or ivermectin subcutaneous injection at 200 µg/kg after 3, 8 and 13 weeks on pasture. This is a regimen often recommended in the UK for anthelmintic prophylaxis. In the third study, an additional group was administered an ivermectin bolus that delivered 50-80 µg/kg/day for 90 days (first year) and 45-80 µg/kg/day for 120 days (second year).

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

depth and wet weight of dung pats over a nine week observation period beginning one week after administration of the third dose in June. The objective of the third study was to evaluate the impact of ivermectin use on dung degradation and pasture quality. Other workers (Holter *et al.* 1994) have challenged several of the conclusions reached by the latter authors, i.e. criteria employed in measuring dung pat disappearance, organic content of paddock soil and earthworm numbers. Accepting these points of criticism and focusing only upon results at the whole paddock level, ivermectin treatment did not appear to adversely impact pasture utilization because there was no evidence of dung build up in the pasture and no evidence that the pasture had to be selectively grazed to avoid rank forage.

Fincher (1992) conducted a bioassay study in the US with ivermectin administered at 0.2mg/kg and observed horn fly emergence reduced by 79-100% for 8 weeks following treatment. Emergence of adult *E. intermedius* and *O. gazella* from brood balls made from dung from treated cattle was reduced for 1-2 weeks. Two species of predaceous Staphylinidae were also evaluated. Progeny of *Philonthus flavolimbatus* exposed to feces from treated cattle were reduced for 1 week following treatment while treatment had no impact on progeny of *P. longicornis*. Similar results were observed by Roncalli (1989) with ivermectin where *O. gazella* larvae failed to develop in dung pats voided on pastures by cattle treated subcutaneously at 0.3 mg/kg 7 and 14 days earlier but not after 21, 28 or 35 days. In contrast, moxidectin administered at 0.2 mg/kg showed no effects upon *O. gazella* or *E. intermedius* viability, brood ball production or progeny development (Fincher and Wang, 1992). Fincher (1996) subsequently evaluated ivermectin pour-on (0.5 mg/kg) in a similar bioassay and observed that emergence of adult horn flies was significantly reduced for 5-6 weeks. Likewise, *E. intermedius* and *O. gazella* adult emergence from brood balls was reduced for 1-2 weeks and 2-3 weeks respectively. Drug had no impact on mean numbers of brood balls produced by either dung beetle species.

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

Floate and Colwell (2001) and Floate et al (2001) evaluated the larvicidal activity of pour-on formulations of doramectin, eprinomectin, ivermectin and moxidectin against horn fly (*Haematobia irritans*), house fly (*M. domestica*) and stable fly (*Stomoxys calcitrans*) by bioassay procedure. Fly eggs or larvae were placed in fecal samples collected weekly from pasture reared cattle following treatment and emerging adult flies were enumerated. All drugs except moxidectin

suppressed horn fly larvae for at least four weeks; moxidectin results at 4 weeks were erratic. Doramectin depressed house fly and stable fly adult populations at 1 and 2 weeks. Reductions at 4 weeks were statistically significant but not convincing. Eprinomectin and ivermectin showed similar effects but results were also erratic. Moxidectin demonstrated the least potent activity particularly against house fly.

Wardaugh and Longstaff (2001) recently conducted a bioassay comparing effects of eprinomectin and moxidectin pour-on formulations, both recently approved for use in Australian dairy cattle. Feces voided 3-70 days after moxidectin treatment had no effect on development or survival of the dung beetle *O. taurus*. However, increased mortality was observed among newly emerged beetles fed feces collected 3 days after eprinomectin treatment and enhanced juvenile mortality occurred with feces collected 1-2 weeks after treatment. The authors observed effects even after beetles exposed to feces collected 1-2 weeks after eprinomectin treatment were placed on feces from non-medicated cows for a further 10 days.

Several workers have employed bioassay procedures to measure insect toxicity of avermectins by adding known drug quantities to fresh feces used to rear insects. Thus, Doherty *et al.* (1994) found horn fly larval survival inhibited by moxidectin and abamectin concentrations of ≥ 128 $\mu\text{g/kg}$ and ≥ 4 $\mu\text{g/kg}$ respectively. *O. gazella* progeny were reduced by 40% and 95% respectively by abamectin incorporated in dung at concentrations of 4-8 $\mu\text{g/kg}$. In contrast, moxidectin reduced progeny only when incorporated into dung at concentrations in excess of 250 $\mu\text{g/kg}$. Gover and Strong (1995) calculated the ivermectin 24hr LC_{50} and LC_{95} for adult *Neomyia cormicina* dung flies to be 0.139 and 0.393 $\mu\text{g/g}$ respectively. For the yellow dung fly, *Scatophaga stercoraria*, Strong and James (1993) calculated the 24hr and 48hr EC_{50} for ivermectin in newly hatched larvae at 0.051ppm and 0.036ppm respectively. A concentration of 0.001ppm prevented adult emergence of 50% of insects and those exposed to drug showed developmental abnormalities in wing morphology at concentrations as low as 0.0005ppm. Clark (1992) earlier had reported morphological changes in *M. vetustissima* e.g. lack of wing symmetry 11 weeks after abamectin treatment. Orton *et al.* (1992) observed ivermectin, eprinomectin and abamectin to have similar OSC_{50} values, e.g. concentrations depressing ovipositing of gravid female blowfly, *Lucilia cuprina* of approximately 13ppm. McCracken and Foster (1993) surveyed invertebrates found in formed pats that were placed on pasture after addition of ivermectin at concentrations of 2, 1 and 0.5 ppm compared with no medication. Pats containing all concentrations of ivermectin markedly reduced fly larvae, e.g. Muscidae compared with control pats, but little effect was noted upon adult *Aphodius* beetles (five species) and unspecialized *Aphodius* larvae.

In several cattle studies cited above, the persistence of ivermectin in formed dung pats was measured by HPLC quantitation. Danish workers (Sommer *et al.*, 1992; Sommer and Steffansen, 1993a) formed pats from dung collected 1-2 days after administering the pour-on formulation at 500 $\mu\text{g/kg}$ or subcutaneous injection at 200 $\mu\text{g/kg}$. Assays at 1, 2, 5 and 13-14 days after treatment showed

peak concentrations of 9.0ppm 1 day after pour-on treatment and 3.9ppm 2 days after subcutaneous injection. Ivermectin from both formulations depleted to similar levels (2.7-2.8ppm) five days after treatment and drug concentrations were low or nondetectable 13-14 days after treatment (reviewed by Herd, 1995; Spratt, 1997 and Edwards *et al.*, 2001). Pats placed on pasture in Denmark in August or in Tanzania at the end of the rainy season (May-June) showed no decrease in residue concentrations after 45 and 14 days, respectively. In contrast, Spanish workers (Lumaret *et al.*, 1993) found the mean concentration of ivermectin in pats formed 2, 4, 7 or 10 days after subcutaneous injection of cattle at 200 µg/kg to decrease below the level of detection (20 µg/kg wet weight) within six days. Formed pats were placed on pasture in southern Spain in the spring during a hot, dry period.

An Australian study (Cook *et al.*, 1996) revealed that the absolute concentration of ivermectin excreted in feces following subcutaneous injection at 0.2 mg/kg was influenced by the volume of feces excreted, which in turn was much greater for grazing animals compared with grain fed animals. Thus, ivermectin levels measured in the feces of pastured cattle were 5 times lower than levels measured in feces of grain fed cattle. This suggests that animals fed a high energy low roughage grain diet voided lower volumes of feces containing higher apparent residue concentrations than cattle fed a high roughage diet. Laffont *et al.* (2001) compared ivermectin excretion kinetics in cattle given a single IV dose to the pour-on formulation at 0.5mg/kg. Half of the latter group were prevented from self grooming while the other half were allowed to groom themselves and penmates. Nearly 70% of the pour-on dose was recovered as parent drug in the feces of the grooming group vs. 6.6% in the non-grooming group indicating that grooming removed a significant portion of the applied dose.

Steel & Hennessy (2001) recently compared fecal excretion of ivermectin, doramectin and moxidectin injectable products in cattle. Ivermectin peak concentrations in feces occurred generally 1-8 days post dose. Peak concentrations were lower and later in pasture fed animals compared to grain fed cattle. Doramectin peak concentrations were observed 4 days post injection and were nearly 2.5 times the peak value for ivermectin. Mean residence time in feces (7.7d) was slightly longer than for ivermectin (6.3d) under similar conditions. Moxidectin peak concentrations were achieved one day after injection and were about 30% higher than ivermectin. Fecal residence time was a mean of 10.7 days. Parent residues of all three drugs was detected in feces 58 days post dose.

Several authors have investigated the impact of avermectins on fecal microbes or fecal dwelling nematodes. In a novel bioassay to assess sublethal effects, Finnegan *et al.* (1997) used fungal sporangia production by the coprophilus fungus *Pilobolus* as an indicator of aphodius beetle activity. The medium percent reduction in sporangia due to beetle activity was significantly lower in feces to which ivermectin pour-on formulation was added at 1 ppm compared to untreated feces. Without beetles, sporulation of *Pilobolus* in feces of a cow treated with the pour-on formulation was reduced 5-15 days after dosing but not earlier. The authors stated that results were preliminary and required follow up for clarification. Barth *et al.* (1994) reported only minimal impact on populations

of dung inhabiting nematodes in pats deposited by cattle 21, 70 and 119 days after administration of an ivermectin sustained release bolus. Dung inhabiting nematodes were identified by species and populations in the drug group were not reduced in numbers except for several *Diplogastridae spp.*

McCracken (1993) expressed concern over the potential for non-targeted species being impacted by avermectin residues in the environment by 3 possible mechanisms: 1) loss of rare insects that may breed exclusively in cow dung. 2) Vertebrates (birds) directly impacted by residues acquired while consuming insects from the dung of treated cattle. 3) Vertebrates (birds and mammals) indirectly impacted by loss of food resource, e.g. dependence on dung inhabiting insects. On the first point, McCracken identifies a reference to *Coleoptera spp.* associated with animal dung or carrion/detritus (15 species associated with cow dung), 3 of which are considered endangered and 1 which is considered rare in the UK. On the second point, McCracken speculates whether or not birds could possibly be poisoned directly through ingestion of dung inhabiting insects if avermectins bioaccumulated. Subsequently, McCracken states in the same report that the potential for direct poisoning of vertebrates through accumulation of avermectins by consumption of invertebrates containing residues would appear to be quite limited and studies at the species level would be required to clarify. Although studies to measure bioaccumulation of avermectins in soil or dung dwelling insects have not been reported, there are ample data demonstrating that avermectins do not bioconcentrate in marine or fresh water plants, invertebrates or fish (Halley et al. 1989 and 1993; Davies *et al.* 1997 and Edwards *et al.*, 2001). On the third point, McCracken identifies a dozen species of birds and several species of bats and small mammals whose diets include insects associated with the dung of livestock. No species appear to depend exclusively upon insects that inhabit cow dung but 4 species were identified for which this is an important food source in the spring or autumn (starlings, rooks, jackdaws and the chough). In a subsequent report, McCracken and Bignal (1998) observed choughs to feed exclusively on leatherjackets (larvae of crane flies) during the summer when feeding on dung insects was expected but insects were not present due to abnormally cool weather.

Concern has also been expressed over the potential for avermectins to impact organisms in the soil habitat which possesses richer biodiversity than dung. A square meter of soil may contain as many as 1000 species represented by microarthropods, nematodes, acari, collembola, diplopoda, earthworms and protozoa (Lavelle 1996). Dung habitats support fewer food webs than most other habitats and are usually limited to species in four or five saprophagous and predaceous trophic levels (Schoenly et al., 1991). Earthworms are prominent members of the soil community and are considered to play a key role in dung dispersion and decomposition. Also, they are reported to be resilient to avermectin effects (Barth et al., 1994; Halley et al., 1989; Madsen, 1990; Wall and Strong, 1987; Wratten et al., 1993.). Only one study (Gunn and Sadd, 1994) has described deleterious effects of avermectins on earthworm growth and reproduction. The latter study was done with heavily supplemented carrier in the test soil. As the control soil did not have any added carrier, it was not possible to determine if the effects observed were actually due to ivermectin or

the carrier itself, or both. Wardhaugh (2001) dismissed the studies conducted above as irrelevant because they employed *Eisenia fetida*, a composting worm not normally associated with cow dung. He considers two recent studies cited below as relevant because they employ species commonly associated with pasture environments. In a recent study (Swedsen et al., 2001, in press), hatchling survival and growth rates of the earthworm, *Lumbricus terrestris*, in treated dung appeared to be similar to worms reared in dung from untreated cattle. The authors concluded that ivermectin and its metabolites had no adverse effects on the survival and growth of *L. terrestris* when exposed through dung under laboratory conditions. Swedsen and Baker (2001 in press) also studied the effects of moxidectin in sheep and cow dung on survival and growth of the deep burrowing earthworm, *Aporrectodea longa*, common in Tasmania; over a 10 week period, no lethal or sublethal effects were observed. Barth et al. (1994) counted numbers of soil dwelling nematodes migrating through dung of cattle 21, 70 and 119 days after animals received an ivermectin sustained release bolus. Nine species of soil inhabiting nematodes were found in the dung of control cattle while the dung of ivermectin treated cattle contained 8 species.

Sherratt et al. (1998) employed two quantitative models to estimate the degree of exposure of dung dependant insects to avermectin residues. Under realistic farming conditions and given the stages of dung insect known to be sensitive to residues, maximum cumulative mortalities of <25% of insect populations on individual farms were predicted to occur. In South Africa, Scholtz and Kruger (1995) compared dung insect communities across similar 80 ha plots, each occupied by 20 cattle for 3 months. All cattle in the treated plot received a single 0.2mg ivermectin injection. All pats included in the study were identified within 24 hr of being voided. Ten natural pats and underlying soil were examined for insects monthly. Dung insect diversity was statistically lower in the ivermectin treated paddocks at one month after treatment but by 2 and 3 months there were no discernable differences between treated and control groups. Two additional studies were conducted by these authors (Kruger and Scholtz, 1998 a & b) to a similar study design to assess impact of ivermectin under drought and high rainfall conditions. Low rainfall preceded the sampling period in the first study and species richness was lower than expected. Insects collected from pats of ivermectin treated cattle showed a reduction in diversity and increases in dominance by some species at one month post treatment and these effects lasted for the 3 month observation period. The latter study was conducted during the same season during 2 years of higher than normal rainfall amounts. No effects in populations were noted in ivermectin treated paddocks at any time in the first year. One week after the second treatment in year 2, a number of beetle species were present in reduced numbers but recovery was complete at later time points (1 and 3 months). Commenting in a related paper (Kruger and Scholtz, 1997), the authors indicate that impacts noted in studies conducted with individual species, e.g. reduction in *E. intermedius*, were not confirmed in the field possibly due to immigration of specimens from adjacent areas that compensated for any adverse effects of the drug.

Hazard Assessment: This section assesses the safety of doramectin in the environment based on the data from this EA and from the doramectin injectable EA (NADA 141-061) plus literature references. Three areas of concern are addressed:

- 1) What is the toxicity of doramectin residues for organisms inhabiting cow dung and what are the impacts on dung degradation? How does doramectin compare with other avermectins as described in the literature?
- 2) What is the toxicity of doramectin residues for organisms inhabiting the soil? How does doramectin compare with other avermectins as described in the literature?
- 3) What effects will field use of doramectin have on susceptible species and organisms higher in the trophic level?

1. Toxicity for dung dependant organisms and impact on dung degradation:

"*In vitro*" toxicity studies are reported in which insect larvae cultured in dung were exposed to measured quantities of parent drug rather than metabolites excreted in feces. For doramectin, the EC_{50} against *O. gazella* was 12.5 ppb and EC_{90} against *H. irritans* was 3 ppb. These values are similar to those reported over the years for other avermectins, e.g. ivermectin, abamectin and eprinomectin. Only moxidectin seems to be significantly less potent in such tests. Thus, the LC_{50} for ivermectin against horn fly and stable fly was 3 ppb and 48 ppb respectively (Schmidt and Kunz, 1980). Abamectin was 100% lethal for *O. gazella* at ≥ 16 ppb whereas moxidectin produced mortality only at ≥ 256 ppb (Doherty *et al.*, 1994). The latter authors also observed *H. irritans* mortality of about 40% at ≥ 4 ppb abamectin and at 512 ppb moxidectin. The 24hr LC_{50} for ivermectin for the dung fly *Neomyia cormicina* was 139 ppb (Grover and Strong, 1995) and 51 ppb for the yellow dung fly *Scatophaga stercoria* (Strong and James, 1993). Sublethal effects were observed in the latter species at ivermectin concentrations of 0.5-1 ppb.

The radiotracer excretion study for doramectin reported in this EA shows residue levels in feces in excess of the values shown above for at least 6-8 weeks causing speculation that dung inhabiting insects could be impacted for several months after dosing. However, bioassays employing the same organisms to measure the interval following dosing that excreted residues cause lethal or sublethal effects indicate that beetles are impacted for only a few weeks and flies for a longer period. For doramectin, a bioassay study reported in this EA employing the dung beetles *O. gazella* and *E. intermedius* and predatory beetle, *P. flavolimbatus* showed mortality to beetles exposed to feces collected for only 1-2 weeks after pour-on treatment. A recent study by Dadour *et al.* (2000) corroborates these observations. Doramectin residues in dung were lethal to *O. binodis* for only 9 days after subcutaneous injection at use dose (0.2 mg/kg). Sublethal effects were observed only at 3 and 6 days post dose. Drug assay of feces revealed a peak residue concentration of 101 ppb, depleting to 80 ppb by day 9. Given lack of lethal or sublethal effects when

beetles were next monitored on day 18, the authors conclude that ≤ 60 ppb doramectin residues in feces have no effect on beetle F_1 emergence.

Bioassay studies conducted with other avermectins against exotic dung beetles were reviewed in the previous section. In summary, ivermectin injectable and pour-on impacted beetles for only 1-2 weeks post dose (Fincher, 1992 and 1996; Roncalli, 1989). Abamectin impacted beetles for 4 weeks post injection (Ridsdill-Smith, 1988); eprinomectin impacted beetles for 1-2 weeks after pour-on treatment (Wardaugh and Longstaff, 2001) and moxidectin showed no effect on beetle viability (Fincher and Wang, 1992, Wardaugh and Longstaff, 2001). Floate (1998) reviewed literature concerning impacts against aphodian beetles. Most species were reduced in numbers for only several days post dose (in agreement with his own observations and those reported in the doramectin injectable EA (NADA 141-061)). Exceptions were *A. vittatus* and *A. finetarius*, where Floate observed inhibition for several weeks, which agrees with literature values for these species.

From the above, it can be concluded that excepting moxidectin which does not appear to be very toxic for beetles, avermectins including doramectin exhibit more potent toxicity when drug substance is added directly to feces containing insect larvae than when the latter are exposed to excreted residues following treatment of cattle. Bioassay procedures conducted in the field in the presence of richer biodiversity are more relevant than "in vitro" tests for assessing insect toxicity. By this procedure, doramectin pour-on has been shown by studies reported in the EA and literature to impact beetles for only 1-2 weeks after pour-on administration.

The more prolonged impact of avermectins on cyclorrhaphan dipteran insects has been well known since the early 1980s, e.g. Miller et al. (1981) and Schmidt (1980). They observed reduced larval survival of pestiferous species (horn fly, face fly and stable fly) and non-pestiferous species (Sphaeroceridae, Sepsidae and *Gymnodia spp.*) 4-8 weeks after ivermectin treatment. Madsen (1990) observed inhibition of house fly more than 2 months after ivermectin injection and Clark (1992) noted asymmetrical wing development in adult bush fly, if larvae were exposed to pats from abamectin treated cattle out to 11 weeks post dose. Floate and Colwell (2001) and Floate *et al* (2001) report that pour-on formulations of ivermectin, doramectin and eprinomectin inhibit pest fly development for 4-8 weeks. Results with moxidectin were erratic, but flies were probably inhibited for 2-4 weeks post dose. Some non-pestiferous species appear to be affected for at least an equal period of time, e.g. Floate (1998). The latter observed reductions in insect populations across taxonomic groups including coprophagous flies and parasitic wasps, some out to 12 weeks after drug administration. The latter data, however, are difficult to interpret because insect populations in pats from treated cattle were compared to insects enumerated from pretreatment control pats rather than control pats that were sampled weekly.

Studies to assess any impact of avermectins against other fecal dwelling organisms are sparse. The literature review above cites one study describing effects against a fecal dwelling fungus (Finnegan *et al.*, 1997) and nematodes

(Barth *et al.*, 1994). Results of the first mentioned study are not easily interpreted because fewer fungal sporangia were observed in feces of cattle 5-10 days after ivermectin treatment but not sooner. "*In vitro*" experiments were no less difficult to interpret because ivermectin pour-on product was used as the source of drug rather than isolated drug substance. In the latter study, nematode populations and species diversity in pats from treated and untreated cattle were essentially the same.

Factors responsible for decomposition, dispersal and disappearance of dung pats were well known before the introduction of avermectins, e.g. Marsh and Campling (1970) and Weir (1971). These factors include trampling by livestock, boring and tunneling by insects to aerate and hydrate the pat, disruption, e.g. by termites, foraging by vertebrates, particularly birds, freeze-thaw cycles, "weathering", e.g. rain and wind, dung dispersion and burial by beetles, disruption by vegetation and ultimately chemical decomposition by cellulose degrading organisms.

Many studies conducted to determine the impact of avermectins on dung degradation have utilized artificially formed pats that have been placed in environments designed to exclude as many of the factors listed above as possible except for exposure to flying insects or soil dwelling invertebrates. Given the likelihood that avermectin treatment would have reduced or eliminated activities of many insects in the pat, it should not be surprising that pats from treated animals often times degraded more slowly. This is particularly true in studies conducted with sustained release formulations or devices where more sustained residue levels would likely be present.

Only recently have researchers, e.g. Floate and Colwell (2001) acknowledged that insects are only one of a suite of factors that affect degradation; other important factors cited by the above authors include mechanical disruption, animal foraging and weather. A number of authors cited in the preceding literature review have concluded that slower degradation of pats from treated cattle is due to fewer insects in these pats, even though other factors known for decades to impact dung degradation were omitted from the studies by design. In a practical context, after more than 20 years of commercial use, there is no evidence that pasture quality is depreciated by avermectins. Three studies conducted in the late '80's and early '90's to determine if avermectin use caused dung accumulation or degraded pasture quality failed to observe any impact. No new studies have been reported.

2. Toxicity for organisms in the soil:

The most important soil dwelling organism at risk are earthworms because they infiltrate dung pats and therefore could be in contact with avermectin residues. A 28 day acute laboratory study against *E. fetida* presented in the doramectin injectable EA (NADA 141-061) revealed an acute LC₅₀ in excess of 1000 mg/kg. A drug concentration of 100 mg/kg impacted burrowing time whereas 16 and 10 mg/kg did not. The 28 day NOEC based on weight gain was calculated to be 2

mg/kg. This compares with an LC₅₀ of 315 mg/kg and NOEC of 12 mg/kg based on weight gain for ivermectin in a 28 day acute toxicity test (Halley *et al.*, 1993).

A subsequent earthworm toxicity study (Gunn and Sadd, 1994) reporting ivermectin sublethal effects on reproduction has raised concern because previous studies had reported earthworms to be resilient to avermectin effects (Barth *et al.*, 1994; Madsen, 1990; Wall and Strong, 1987; Wratten *et al.*, 1993.). The 14 day LC₅₀ of 15.8 mg/kg reported by Gunn and Sadd that is lower than reported previously may have been due to the high concentration of excipients added with the ivermectin since drench product rather than drug substance was used in this study. More importantly, the non-lethal effects reported by Gunn and Sadd (reduced cocoon production) were reported at 21 days rather than more demanding determination of reproductive effects as investigated in the doramectin study (Section 8 A 5-6). Gunn and Sadd reported 4 mg/kg to be the lowest dose tested that statistically impacted cocoon production; a NOEC was not established. This compares with the doramectin study where 1.6mg/kg was the lowest dose that statistically reduced juvenile production; a NOEC of 0.89 mg/kg was computed. The latter drug concentrations are over 1000-2000 times the predicted environmental concentration of doramectin and residues in soil.

3. Effects of doramectin use on susceptible species and organisms in higher trophic levels.

Information contained in the doramectin injectable EA (NADA 141-061) and summarized in Section 8C1c supports the conclusion that species of dung beetles native to the US will not be threatened by use of doramectin in pastured cattle. Likewise, recent reports summarized in Section 8C1c indicate that exotic dung dispersing beetles native to Africa and introduced into the Southern US from California east through Texas, Georgia, South Carolina and Florida in the 1970s and 1980s are not only well-established but are rapidly expanding their habitats south through Mexico and north into the plain states, Midwest and Northeastern states. Given that the latter beetles have been found at all trapping sites selected for investigation, e.g. Flanders *et al.* (2000) and some species, e.g. *O. gazella* are spreading "at an astounding pace" (Hoebeke and Beucke, 1997), there is no evidence that populations have been threatened by avermectin use.

A survey (EA Section 7b) conducted by Pfizer of doramectin pour-on use across the US for years 1999-2001 reveals that peak usage occurs in the months of March-May and Sep-Nov. Usage is much lower during the peak period of dung beetle breeding across the Southern states of June-Sep. A local survey of avermectin sales to cow-calf operators in one Texas county and 2 Florida counties reveals patterns of daily drug usage. Results are similar to those obtained for a similar survey conducted for the doramectin injectable EA (NADA 141-061). Drug purchase and presumably drug use occurred evenly throughout the 90 day monitoring period, suggesting that blocks of adjacent herds would not likely be treated simultaneously. Thus, residues that could potentially impact local insect populations would not be broadcast simultaneously over a large geographic region.

Many dipteran (fly) species are more susceptible to avermectin residue toxicity than insects of the order Coleoptera (Summer et al., 1992, Floate, 1998). Recently Sherratt et al. (1995) described a quantitative model for estimating the impact of avermectin usage on dipteran populations. Realistic management conditions were considered and scenarios were constructed in which herds were either turned out to pasture in April or maintained on pasture all year. The model presupposed that all animals in the herd were treated in April or all first year animals only were treated in April, May, September or November. Data analysis indicates that instantaneous impacts on selected species could approach 100%, but maximum cumulative impacts would range from 15-25% of total populations.

Dung dependant beetle and fly populations were quantified in 3 studies conducted in identical 80 ha plots, each occupied for 3 months by 20 pasture cattle. All cattle in one plot received ivermectin at the beginning of the study while the second plot was untreated. The first study (Scholz and Kruger, 1995) conducted under average rainfall amounts showed a significant drop in insect diversity in the ivermectin plot during the first month with recovery in the second and third months. Two additional studies were conducted by these authors (Kruger and Scholtz, 1998a & b) to a similar study design to assess impact of ivermectin under drought and high rainfall conditions. Low rainfall preceded the sampling period in the first study and species richness was lower than expected. Insects collected from pats of ivermectin treated cattle showed a reduction in diversity and increases in dominance by some species at one month post treatment and these effects lasted for the 3 month observation period. The latter study was conducted during the same season during 2 years of higher than normal rainfall amounts. No effects in populations were noted in ivermectin treated paddocks at any time in the first year. One week after the second treatment in year 2, a number of beetle species were present in reduced numbers but recovery was complete at later time points (1 and 3 months).

To put into context the extreme habitat disruption necessary to impact ecosystems and alter species distributions, even the process of slash-and-burn agriculture and complete mechanical forest clearance, reduces soil biota richness (species diversity) by only 60% relative to nearby undisturbed sites. Impacts on trophic structure e.g. proportion of plant parasites, bacterial feeders, predators, etc were also small (Bloemers et al., 1997).

McCracken (1993) expressed concern over the possibility of rare or endangered dung dependant insects becoming extinct as a result of avermectin use in livestock. This supposition is based on assumptions that have not been investigated that avermectin use (from temporal and geographic perspectives) impacts insects of concern and if so, that populations reduced by drug use would fail to recolonize. McCracken expressed further concern over potential for indirect effects of avermectin use, e.g. reducing important invertebrate food sources for birds, bats and some small mammals. Several birds and especially the chough were identified as particularly dependant on this food source during the spring and late summer/early autumn. In subsequent papers, McCracken and Foster (1994) state that in Scotland where choughs were observed, the

best feeding opportunities for dung dependant insects were in summer (adult beetles and fly larvae) and late fall (Aphodius beetles); however, most avermectin use in these climes is in the spring. In a subsequent report, McCracken and Bignal (1998) observed choughs to feed exclusively on leatherjackets (larvae of crane flies) during the summer when feeding on dung insects was expected. The explanation offered was that spring came very late and dung insect populations were not available. Thus the chough is not dependant on dung insects during the spring and summer and readily exploits alternative food sources when necessary without losses in population.

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: As with other avermectins, doramectin is excreted in the dung of treated animals and can inhibit the reproduction and growth of pest and beneficial insects that use dung as a source of food and for reproduction. The magnitude and duration of such effects are species and life-cycle specific. When used according to label directions, the product is not expected to have an adverse impact on populations of dung-dependent insects.

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 aquatic organisms. Do not permit cattle to enter lakes, streams, or ponds for at least 6 hours after treatment. Do not contaminate water by direct application or by the improper disposal of drug containers. Dispose of containers in an approved landfill or by incineration.

11. ALTERNATIVES TO THE PROPOSED ACTION

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.

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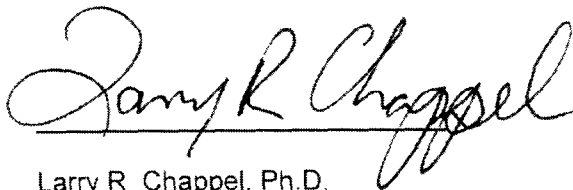
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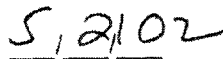
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13. CERTIFICATION

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

A handwritten signature in cursive script, reading "Larry R. Chappel". The signature is written in dark ink and is positioned above a horizontal line.

Larry R. Chappel, Ph.D.
Director, Veterinary Medicine
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A handwritten date "5/2/02" in cursive script, positioned above a horizontal line.

Date

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* Reprints filed with the Doramectin Injectable EA (NADA 141-161)

Appendix A-1

Material Safety Data Sheet



MATERIAL SAFETY DATA SHEET

SECTION 1 - IDENTIFICATION OF SUBSTANCE AND COMPANY

Pfizer Animal Health
812 Springdale Drive
Exton, PA 19341

Emergency telephone 1-800-228-5635
Hours of operation 24 Hours
Telephone 1-800-877-6250

Product name	Doramectin
Chemical family	Antiparasitic macrocyclic lactone
Synonyms	B1 Cyclohexyl Avermectin; Doramectin
Therapeutic use	Antiparasitic (veterinary); endectocide
Chemical name	25-cyclohexyl 5-O-demethyl-25-de(1-methylpropyl) avermectin A1a

SECTION 2 - COMPOSITION

<u>Ingredient</u>	<u>CAS Number</u>	<u>Amount</u>
Doramectin*	117704-25-3	100 %

*Hazardous

Note: Ingredients indicated as hazardous have been assessed under U.S. OSHA Hazard Communication Standard for workplace safety (29 CFR 1910.1200).

SECTION 3 - HAZARDS IDENTIFICATION

Signal word	CAUTION!
Statements of hazard	MAY BE HARMFUL IF SWALLOWED MAY BE A REPRODUCTIVE HAZARD (BASED ON ANIMAL DATA) DANGEROUS FOR THE ENVIRONMENT.
Eye effects	Not an eye irritant (based on animal data).
Skin effects	Not a skin irritant (based on animal data).
Inhalation effects	Not known
Ingestion effects	May be harmful if swallowed

SECTION 4 - FIRST AID MEASURES

Skin	Wash skin with soap and water. Remove contaminated clothing and shoes. Wash clothing and thoroughly clean shoes before reuse. If irritation occurs or persists, get medical attention.
Eyes	Immediately flush eyes with water for at least 15 minutes. If irritation occurs or persists, get medical attention.
Inhalation	Remove to fresh air. If discomfort persists, get medical attention.
Ingestion	If swallowed, get medical attention. Do not induce vomiting unless directed by medical personnel. Never give anything by mouth to an unconscious person.

SECTION 5 - FIRE FIGHTING MEASURES

General hazard	Toxic gases may be emitted in fires of this substance. See Hazardous combustion products, below.
Fire fighting instructions	Wear approved positive pressure, self-contained breathing apparatus and full protective turn out gear. Use caution in approaching fire.
Extinguishing media	Water, carbon dioxide, dry chemical or foam.
Flash point	Not applicable
Autoignition	Not applicable (N/A)
Minimum explosive concentration for dust/vapor	0.025 oz/ft ³
Flammability limits	Not applicable (N/A)
Hazardous combustion products	Emits toxic fumes of carbon monoxide, carbon dioxide and oxides of nitrogen.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Small spill	Contain the source of the spill or leak. Scoop spilled material into a labeled container for recovery or disposal. Clean spill area thoroughly with detergent and water.
Large spill	Review Sections 3, 8 and 12 before proceeding with clean up. Use appropriate containment to avoid environmental contamination. Scoop or shovel spilled material into a labeled container for disposal. Avoid generating airborne dust. Close container and move it to a secure holding area. Prevent discharge to drains.

SECTION 7 - HANDLING AND STORAGE

General handling	Do not generate airborne dust or expose to ignition sources. Ground and bond all bulk transfer equipment. Keep away from heat. Use with adequate ventilation. Avoid contact with eyes, skin and clothing. Avoid breathing dust. When handling, use proper personal protective equipment as specified in Section 8.
Storage conditions	Store out of direct sunlight in a cool, well ventilated, dry area. Store in light-resistant containers. Keep container tightly closed when not in use.
Temperature range for storage	4 °C

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure limits			
<u>Compound</u> Doramectin	<u>Issuer</u> Pfizer	<u>Type</u> TWA 8 HR	<u>OEL</u> 0.2 mg/m ³
Exposure information	No Short Term Exposure Limit (STEL) has been established		
Measurement method	DORAMECTIN CAM-JWT-93-08 (contact Pfizer for additional details)		
Ventilation	Keep airborne contamination levels below the Exposure limits listed above in this section. General room ventilation is adequate unless the process generates dust or fumes. Do not use in a confined space.		
Respiratory protection	If the applicable Occupational Exposure Limit (OEL) is exceeded, wear an appropriate respirator with a protection factor sufficient to control exposures to below the OEL.		
Eye protection	Safety glasses or goggles.		
Skin protection	Use protective clothing (uniforms, lab coats, disposable coveralls, etc.) in both production and laboratory areas.		
Hand protection	Wear latex or other impervious gloves if skin contact is possible.		

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Physical form	Powder
Color	White
Molecular weight	809.72
Molecular formula	C ₂₆ H ₄₀ O ₄
pH	Not known
Boiling point	Not applicable (N/A)

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SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES ... continue

Melting point	160.5 - 162.2 °C
Pour point	Not applicable (N/A)
Density	No data available
Vapor pressure	< 0.00000007 l at 30 - 35 °C
Water solubility	Doramectin is insoluble in water (25 mg/l at 25 °C)
Solvent solubility	Freely soluble in most polar organic solvents.

SECTION 10 - STABILITY AND REACTIVITY

Reactivity	Stable
Conditions to avoid	Heat and light
Incompatibilities	Strong acids and bases
Hazardous decomposition products	No data available - See Section 5 - under Hazardous combustion products
Hazardous polymerization	Will not occur
Oxidizing properties	No data available
Explosive properties	Dust of this material has low sensitivity to electrical ignition. Minimum ignition energy is between 100 to 500 mJ

SECTION 11 - TOXICOLOGY INFORMATION

Acute toxicity				
<u>Compound</u>	<u>Type</u>	<u>Route</u>	<u>Species</u>	<u>Dosage</u>
	LD ₅₀	Oral	Rat (M)	1000 - 2000 mg/kg
	LD ₅₀	Oral	Rat (F)	500 - 1000 mg/kg
	LD ₅₀	Oral	Rat (M)	50 - 100 mg/kg
	LD ₅₀	Oral	Rat (F)	100 - 200 mg/kg
Eye	Not irritating based on animal data.			
Skin	Not irritating based on animal data.			
Inhalation	No data available			

SECTION 11 - TOXICOLOGY INFORMATION continued

Ingestion	The differences seen in the acute oral LD50 ranges listed above demonstrate the effect that varying the vehicle can have on the toxic potential of doramectin. When administered to rats in an aqueous suspension, the LD50 ranges were 500 - 100 mg/kg for females and 1000 to 2000 mg/kg for males. When administered in sesame oil, a non aqueous vehicle, the acute toxicity was greater with LD50 ranges of 50 - 100 mg/kg for female rats and 100 to 200 mg/kg for male rats. Because the human digestive tract is primarily an aqueous environment, it may be assumed that the more relevant vehicle for assessing occupational exposure would be the aqueous suspension.
Mutagenicity	No evidence of mutagenicity was observed for doramectin when tested in vitro and in vivo in the following assays: the Ames test, the mouse lymphoma L5178Y assay and the unscheduled DNA synthesis (UDS) assay in cultures of rat hepatocytes.
Subchronic effects	Repeat-dose and subchronic oral toxicity studies of doramectin were conducted in rats at doses up to 8 mg/kg for 3 months and in dogs at doses up to 4 mg/kg for 1 month or 2 mg/kg for 3 months. In rats, no evidence of drug-related toxicity other than increased absolute and relative liver weight was seen in high-dose females. In a 1-month study in dogs, decreased food consumption and body weight was seen in all treated animals, but was most pronounced at high dose (4 mg/kg/day). High-dose males also exhibited tremors, salivation and ataxia; emesis was observed in high-dose females. During the two 3-month studies in dogs, no drug-related effects were seen on body weight, food consumption, vital signs, serum chemistry, hematology or urinalysis values. A dose-dependent mydriasis (pronounced or abnormal pupillary dilation) was the primary clinical observation in these treated animals; a no-effect level (NOEL) of 0.1 mg/kg/day was established for this effect.
Chronic toxicity	See Chronic effects/Carcinogenicity below.
Chronic effects/ carcinogenicity	No carcinogenic data available. However, the carcinogenic potential of a structurally related avermectin has been investigated in rodents. No evidence of carcinogenicity was seen in these studies.
Carcinogen status	Not listed as a carcinogen by IARC, NTP or US OSHA.
Reproductive effects	No reproductive effects were observed in a two-generation oral toxicity study in rats.
Teratogenicity	No evidence of drug-related maternal toxicity, embryotoxicity or teratogenicity was seen in mice or rats at doses up to 6 mg/kg. However, increased embryomortality was seen in mice at 6 mg/kg/day. Delayed developmental abnormalities were seen in the rabbit at 1.5- and 3 mg/kg/day. Also, fetotoxicity was seen in the rabbit at a maternally toxic dose 3 mg/kg/day.

SECTION 11 - TOXICOLOGY INFORMATION ... continued

At increased risk from exposure This material has been shown in rats to be excreted in milk and, as a result, to cause toxicity in young pups; nursing mothers should exercise caution regarding exposure. While it was not teratogenic, at higher doses it has also been shown to cause delayed development of rabbit fetuses; therefore, pregnant women and females planning to have a child should also exercise caution regarding exposure.

SECTION 12 - ECOLOGICAL INFORMATION

Environmental overview As with other members of the avermectin family, doramectin is highly toxic to fish and certain aquatic organisms. However, once in contact with soil, it is tightly bound and does not readily desorb. It is unlikely to reach groundwater and is also biodegradable by soil microflora.

Aquatic toxicity

<u>Compound</u>	<u>Type</u>	<u>Species</u>	<u>Dosage</u>
	EC50	Daphnia magna	0.1 ppb
	LC50-96h	Bluegill Sunfish	1.1 ppb
	LC50-96h	Rainbow Trout	5.1 ppb
	LC50	Daphnia magna	< 1 mg/L in NPDES
	LC50	Mysid Shrimp	< 1 mg/L in NPDES
	LC50	Minnow	< 1 mg/L in NPDES
	LC50	Sheepshead Minnow	1 mg/L in NPDES
	LC50	Green algae	10 mg/L in NPDES

Partition coefficient 25704 at 20 °C

SECTION 13 - DISPOSAL INFORMATION

Disposal procedure Do not dispose of even small amounts in the sanitary sewer, stormwater sewer, lakes, streams, or ponds. Incineration is the recommended method of disposal for this material. Federal, State, or Local environmental regulations and Site conditions may affect proper disposal options.

SECTION 14 - TRANSPORTATION INFORMATION

Proper shipping name	Environmentally hazardous substance, solid, n.o.s. (doramectin)
Identification number	UN 3077
General shipping instructions	Marine pollutant
IMDG hazard class	9
IMDG packing group	III

000021 - Doramectin
Rev. Supp. data: 11/00, Version 2.2.0

Printed by:
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SECTION 14 - TRANSPORTATION INFORMATION, continued

IATA hazard class	9
IATA packing group	III

SECTION 15 - REGULATORY INFORMATION

EU Classification	Toxic to Reproduction: Category 3; Dangerous for the Environment
EU Labelling	Xn; N
Risk phrases	R22 - Harmful if swallowed. R50/53 - Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. R63 - Possible risk of harm to the unborn child. R64 - May cause harm to breast fed babies.
Safety phrases	S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection. S57 - Use appropriate container to avoid environmental contamination.
TSCA status	Yes
SARA section 302	No
SARA section 313	No

SECTION 16 - OTHER

Disclaimer	Pfizer Inc believes that the information contained in this Material Safety Data Sheet is accurate, and while it is provided in good faith, it is without a warranty of any kind, expressed or implied.
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MATERIAL SAFETY DATA SHEET

SECTION 1 - IDENTIFICATION OF SUBSTANCE AND COMPANY

Pfizer Animal Health 812 Springdale Drive Exton, PA 19341	Emergency telephone 1-800-228-5635 Hours of operation 24 Hours Telephone 1-800-877-6250
Product name	Doramectin pour-on solution
Trade names	DECTOMAX® pour-on solution
Chemical family	Avermectin macrocyclic lactone
Synonyms	Not applicable (N/A)
Therapeutic use	Antiparasitic (veterinary); endectocide
Chemical name	Mixture

SECTION 2 - COMPOSITION

<u>Ingredient</u>	<u>CAS Number</u>	<u>Amount</u>
Doramectin*	117704-25-3	Proprietary
Cetearyl octanoate*	Not assigned	Proprietary
Isopropanol*	67-63-0	Proprietary

*Hazardous

Note: Ingredients indicated as hazardous have been assessed under US OSHA Hazard Communication Standard for workplace safety (29 CFR 1910.1200).

SECTION 3 - HAZARDS IDENTIFICATION

Signal word	WARNING!
Statements of hazard	FLAMMABLE LIQUID AND VAPOR. MAY BE HARMFUL IF SWALLOWED OR INHALED. MAY BE A REPRODUCTIVE HAZARD (BASED ON ANIMAL DATA). DANGEROUS FOR THE ENVIRONMENT.
Eye effects	Irritation may occur following direct contact. Symptoms might include redness, swelling, discharge, blurred vision, pain or permanent eye damage.
Skin effects	Prolonged or repeated contact may cause defatting and drying of the skin.
Inhalation effects	Inhalation of large amounts of isopropanol may be harmful. See 'Other potential health effects', below.

01703 - Doramectin pour-on solution
Revision: 04/01/00 Version: 2.0/0

Pfizer Inc.
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SECTION 3 - HAZARDS IDENTIFICATION ... continued

Ingestion effects	Harmful if swallowed. See 'Other potential health effects', below.
Other potential health effects	Signs and symptoms of isopropanol overexposure may include headache, dizziness, drowsiness, and loss of consciousness.
NOTE:	This document has been prepared in accordance with the U.S. OSHA Hazard Communication Standard, which requires the inclusion of all known hazards of the product or its ingredients regardless of the potential risk. The precautionary statements and warnings included were selected with the anticipated use of the product in mind, but may not apply in all cases. Your needs may vary depending upon the potential for exposure in your workplace. If your workplace is regulated by OSHA, this document must be used as a part of a complete hazard-training program.

SECTION 4 - FIRST AID MEASURES

Skin	Wash skin with soap and water. Remove contaminated clothing and shoes. Wash clothing and thoroughly clean shoes before reuse. If irritation occurs or persists, get medical attention.
Eyes	Immediately flush eyes with water for at least 15 minutes. Get medical attention.
Inhalation	Remove to fresh air. If not breathing, give artificial respiration. Get medical attention immediately.
Ingestion	Get medical attention immediately. Do not induce vomiting unless directed by medical personnel. Never give anything by mouth to an unconscious person.

SECTION 5 - FIRE FIGHTING MEASURES

General hazard	Flammable liquid. Vapors may form explosive mixture with air.
Fire fighting instructions	Wear approved positive pressure, self-contained breathing apparatus and full protective turn out gear. Evacuate area and fight fire from a safe distance. Dike and collect water used to fight fire.
Extinguishing media	Powder, alcohol-resistant foam, large quantities of water, carbon dioxide.
Flash point	37.4 °F
Autoignition	425 °C
Hazardous combustion products	Emits toxic fumes of carbon monoxide, carbon dioxide and oxides of nitrogen.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Small spill	Contain the source of the spill or leak if it is safe to do so. Use non-combustible material to absorb spill; then place in a suitable, labeled recovery container. Clean spill area thoroughly.
Large spill	Review Sections 3, 8 and 12 before proceeding with clean up. Contain the source of the spill or leak if it is safe to do so. Dike, pump, or use non-combustible material to absorb spill; then place in a suitable, labeled recovery container. Put saturated absorbent material into a labeled container. Close container and move it to a secure holding area.

SECTION 7 - HANDLING AND STORAGE

General handling	Eliminate possible ignition sources (e.g., heat, sparks, flame, impact, friction, electricity), and follow appropriate grounding and bonding procedures. Use only in a well-ventilated area. Do not get in eyes. Avoid contact with skin and clothing. Do not breathe vapor or mist.
Storage conditions	Store out of direct sunlight in a cool, well-ventilated dry area. Store above freezing point (-75 °F). Protect from light. Keep container tightly closed when not in use.
Temperature range for storage	-30 °C

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure limits	
<u>Compound</u>	<u>Issuer</u> <u>Type</u> <u>OEL</u>
Doramectin	Pfizer TWA 8 HR 0.2 mg/m ³
Isopropanol	ACGIH TWA 8 HR 200 ppm
	ACGIH STEL 400 ppm
	OSHA TWA 8 400 ppm
Measurement method	Doramectin: C-AM JW7-93-08 (contact Pfizer for additional details)
Ventilation	Engineering controls should be used as the primary means to control exposures. Good general ventilation should be sufficient to control airborne levels. For laboratory use, handle in a lab hood.
Respiratory protection	If the applicable Occupational Exposure Limit (OEL) is exceeded, wear an appropriate respirator with a protection factor sufficient to control exposures to below the OEL.
Eye protection	Chemical splash goggles are recommended if eye contact is possible.
Skin protection	Use protective clothing (uniforms, lab coats, disposable coveralls, etc.) in both production and laboratory areas.
Hand protection	Chemical protective gloves

000000 - Doramectin (a.i.) 500 g/kg
Revised: 06/01/2007 Version 2.0

Pfizer Inc.
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SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Flammability limits	Lower limit: 2 - Upper limit: 12
Physical form	Liquid
Color	Colorless or Blue
Clarity	Clear
Odor	Characteristic odor of isopropanol
Molecular weight	Mixture
Molecular formula	Mixture
pH	No data available
Boiling point	183 °F
Melting point	Not applicable (N/A)
Density	No data available
Vapor pressure	No data available
Water solubility	Doramectin is insoluble (25 ppb @ 25 °C)
Solvent solubility	Doramectin is freely soluble in methylene chloride or methanol, and soluble in isopropanol
Additional information	Specific gravity = 0.796 - 0.799 @ 25 °C [DECTOMAX pour-on solution]

SECTION 10 - STABILITY AND REACTIVITY

Reactivity	Stable
Conditions to avoid	Direct sunlight, heat, sparks or open flames
Incompatibilities	Strong oxidizers
Hazardous decomposition products	No data available
Hazardous polymerization	Will not occur
Explosive properties	Flammable liquid

SECTION 11 - TOXICOLOGY INFORMATION

Acute toxicity Compound

Type	Route	Species	Dosage
LD ₅₀	Oral	Rat (M)	1000 - 2000 mg/kg
LD ₅₀	Oral	Rat (F)	500 - 1000 mg/kg
LD ₅₀	Oral	Rat (M)	50 - 100 mg/kg
LD ₅₀	Oral	Rat (F)	100 - 200 mg/kg

Eye

Evidence of moderate to severe irritation was observed when isopropanol was tested in the standard Draize test at doses of 1.0 or 100 mg/24H in the rabbit.

Skin

The acute dermal LD₅₀ for isopropanol in rabbits is reported to be 12,800 mg/kg.

Inhalation

The acute LC₅₀ for isopropanol in rats is reported to be 16,000 ppm for 8-hours.

Ingestion

Acute oral LD₅₀s for the active ingredient are listed above in the table. The differences seen in the acute oral LD₅₀ ranges listed above demonstrate the effect that varying the vehicle can have on the toxic potential of doramectin. When administered to rats in an aqueous suspension, the LD₅₀ ranges were 500 - 100 mg/kg for females and 1000 to 2000 mg/kg for males. When administered in sesame oil, a non aqueous vehicle, the acute toxicity was greater with LD₅₀ ranges of 50 - 100 mg/kg for female rats and 100 to 200 mg/kg for male rats. Because the human digestive tract is primarily an aqueous environment, it may be assumed that the more relevant vehicle for assessing occupational exposure would be the aqueous suspension.

Mutagenicity

No evidence of mutagenicity was observed for doramectin when tested in vitro and in vivo in the following assays: the Ames test, the mouse lymphoma L5178Y assay and the unscheduled DNA synthesis (UDS) assay in cultures of rat hepatocytes.

Subchronic effects

Repeat dose and subchronic oral toxicity studies of doramectin were conducted in rats at doses up to 8 mg/kg for 3 months and in dogs at doses up to 4 mg/kg for 1 month or 2 mg/kg for 3 months. In rats, no evidence of drug related toxicity other than increased absolute and relative liver weight was seen in high dose females. In a 1-month study in dogs, decreased food consumption and body weight was seen in all treated animals, but was most pronounced at high dose (4 mg/kg/day). High dose males also exhibited tremors, salivation and ataxia, emesis was observed in high dose females. During the two 3-month studies in dogs, no drug related effects were seen on body weight, food consumption, vital signs, serum chemistry, hematology or urinalysis values. A dose dependent mydriasis

continued

Subchronic effects/continued	(pronounced or abnormal pupillary dilation) was the primary clinical observation in these treated animals; a no-effect level (NOEL) of 0.1 mg/kg/day was established for this effect.
Chronic effects/carcinogenicity	No carcinogenic data available. However, the carcinogenic potential of a structurally related avermectin has been investigated in rodents. No evidence of carcinogenicity was seen in these studies.
Carcinogen status	None of the components of this formulation is listed as a carcinogen by IARC, NTP or OSHA.
Reproductive effects	No reproductive effects were observed in a two-generation oral toxicity study in rats.
Teratogenicity	No evidence of drug-related maternal toxicity, embryotoxicity or teratogenicity was seen in mice or rats at doses up to 6 mg/kg. However, increased embryomortality was seen in mice at 6 mg/kg/day. Delayed developmental abnormalities were seen in the rabbit at 1.5 and 3 mg/kg/day. Also, fetotoxicity was seen in the rabbit at a maternally toxic dose 3 mg/kg/day.
At increased risk from exposure	This material has been shown in rats to be excreted in milk and, as a result, to cause toxicity in young pups; nursing mothers should exercise caution regarding exposure. While it was not teratogenic at higher doses it has also been shown to cause delayed development of rabbit fetuses; therefore, pregnant women and females planning to have a child should also exercise caution regarding exposure.

SECTION 12 - ECOLOGICAL INFORMATION

Environmental overview		As with other members of the avermectin family, DEC TOMAX is highly toxic to fish and certain aquatic organisms. However, once in contact with soil, it is tightly bound and does not readily desorb. It is unlikely to reach groundwater and is also biodegradable by soil microflora.	
Aquatic toxicity			
<u>Compound</u>	<u>Type</u>	<u>Species</u>	<u>Dosage</u>
	LC50-48h	Daphnia magna	0.1 ppb
	LC50-96h	Bluegill Sunfish	1.1 ppb
	LC50-96h	Rainbow Trout	5.1 ppb
Partition coefficient	25704 at 25 °C		

SECTION 13 - DISPOSAL INFORMATION

Disposal procedure	Do not dispose of even small amounts in the sanitary sewer, stormwater sewer, lakes, streams, or ponds. Incineration is the recommended method of disposal for this material. Treatment, storage, transportation and disposal must be in accordance with applicable Federal, State, and Local regulations.
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SECTION 14 - TRANSPORTATION INFORMATION

Proper shipping name	Flammable liquid n.o.s. (contains isopropanol)
Identification number	UN 1993
U.S. DOT hazard class	3
U.S. DOT packing group	II
U.S. DOT labeling requirements	FLAMMABLE LIQUID
IATA hazard class	3
IATA packing group	II

SECTION 15 - REGULATORY INFORMATION

EU Classification	Flammable; Toxic to Reproduction; Category 3; Dangerous for the Environment
EU Labelling	F; Xn; N
Risk phrases	R11 - Highly flammable R50/53 - Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. R63 - Possible risk of harm to the unborn child. R64 - May cause harm to breastfed babies.
Safety phrases	S16 - Keep away from sources of ignition - No smoking S36/37/39 - Wear suitable protective clothing, gloves and eye face protection. S57 - Use appropriate container to avoid environmental contamination
TSCA status	Not listed
SARA section 302	No
SARA section 313	No
Other	This product has been classified in accordance with the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR.

SECTION 16 - OTHER

Disclaimer

Pfizer Inc believes that the information contained in this Material Safety Data Sheet is accurate, and while it is provided in good faith, it is without a warranty of any kind, expressed or implied.

Appendix A - 2

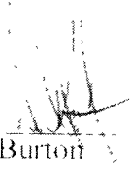
Certification of Compliance - Pour-On Solution Manufacturing Site

Pfizer Inc.
One Pfizer Way
Lee's Summit, Missouri 64086-7000
Tel: 816 820 8880

Global Manufacturing

May 2, 2002

This is to certify that when the Doramectin 0.5% Pour-On solution is produced, the Pfizer Inc plant at Lee's Summit, Missouri will be in compliance with all applicable federal, state, and local emissions and occupational safety requirements, and is expected to remain in compliance.



David A. Burton
Site Leader

Appendix B
Data Summary Charts

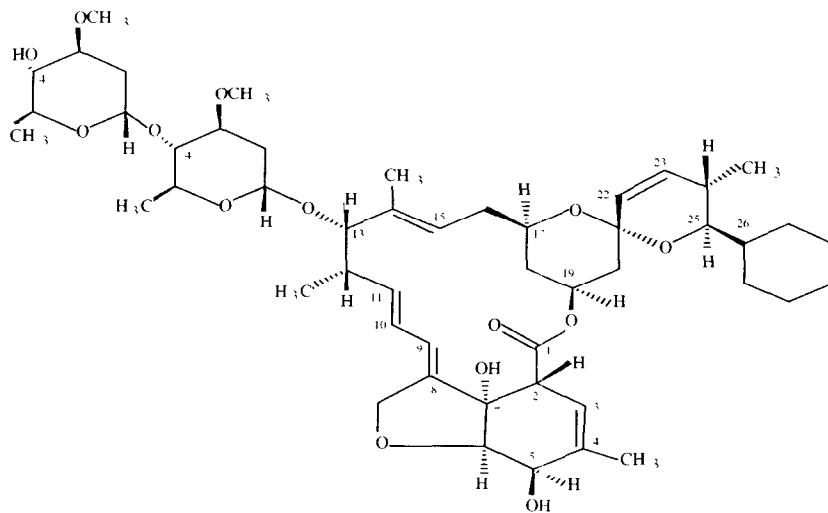
APPENDIX B

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

Fecal Sorption: Cattle feces	<u>Kd</u>	<u>Koc</u>
	15,600	34,100

Photodegradation: Half-life (hours) 4.45

Biodegradation in Soil:

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

ACUTE AND SUBACUTE TOXICITY STUDIES

TERRESTRIAL ORGANISMS

<u>ORGANISM</u>	<u>ENDPOINT</u>
Soil Microbes	Minimum Inhibitory Concentration (µg/ml)
<i>Clostridium perfringens</i>	40
<i>Aspergillus flavus</i>	600
<i>Pseudomonas aeruginosa</i>	800
<i>Nostoc</i>	60
<i>Chaetomium globosum</i>	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	
<i>Eisenia fetida</i>	28 day LC ₅₀ >1000 ppm
28 day acute study	LOEC, weight gain 4 ppm NOEC, weight gain 2 ppm

E. fetida 56 day
sublethal effects & reproductive output

sublethal effect
(delayed burrowing time)
= 17 mg/kg
NOEC = 0.89 mg/kg
(based on fecundity)

Enchytraeus albidus 42 day
sublethal effects and reproductive output

NOEC = 13 mg/kg
(based on fecundity)

Bobwhite Quail

Acute Oral LD₅₀
> 2000mg/kg

Dung Dwelling Insects

LC₉₀(ppb)

H. irritans
O. gazella

3
38.2

Effect of dung residues on viability

O. gazella

progeny production reduced by dung
residues up to 14 days

E. intermedius

progeny production reduced by dung
residues up to 14 days

P. flavolimbatus

progeny production reduced by dung
residues up to 7 days

AQUATIC ORGANISMS

<u>ORGANISM</u>	<u>ENDPOINT</u>		
	<u>LC₅₀</u>	<u>NOEC</u>	<u>LOEC</u>
Freshwater Algae	---	ND*	---
Water flea (<i>Daphnia</i>)	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

Report Summary: TISSUE DEPLETION AND EXCRETION OF
DORAMECTIN BY POUR-ON TREATED CATTLE

Study Number: 1535N-60-94-165

Test Species: Edible tissues, hide and excreta from medicated cattle

Summary of Experimental Design: Four cattle (two male castrates and two females) with a mean weight of 182.6 Kg received a single 500 µg/Kg dose of [³H] doramectin formulated in the commercial vehicle by pour-on application along the entire length of the dorsal midline. Collections of urine and feces were made over 24 hr periods beginning one day before dosing and for 14 days after dosing; feces were also collected on days 21, 35, 42, 49 and 56 days post dose. Cattle were slaughtered at 56 days for collection of liver, kidneys, *semimembranosis* muscle, the *longissimus-dorsi* muscle underlying the site of application along the midline of the back, perirenal fat and hide (with hair intact) from the entire length of the pour-on area in three horizontal strips from the dorsal mid-line to the bottom of the ribs. Two nonmedicated cattle were also slaughtered and samples of hide and edible tissues were collected for use as assay controls.

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

Summary of Results: Cattle were confined to metabolism cages for the first 14 days after dosing and total residues in feces fluctuated daily from 0.5-69 ng/g. After day 14, cattle were confined to pens except when returned to metabolism cages one day per week for collection of urine and feces. At 21 days post dose, total residues peaked at 156 and 270 ng/g for females and males respectively; by 56 days, residues had depleted to 7.4 and 3.9 ng/g for females and males respectively (Tables 1 and 2). Over 56 days, the amount of the dose excreted in feces was 36% for females and 39% for males (Table 3). Little of the dose (0.04% or less) was found in urine. The highest concentration of radiotracer on hide and hair was along the midline or site of application. In one case, the residues found on the pour-on site were 755ng/g and fell to <19ng/g within 9 inches of the midline. The amount of doramectin residues remaining on the hide and hair was estimated to be <<1% of the administered dose. Tissue concentrations of total doramectin residues at 56 days were highest in fat (17±10ng/g) and liver (9±6ng/g) followed by kidney (2.2±1.6ng/g) and muscle (0.9±0.5ng/g). Doramectin was the most abundant residue in all tissue examined. Radiotracer profiles of fecal extracts indicated that >75% of the residue was doramectin. Only one metabolite identified as doramectin de-methylated in the disaccharide portion of the molecule and accounting for approximately 10% of the radiotracer was observed.

Table 1 Doramectin residue excretion summary of pooled feces from female cattle. (Table 5, report 1535N-60-94-165)

Time Post-dose (days)	Doramectin Total Residues ng/g	Total (Kg) Feces Collected	Excretion Rate mg drug per day
1	7.01	11.96	0.0838
2	24.4	15.61	0.381
3	37.0	14.86	0.550
4	29.6	16.11	0.447
5	34.3	14.80	0.508
6	27.7	19.30	0.535
7	31.6	14.94	0.472
8	27.0	19.80	0.535
9	35.6	22.56	0.803
10	30.7	21.17	0.650
11	19.0	21.42	0.407
12	34.4	21.28	0.732
13	46.2	16.75	0.774
14	52.6	18.83	0.990
21	156.0	16.16	2.52
35	54.8	19.79	1.08
42	50.8	21.20	1.08
49	20.8	23.08	0.480
56	7.40	19.32	0.143

Table 2 Doramectin residue excretion summary of pooled feces from male cattle. (Table 6, report 1535N-60-94-165)

Time Post-dose (days)	Doramectin Total Residues ng/g	Total (Kg) Feces Collected	Excretion Rate mg drug per day
1	0.46	13.64	0.00627
2	14.1	14.34	0.202
3	59.1	13.93	0.823
4	68.8	13.30	0.915
5	43.0	17.51	0.753
6	33.8	19.49	0.659
7	24.9	19.03	0.474
8	17.4	17.61	0.306
9	19.8	17.77	0.352
10	17.7	16.22	0.287
11	15.5	16.27	0.252
12	18.3	14.10	0.258
13	21.9	13.86	0.304
14	44.2	13.83	0.611
21	270.0	16.93	4.57
35	52.0	22.34	1.16
42	23.2	26.93	0.625
49	13.7	21.82	0.299
56	3.9	31.37	0.122

Table 3 Dose material balance feces. (Table 4, report 1535N-60-94-165)

Pooled	mg Male Dose	mg Female Dose
Total doramectin administered	195	175
Total dose excreted	76	63
Percent of dose excreted	39%	36%

Water Wash off of Doramectin from Pour-On Treated Cattle

Report Summary: WATER WASH-OFF OF DORAMECTIN FROM POUR-ON TREATED CATTLE

Study Number: 1535N-60-94-164

Test Species: Wash-off from medicated cattle

Summary of Experimental Design: Four female cattle with a mean weight of 179.2 Kg received a single dose of 500 µg/Kg [³H] doramectin formulated in the commercial vehicle by pour-on application along the entire length of the dorsal midline. Three hours after dosing, animals were placed individually in metabolism cages and 12 L of tap water was evenly sprayed over the backs of each animal for a period of 20 minutes. After a further 15 minutes, cattle were removed from the cages, water was collected and cages were each rinsed with 1 L of 95% ethanol which was also collected for assay.

Summary of Results: Water samples were diluted with THF to prevent the adhesion of doramectin to flasks or pipette surfaces. Water and ethanol samples were analyzed by liquid scintillation counting for [³H] content. Of the 85-95 mg of doramectin applied to each animal, between 4.5-11 mg was recovered in the water and ethanol washes, indicating that a mean of 8.5% of the dose (5.3-12.8%) was washed off when cattle were exposed to a simulated 20 minute rainfall 3 hours after the dose was applied.

Effects of Doramectin Pour-On on Three Species of Dung Inhabiting Insects

Report Summary: EFFECTS OF DORAMECTIN POUR-ON ON THREE SPECIES OF DUNG INHABITING INSECTS

Study Number: 1430C-60-95-212

Test Species: *Euoniticellus intermedius* and *Onthophagus gazella* (dung beetle), *Philonthus flavolimbatus* (predatory beetle)

Summary:

A study was conducted to evaluate the insecticidal persistence in dung of doramectin administered topically to cattle at a dosage of 500 µg/kg (1 mL/10 kg) against two species of dung burying Scarabaeidae: *Euoniticellus intermedius* and *Onthophagus gazella*, and the predaceous Staphylinidae: *Philonthus flavolimbatus*. Ten cattle were randomly allocated to a saline- or a doramectin-treated group (each of 5 animals) in a tiered manner based on day -7 body weights. Bioassays were conducted in the laboratory on feces collected from each animal weekly for eight weeks following treatment for *E. intermedius* and *O. gazella*, and for six weeks for *P. flavolimbatus*. For all three beetles species, exposure to dung from saline- or doramectin-treated animals had no effect on viability or mating of breeding pairs of beetles. Brood ball production by the scarab beetles was not significantly different between groups at any time posttreatment. For *E. intermedius* and *O. gazella*, there were significantly fewer progeny produced by beetles exposed to dung from doramectin-treated cattle at days 7 and 14 ($P < 0.0280$). For *P. flavolimbatus*, there were significantly fewer progeny produced by beetles exposed to dung from doramectin-treated cattle at day 7 ($P = 0.0009$). There was no significant difference in progeny counts for scarab beetles at days 21, 28, 35, 42, 49 and 56, and for predacious beetles at days 14, 21, 28 and 35, suggesting that any excreted residues at these times were below lethal concentrations.

Table 1. Number of progeny of *Euoniticellus intermedius*, recovered from dung of saline- or doramectin-treated cattle. Means and ranges from 5 animals per treatment.

Days Post-dose	Number of Animals	Saline-treated Cattle		Doramectin-treated Cattle		P. Value
		Mean	Range	Mean	Range	
0	5	17	0-35	22	11-30	0.3902
7	5	25	19-31	1	0-3	0.0001
14	5	27	10-48	14	0-24	0.0280
21	5	22	16-29	16	10-20	0.3194
28	5	16	7-29	22	11-28	0.3363
35	5	20	11-26	20	12-35	0.9176
42	5	32	26-39	25	15-38	0.2578
49	5	33	28-36	26	19-32	0.2723
56	5	28	19-39	28	1-47	0.9176

Table 2. Number of progeny of *Onthophagus gazella*, recovered from dung of saline- or doramectin-treated cattle. Means and ranges from 5 animals per treatment.

Days Post-dose	Number of Animals	Saline-treated Cattle		Doramectin-treated Cattle		P. Value
		Mean	Range	Mean	Range	
0	5	16	9-33	11	2-17	0.4976
7	5	44	11-56	0	0	0.0001
14	5	29	14-44	2	0-7	0.0005
21	5	8	0-20	7	0-22	0.8919
28	5	34	20-48	27	2-42	0.3037
35	5	52	27-66	55	43-64	0.7650
42	5	36	12-53	35	26-43	0.9566
49	5	14	9-22	27	14-54	0.0956
56	5	43	32-53	29	13-42	0.0722

Table 3. Number of progeny of *Philonthus flavolimbatus*, recovered from dung of saline- or doramectin-treated cattle. Means and ranges from 5 animals per treatment.

Days Post-dose	Number of Animals	Saline-treated Cattle		Doramectin-treated Cattle		P. Value
		Mean	Range	Mean	Range	
0	5	17	3-24	17	11-25	0.8722
7	5	18	13-25	0	0	0.0009
14	5	10	0-20	4	1-8	0.1886
21	5	10	0-26	18	10-28	0.1215
28	5	18	5-24	21	0-33	0.4953
35	5	21	8-30	16	13-21	0.2634
42	5	21	3-35	33	22-38	0.0232

Acute Oral Toxicity of Doramectin in Bobwhite Quail

Report Summary: ACUTE ORAL TOXICITY (LD₅₀) OF DORAMECTIN IN BOBWHITE QUAIL

Study Number: PFZ 537

Test Species: Bobwhite Quail (*Colinus virginianus*) male and females 182-207 g body weight

Summary of Experimental Design: Treatment groups consisted of 5 male and 5 female young adults aged at least 16 weeks and between 182 and 207 g body weight. Birds were housed by sex in tiered cages and received a single oral dose of doramectin suspended in corn oil by intubation at either 500, 1000 or 2000 mg/kg. Aliquots of dosing samples were assayed immediately after preparation to determine homogeneity and concentration of doramectin. Birds were observed daily for 14 days after dosing and any mortality or clinical signs were recorded. Weight gain and feed consumption were determined at weekly intervals.

Summary of Results: Assay of dosing suspensions indicated that doramectin was homogeneously distributed in the vehicle and doses administered were within 98% of nominal concentrations. There were no mortalities. Clinical signs of toxicity, including subdued behavior and unsteadiness, were observed in one bird each at 500 and 1000 mg/kg and in two birds at 2000 mg/kg. Slight weight loss was observed in females dosed at 1000 mg/kg and in both sexes at 2000 mg/kg for the first week after dosing. Otherwise, body weight changes were no different from controls. Food consumption was slightly reduced in males receiving 2000 mg/kg for the first week after dosing. Otherwise, food consumption was no different from controls. Males and females receiving 2000 mg/kg doramectin were necropsied at 14 days post-dose along with controls and no abnormalities were detected by macroscopic examination.

Results indicated that the acute oral LD₅₀ value of doramectin for the Bobwhite quail lies in excess of 2000 mg/kg.

An Acute Dermal Irritation Study in Albino Rabbits

Report Summary: AN ACUTE DERMAL IRRITATION STUDY IN ALBINO RABBITS

Study Number 95 - 657- 30

Test Species Albino rabbit (New Zealand White)

Summary of Experimental Design: Two male and two female adults with bodyweights ranging from 3.96 - 4.28 kg were housed individually in stainless steel wire cages. Hair on the back of each rabbit was removed with an electric clipper and 0.5 ml doses were applied to 1 inch square gauze pads which were held in continuous contact with unabraded skin for 4 hours. Each rabbit was exposed to the pour-on formulation containing the ingredients listed on p. 6 of the EA as well as to pour-on formulation not containing dye and to a dye-containing placebo (vehicle) solution. Rabbits were observed daily for clinical signs of systemic toxicity and for changes in appearance or behavior and their food consumption was evaluated. Individual body weights were recorded prior to dosing and prior to euthanasia on day 4. At 1, 2, 4, 48 and 72 hours after exposure, each application site was examined for any gross changes and the degree of erythema and edema was assessed according to the Draize System (Scale of 0 - 4)

Summary of Results: No clinical signs of toxicity were noted in any of the rabbits and there was no effect on body weight. Very slight erythema but no edema was noted at one hour following exposure to both pour-on formulations (dye containing and dye absent) and placebo. Erythema subsided completely within 1 - 2 days from most sites but very slight erythema remained present at several sites at study termination. Additionally some superficial fissuring of the skin became apparent at 1 - 2 sites receiving either doramectin-containing formulation or the placebo 2 - 3 days after dosing.

<u>Treatment</u>	<u>Time After Application (hr)</u>	<u>Mean Value (0 - 4)</u>	
		<u>Erythema</u>	<u>Edema</u>
Dye-Containing Doramectin Solution	1	1.0	0.0
	24	0.75	0.0
	48	0.50	0.0
	72	0.25	0.0
Dye Free Doramectin Solution	1	1.0	0.0
	24	0.75	0.0
	48	0.50	0.0
	72	0.50	0.0
Dye-Containing Placebo Solution	1	1.0	0.0
	24	0.5	0.0
	48	0.25	0.0
	72	0.25	0.0

Eisenia fetida, Sublethal Effects And Reproductive Output

Study Summary

An Assessment of the Effects of Doramectin on the Reproductive Output and Other Sub-lethal End Points of the Earthworm *Eisenia fetida*
Pfizer Protocol Number 2409A-60-01-022
(T R Wilbury Study Number : 2183-PF)

An assessment of the effects of doramectin on the reproductive output and other sublethal endpoints of the earthworm, *Eisenia fetida* was conducted. The test system was artificial soil (70% sand, 20% kaolin clay, 10% finely ground sphagnum peat moss). Each treated or control sample consisted of a 0.5 gallon glass jar containing 655 g hydrated artificial soil (equivalent to 505 g dry weight) to which ten adult earthworms (mean weight 537 mg/adult worm) were added.

The worms were exposed to a geometric series of seven test concentrations of doramectin (94.3% active ingredient) and a negative control (artificial soil). The nominal concentrations of doramectin were 0.50, 0.89, 1.6, 2.9, 5.1, 9.3 and 17 mg a.i./kg on a dry weight basis. Eight control replicates and four replicates per treatment were tested.

The treated and control samples were incubated for 56 days, at temperatures ranging from 19.1 to 22°C, and illuminated with artificial light on a 16 hours light/8 hours dark cycle and a light intensity of approximately 780 lux. During the study moisture content of the doramectin-treated samples ranged from 23-26 %, and the pH of the samples ranged from 5.3 - 6.1.

A 56-day soil toxicity test was concurrently conducted using carbendazim (97%) as a reference toxicant. Nominal concentrations of carbendazim were 0.099, 0.99 and 9.9 mg a.i./kg tested in duplicate. The test was conducted in a manner identical to the doramectin test with which it shared the controls (separate untreated controls were not prepared). During the course of the study the moisture content of the carbendazim-treated samples ranged from 21-25% and the pH from 5.5 - 6.0.

At Day 1 and at weekly intervals thereafter up to Day 28 (the end of the adult exposure phase), the worms were fed with approximately 5 grams of dried ground horse manure. The manure was spread on the surface of each soil in each test vessel and moistened with 5 ml of water.

The number of adult worms and sublethal effects (inability to burrow, immobility, open wounds, color change etc) were determined after 28 days exposure and the weight of adult earthworms was determined on Days 0 and 28. The number of juvenile worms produced was determined after 56 days of exposure.

Survival of adult worms exposed to seven doramectin concentrations ranged from 93-100% after 28 days of exposure. All surviving adult worms in control samples and those exposed to 0.50, 0.89, 1.6, 2.9, 5.1, and 9.3 mg/kg doramectin burrowed into the soil within 15 minutes on Day 0 and Day 28. Adult earthworms exposed to 17 mg/kg burrowed into soil within 15 minutes at Day 0 but required 25 to > 30 minutes to burrow on Day 28. No other sublethal effects were observed during the test. Adult worm weight loss averaged 29% in the controls and 15-36% in the doramectin-treated samples after 28 days. At the conclusion of the test (Day 56) juvenile production in the control and at 0.50, 0.89, 1.6, 2.9, 5.1, 9.3 and 17 mg/kg averaged 239, 228, 220, 165, 74, 4 and <1 and 1 juveniles, respectively. Exposure of adult earthworms to doramectin resulted in a no observed effect concentration (NOEC) of 0.89 mg a.i./kg based on fecundity data (the number of juveniles produced).

Results of the 56-day soil toxicity test conducted with the reference toxicant carbendazim (97%) at nominal concentrations of 0.099, 0.99 and 9.9 mg /kg showed that after 28 days exposure there was 100% survival of adult earthworms at all concentrations tested. All surviving adult worms burrowed into soil within 15 minutes on Day 0 and Day 28. Adult worm weight loss averaged 25 - 35% after 28 days in the treated samples and 29% in controls. At Day 56, juvenile production at 0.099, 0.99 and 9.9 mg/kg averaged 209, 161 and 26 juveniles respectively as compared to 239 in the controls. The NOEC for earth worms exposed to carbendazim was 0.099 mg a.i./kg based on the number of juveniles produced.

Enchytraeus albidus, Sublethal Effects And Reproductive Output

Study Summary

An Assessment of the Effects of Doramectin on the Reproductive Output and Other Sub-lethal End Points of the Enchytraeid worm *Enchytraeus albidus*
Pfizer Protocol Number 2409A-60-01-021
(T R Wilbury Study Number : 2191-PF)

An assessment of the effects of doramectin on the reproductive output and other sublethal endpoints of the enchytraeid worm, *Enchytraeus albidus* was conducted. The test system was artificial soil (70% sand, 20% kaolin clay, 10% finely ground sphagnum peat moss). Each treated or control sample consisted of a 250 mL beaker containing 24.5 g hydrated artificial soil (equivalent to 20 g dry weight) to which ten worms (length approximately 10.4 mm/worm) were added.

The worms were exposed to a geometric series of six test concentrations of doramectin (94.4% active ingredient) and a negative control (artificial soil). The nominal concentrations of doramectin were 13, 24, 43, 77, 140 and 250 mg a.i./kg on a dry weight basis. Eight control replicates and four replicates per treatment were tested. One additional sample without worms was prepared for the control and each test concentration and used to monitor pH and moisture at the start and at the end of the test (Day 42). During the study soil moisture content was maintained at approximately 20%, and the pH of the samples ranged from 5.9 - 6.4.

A 42-day soil toxicity test was concurrently conducted using carbendazim (97%) as a reference toxicant. Nominal concentrations of carbendazim were 0.10, 1.0 and 10 mg a.i./kg tested in duplicate. The test was conducted in a manner identical to the doramectin test with which it shared the controls (separate untreated controls were not prepared). During the study soil moisture content ranged from 18-20% and the pH of the samples ranged from 6.0 - 6.2.

The treated and control samples were incubated for 42 days, at a temperature of $20 \pm 2^{\circ}\text{C}$, and illuminated with artificial light on a 16 hours light/8 hours dark cycle and a light intensity of approximately 660 lux. The worms were fed 50 mg of finely ground rolled oats at Day 0 and 25 mg on Days 7, 14, 21 and 35. The number of adult worms and sublethal effects (inability to burrow, immobility, open wounds, color change etc) were determined after 21 days exposure and the number of juvenile worms produced was determined after 42 days of exposure.

After 21 days of exposure to doramectin, average adult survival was 99, 98, 98, 98, 98, 95, and 83% at 0 (control), 13, 24, 43, 77, 140 and 250 mg /kg. No sublethal effects were observed during the test. At the conclusion of the test (Day 42) juvenile production in the 0 (control), 13, 24, 43, 77, 140 and 250 mg /kg averaged 57, 43, 32, 30, 15, 2 and <1 juveniles, respectively.

Based on fecundity data (the number of juveniles produced) exposure of *Enchytraeus albidus* to doramectin resulted in a no observed effect concentration (NOEC) of 13 mg a.i./kg.

After 21 days of exposure to carbendazim there was 90 - 100% survival of adult worms at all tested concentrations. At Day 42, juvenile production at 0.10, 1.0 and 10 mg/kg averaged 27, 18 and 5 juveniles, respectively as compared to 57 in the controls. The NOEC and EC50 for worms exposed to carbendazim was < 0.10 mg a.i./kg based on the number of juveniles produced.