Environmental Assessment

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

Pfizer Inc

August 1996

ENVIRONMENTAL ASSESSMENT

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1. DATE: August 2, 1996

2. APPLICANT: Pfizer Inc
(Sponsor #000069)

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

4. DESCRIBE THE PROPOSED ACTION:

A. Requested Approval and Need for the Action

Pfizer Inc is filing a New Animal Drug Application requesting approval for the use of doramectin 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 500 µg 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*, 15S, 17a*R*, 20*R*, 20a*R*, 20b*S*)-6'-cyclohexyl-5',6.6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20.20b-dihydroxy-5',6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*, 13*H*, 17*H*-furo-[4,3,2-[*pq*][2.6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-7-yl 2.6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-*a*-*L*-*arabino*-hexopyranosyl)-3-*O*-methyl-*a*-L-*arabino*-hexopyranoside

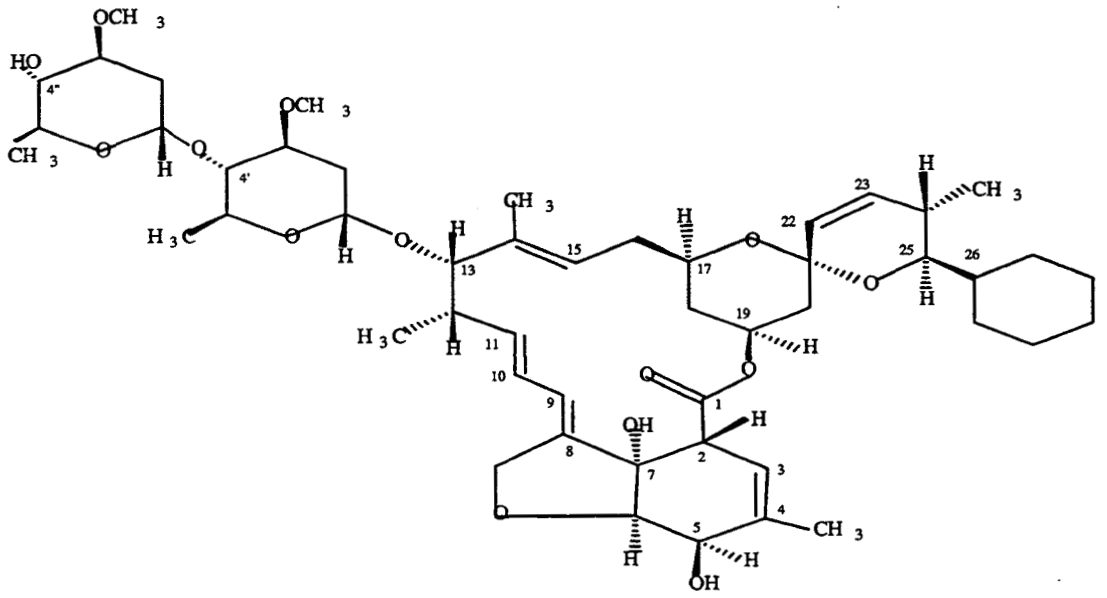
CAS Registry Number: 117704-25-3

Pfizer Code Number: UK-67,994

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

Molecular Weight: 899.13

Structural Formula:



B. Other 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 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 exposure limit set by Pfizer.

C. Introduction of Substances as a Result of Use

1. Doramectin Administration to Cattle

Doramectin will be administered to both pastured and feedlot cattle. Since the latter represent a denser population, they will be used to estimate upper limits for the amount and concentration of doramectin introduced into the environment. The average amount of drug administered to a single animal can be estimated as follows. Feedlot cattle will most commonly be treated shortly after arrival at the feed lot. Assuming the average body weight of 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, 28, 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 kg wet waste/day x 130 days = 3510 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:

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

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 1.5×10^3 kg/m^3, 15 cm of soil weighs about 9.1×10^5 kg/acre; therefore, using an average rate of incorporation of 15 tons (13.6 metric tons) manure/acre/year, use of aged manure containing 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

It is estimated that use of doramectin pour-on formulation for the therapy of parasitic infections of cattle could result in up to approximately 1.7 metric tons of doramectin being introduced into the environment

annually. This estimate is based on the amount of drug needed to medicate a single animal and the number of animals likely to be medicated over the period of a year.

The 1994 USDA survey indicates that approximately 34.9 x 10^6 beef cows and approximately 31.3 x 10^6 calves and stockers were on pasture and approximately 25 x 10^6 cattle were processed through feedlots. Use tracking survey information (Doane, 1992-1995) was reviewed for the southern US to determine the total number of cattle treated with ivermectin and the proportion dosed with the pour-on formulation (see also Section 6.C.7.b). Survey information indicates that during the second and fourth quarters of the year as many as 20% of the cattle on pasture were treated with ivermectin while as many as 10% of the population were treated in each remaining quarter. Across the southwestern and southeastern U.S. respectively, pour-on accounted for 45-50% and about 65% of total ivermectin usage per year during 1994 and 1995. Assuming conservatively that pour-on represents 65% of total ivermectin usage and that ivermectin use is a close proxy of the maximum doramectin pour-on usage and further assuming a similar rate of treatment across the entire U.S., approximately 13.7 x 10^6 beef cows and 12.4 x 10^6 calves and stockers would be treated over the entire year. Furthermore, if as many as 25% of feedlot cattle were treated with ivermectin, 65% of these would receive pour-on; therefore, an additional 4 x 10^6 cattle would be treated over the entire year. Assuming a dose level of 0.5 mg/kg and average body weights for beef cows, calves/stockers and feedlot cattle at time of treatment of 432 kg, 145 kg and 300 kg, respectively, animals would receive 216, 72.5 and 150 mg doramectin, respectively. Therefore, treatment of the number of cattle indicated above would result in use of approximately 4460 kg doramectin:

216 mg/beef cow x 13.7 x 10^6 beef cows = 2.959 x 10^9 mg or 2959 kg

72.5 mg/calf-stocker x 12.4 x 10^6 calves-stockers = 8.99 x 10^8 mg or 899 kg

150 mg/feedlot cow x 4 x 10^6 feedlot cattle = 6.0 x 10^8 mg or 600 kg

Total = 4458 kg

Since only about 38% of the administered doramectin is excreted, treatment of these numbers of cattle would result in excretion of approximately 1700 kg of doramectin:

4458 kg x 0.38 = 1694 kg or approximately 1.7 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 is the only avermectin approved for use in the U.S. It is believed that introduction of doramectin would be unlikely to increase overall usage of avermectins. Thus, it is assumed that current ivermectin usage is a close proxy of the maximum doramectin usage. Survey conclusions follow.

1) Regional Survey: 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.

2) Local Surveys: Surveys of large practices in Texas and Florida indicates that considerable deworming activities take place during the calving periods of March-May and September-November. Based on veterinary testimony, operators treat nearly all their cattle at one time to a maximum rate of 200-250 per day. Also based on veterinary testimony and daily sales (and presumably use), treatment of local herds under the care of a single veterinarian occurs throughout the 3 month period rather than in a more compressed time period. In Matagorda county Texas, 19 operators (4%) purchased sufficient ivermectin over 90 days to treat 27% of the county beef cow population. In contrast, in two Florida counties surveyed, 4-5% of the operators purchased sufficient ivermectin over 90 days to treat 2-3% of their respective counties beef cow populations. The survey suggests that herds in adjacent pastures would not be treated simultaneously; therefore, over a season, the total number of pats containing residues in a pasture or adjacent pastures would be a small percentage of total pats. Pats containing residues at concentrations likely to impact dung beetle progeny based on the results of a bioassay study (see 8.A.6) would be limited to areas traversed by treated herds for the first 1-2 weeks post dose.

Usage tracking survey information (Doane, 1992-1995) for the southwest and southeast regions of the US was reviewed to determine from the number of cattle treated with ivermectin, the proportion dosed with the pour-on formulation. During 1994 and 1995, pour-on accounted for 45-50% of total ivermectin usage in the southwest region and for approximately 65% of ivermectin usage in the southeast region over the two year period. On a quarterly basis, pour-on usage was fairly constant in both regions except for 3Q95 in the Southwest where it increased from about 40% to 67%. In the Southeast, pour-on usage increased during 4Q94 to 74% but returned to 60-67% the next 4 quarters.

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 4 million feedlot cattle annually with pour-on (Section 6.C.7.a) would produce 5.2 million tons of aged waste containing residues:

1.3 tons/animal/year x 4 million animals = 5.2 million tons/year

At an application rate of 13.6 metric tons of aged manure per acre, this manure would be dispersed over about 3.82×10^5 acres:

5.2 million tons ÷ 13.6 tons/acre = 3.82×10^5 acres

Medication of 13.7 million beef cows and 12.4 million calves and stockers on pasture annually with pour-on (Section 6.C.7.a) would expose a total of 117.5 million pasture acres to residues in dung pats, assuming a stocking density of 9 acres/cow-calf pair or 4.5 acres per animal (finding of local survey reported in the doramectin injectable EA, NADA 141-061):

26.1 million pasture cattle x 4.5 acres per animal = 117.5×10^6 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 ± 0.01°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-photodegradate 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% $^{14}CO_2$ 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 mg/kg]/15,600 = 3.0×10^{-6} 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:

Amount removed = (volume of runoff per acre)(concentration in runoff)
= (1/12 acre-ft)(1.233×10^6 L/acre-ft)(3.0×10^{-6} mg/L) = 0.31 mg

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

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

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

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

If the initial concentration of doramectin in manure-amended soil is 0.81 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 x 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:

Number of successive reapplications	Concentration (ppb) of doramectin residues in soil
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), $C_w = (8.4 \times 10^{-4}$ mg/kg)/289 = 2.9×10^{-6} 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 m^2/acre = 8094 m^3 x 1000 L/m^3 = 8.1×10^6 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 = 8.1×10^6 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 = 1.5×10^3 kg/m^3, therefore:

$m = [0.05\ m \times 1\ acre \times (4047\ m^2/acre)] \times (1.5 \times 10^3\ kg/m^3) = 3 \times 10^5$ 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:

$C_w = A_w/V = 1.93\ mg/(8.1 \times 10^6 L) = 2.4 \times 10^{-7}$ mg/L or 0.24 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 mg/73.4 mg x 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), θ = 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 cm x R_f), consistent with the results obtained. To extrapolate to field conditions, if half the volume from a 25.4 cm (10 in) rainfall percolates to the water table, the applied doramectin will move only 0.17-0.29 cm (0.5 x 25.4 cm x R_f); even 10 times this amount of rainfall (i.e., 100 inches) would not lead to significant movement of doramectin though the soil.

Given the low concentration of doramectin in soil following repeated application of cattle feedlot manure (0.84 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:

Compartment	Maximum Expected Concentration	EA Section
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:

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

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

Perennial ryegrass was the most sensitive of the 6 species exposed to doramectin, with an NOEC of 1.6 mg A.I./kg and an LOEC of 3.3 mg A.I./kg, based on the effects observed on root elongation.

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. Earthworms

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

5. Immature Dung Beetles and Horn Flies

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

6. 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 Scarabaedae, *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.

7. 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 autumalis* were reduced in pats from treated cattle. Predatory beetles, primarily larval *Sphaeridium* spp. and adult *Staphylinidae* were also reduced at 4 days but not at 28 days.

8. 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 EC_{50} concentrations and NOECs are as follows:

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

3. Bluegill Sunfish

Acute toxicity of doramectin for bluegill sunfish (*Lepomis macrochirus*) was measured under static conditions. The 96 hour LC_{50} is 11 ppb and the NOEC is 2.3 ppb.

4. Rainbow Trout

Acute toxicity of doramectin for rainbow trout (*Onchorhynchus mykiss*) was measured under static conditions. The 96 hour LC_{50} is 5.1 ppb and the NOEC is 2.5 ppb.

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

1. Terrestrial Species

a. Soil Dwelling

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 accounts for the very small residual amount of drug that may remain from previous annual fertilizations. This maximum predicted concentration in soil is not expected to have an adverse effect on non-target, soil dwelling terrestrial species. Minimum inhibitory concentrations of doramectin were 40 ppm or above for soil microorganisms tested, nearly 5×10^4 times the soil PEC. The NOEC for earthworms was 2 ppm, a level that exceeds the soil PEC by 2.4×10^3 times; no lethal effects were observed for earthworms at concentrations up to 1000 ppm, 1.2×10^6 times the soil PEC. 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. Dung Dwelling

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 autumalis* were reduced in pats from treated cattle. Predatory beetles, primarily larval *Sphaeridium* spp. and adult *Staphylinidae* were also reduced at 4 days but not at 28 days, probably due to the absence of flies upon which they feed at the early time point rather than any drug effect.

Ecology of Dung Beetles in the U.S.: Concern has been expressed that use of avermectins in pasture cattle in the U.S. may adversely affect dung 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) describes the ecology of dung beetles, e.g. geographic and temporal distribution, mobility, dung preference and breeding period. 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 potential use survey). The conclusions of the ecology study are reiterated below. They will be used in developing a hazard assessment concerning use of the pour-on for treatment of pastured cattle.

Conclusions: Species of dung beetles native to the U.S. will not be threatened by use of doramectin in pastured cattle, and therefore need not be included in the hazard assessment. This is principally because

native species do not appear to be dependent upon cattle dung as an exclusive food source. Moreover, the habitat of many native species is widespread and includes regions of the country with relatively few pastured cattle, e.g. the northeastern states. Also, the breeding period of most native species extends from Spring through Fall and is not necessarily limited nor coincidental with periods of high drug use. Taken collectively, considerable segments of the native population would not encounter residues and attendant survival risks because they either do not feed on cattle dung or their reproductive period includes times of the year when fewer cattle are excreting residues. Beetle populations not exposed to residues would compensate for any decrease in reproductive potential among native populations feeding on dung from treated cattle.

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

Potential Effects of Doramectin Treatment on Dung Degradation: Concern has been raised, i.e. Strong, 1992, that treatment of cattle with avermectins (such as doramectin) might delay the degradation of dung pats on pasture due to the insecticidal activity of residues excreted in dung. 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). Conclusions from this literature review are summarized below. They will be considered in relationship to doramectin exposure resulting from pour-on administration in the hazard assessment that follows.

Conclusions: Larval development of dung dependent dipteran and coleopteran species is impacted to varying degrees by avermectin treatment of cattle. Studies in which dung was collected following avermectin treatment, formed into artificial pats and placed on pasture for insect colonization were useful in determining the relative sensitivity of insect groups to avermectins and the duration of insecticidal activity exerted by various drug formulations. In general, larval development of cyclorrhaphan diptera was inhibited for the longest period of time; nematoceran diptera, scarabaeinan beetles and aphodian beetles were

impacted for decreasing periods of time in that order. Studies in which known concentrations of ivermectin were added to formed pats provided only limited additional information. For example, ivermectin concentrations between 0.5-2 ppm had no effect on aphodids but markedly reduced fly larvae. In another study, scarabaeidan larvae (presumably aphodids) were unaffected by 0.125 ppm ivermectin, while larval development was inhibited by 0.25 and 0.5 ppm drug. Where two or more dosage forms were compared in the same study, the bolus inhibited development for the longest period of time followed by the injectable formulation. The pour-on formulation was inhibitive for the least amount of time. The persistence of avermectin in excreted dung pats appears to be influenced by climate with drug disappearing most rapidly under hot, dry conditions. Since insects colonize pats immediately after defecation and find them much less attractive after 1-2 days, the persistence of drug in the dung probably has little impact on pat colonization except for beetle species that have been observed to preferentially colonize dung from avermectin treated cattle.

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

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

Hazard Assessment: This section considers whether or not the use of doramectin pour-on in pastured cattle threatens exotic dung beetles that have been introduced into the southern United States. The doramectin injectable EA (NADA 141-061) presented a similar hazard assessment concerning use of doramectin injectable in pastured cattle. This assessment considers information provided in previous sections as follows: 1) the toxicity of doramectin for dung beetles, specifically, the

EC_{50} and EC_{90} of doramectin for the introduced species, *O. gazella* (Section 8.A.5) and effects on 2 species of dung beetles (*O. gazella* and *E. intermedius*) and one species of predatory beetle (*P. flavolimbatus*) exposed to feces of cattle administered the pour-on (Section 8.A.6), 2) the excretion of doramectin by cattle following administration of the pour-on formulation (Section 6.C.2), 3) the ecology of dung beetles (Section 8.C.1.b), and 4) the spatial and temporal introduction of doramectin residues into the southern U.S., regionally and locally (Section 6.C.7.b).

1) <u>The Toxicity of Doramectin and Excreted Residues for Dung Beetles:</u>

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

Adult pairs of *O. gazella, E. intermedius* and *P. flavolimbatus* were exposed to cattle feces 7, 14, 21, 28, 35, 42 and 56 days after cattle were treated with doramectin pour-on at 0.5 mg/kg. No effects were observed on either viability of adults or upon mating or brood ball production. Numbers of *O. gazella* and *E. intermedius* progeny recovered from feces collected 7 and 14 days after treatment were significantly reduced compared to the saline control. *P. flavolimbatus* progeny were reduced only when exposed to feces collected at 7 days.

Several bioassays have been published for other avermectins but only for injectable rather than pour-on formulations. A study conducted in the US determined the impact of ivermectin administered at 0.2 mg/kg against the same three beetle species (Fincher, 1992). Results were identical to those obtained with doramectin pour-on. 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). In western Australia, (Ridsdill-Smith, 1988), dung collected from cattle treated with abamectin at 0.2 mg/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 levamesol 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.

2) Doramectin Excretion by Cattle Following Pour-on Administration:

Following administration of tritiated doramectin pour-on to cattle at 0.5 mg/kg, the concentration of total residues in pooled feces of male and female cattle exceeded the EC_{90} for *O. gazella* for 35-42 days post dose. However, results of the more direct bioassay study described above indicated that residues were excreted at concentrations sufficient to impact dung beetle development for only 1-2 weeks post dose; beetles exposed to feces collected 3-8 weeks post dose exhibited no adverse effects. A recently published 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 as utilized for the doramectin radiotracer excretion study voided lower volumes of feces containing higher apparent residue concentrations than cattle fed a high roughage diet, as in the case of the doramectin bioassay study where cattle received only alfalfa cubes and water.

3) The Ecology of Dung Beetles:

Many species of dung beetle native to the U.S. were precluded from the hazard assessment because they do not utilize cattle feces as a food source to any significant extent and, therefore, would not be exposed to drug. This would include most members of the subfamily Aphodiinae and nearly all Geotrupinae. Among the former, only a group of 11-12 *Aphodius* spp. accidentally introduced from Europe are commonly found in cow dung. However, these species would not be threatened because they are very broadly distributed throughout the U.S. including regions such as the northeast where only modest numbers of beef cattle are reared on pastures. If local beetle populations were disrupted, their rapid spreading rate (as recently documented by Lobo, 1994) would ensure repopulation of depleted areas. Moreover, studies in which doramectin was administered to pastured cattle (see doramectin injectable EA [NADA 141-061]) suggest that aphodids are not very sensitive to doramectin. Studies where ivermectin was administered to pastured cattle suggest the same thing (Madsen et. al., 1988 and 1990; Strong and Wall, 1994; Sommer et. al., 1992). For example, dung collected from cattle treated 4 days earlier with doramectin had no effect on numbers of aphodid adults or larvae. Dung collected from cattle treated with ivermectin inhibited larval development for only 1-2 days except for one study where inhibition for 1-2 weeks was reported.

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

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

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

4) Spatial and temporal Introduction of Doramectin Residues into the Southern US Regionally and Locally as a Consequence of Pour-on Administration:

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

The regional survey indicated that from 1992-1994, no more than 20% of the total pastured cattle population were treated with ivermectin per quarter in the southeastern or southwestern regions, respectively. Based on 1994-95 survey data, pour-on accounted for a maximum of 65% of total ivermectin usage; therefore, no more than 13% of the pastured cattle population received ivermectin pour-on per quarter. Viewed conversely, at least 87% of the total population from either

region was not treated with pour-on in any given quarter. Amongst ivermectin treated cattle, 16-20% were dosed with the pour-on in either the second or fourth quarters and even fewer received this formulation in the first and fourth quarters.

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

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

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

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

1983). *E. intermedius*, another introduced species, was observed in central Texas in May-June and in September (Blume, 1984).

In Florida, the introduced species *O. taurus* and *O. depressus* are active and reproducing from March-October (Fincher and Woodruff, 1975; Woodruff, 1973). *O. gazella* has been observed in Florida in August and is probably active and reproducing through October (Hunter and Fincher, 1985). Therefore, the breeding season for introduced beetles only partially overlaps periods in which ivermectin is used more frequently, i.e. March-May in central Florida and September-November in southern Florida. However, from June-September, when beetles are reproducing, ivermectin monthly use averages only 3-6% of the yearly total, with pour-on representing at most 65% of this, indicating that most pastures would not contain drug residues.

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

Nevertheless, there may be pasturing situations where pats are dropped in less accessible areas and, therefore, remain largely undisturbed, e.g. woodland pastures. In these cases, dung dispensing insects may be absent from pats dropped by treated cattle for 1-2 weeks post dose.

Overall conclusions: Dung pats dropped by cattle for 1-2 weeks after treatment with doramectin pour-on likely contain sufficient drug to prevent development of some species of dung beetles. Under certain conditions, the dung pats may also require significantly longer periods of time to degrade. However, many factors appear to be involved in dung degradation and under conditions that simulate actual pasture use, avermectins including doramectin have not been shown to adversely impact grazing efficiency of pasture.

It appears unlikely that native dung beetles would be adversely impacted by use of doramectin. Many species simply do not utilize cattle dung as a food source, or if they do, they also utilize other sources of dung. Some species of dung beetles e.g. aphodids do not appear to be very sensitive to avermectins including doramectin, and if impacted it is only those utilizing pats voided a few days after treatment. Exotic species appear to be more sensitive to avermectins and also appear to be the most dependent on cattle dung as a sole food source. In the U.S., these species are found only in Hawaii and the southern states. In the latter region, they are active and breeding from approximately May through October, with peaks from June-August. Native species, in contrast, are

often distributed over much larger habitats and tend to be active and breeding most months of the year except for winter months in the more northern reaches of their habitats. Dung beetles are winged insects and are strong fliers. Where they have been tracked, they are capable of covering considerable distances and have expanded their niches by 50-80 km per season in Australia (Lee, 1979) and up to 32 km per year in the U.S. (Fincher et. al., 1983).

Tracking ivermectin use (which serves as a proxy for doramectin use) reveals that no more than 13-16% of pastured cattle in southern states where exotic beetles occur are treated with the pour-on formulation per quarter. Treatment of individual herds occurs randomly across each county. Although each operator treats essentially all cows and many calves, the probability of simultaneous treatment of a block of adjacent herds is remote. Therefore, although exotic beetles that ingest dung voided by cows 1-2 weeks after treatment may be impacted, sufficient dung from nontreated cattle is available locally to prevent extinction of the species or even significant disruption of local populations. Further, breeding activities of exotic beetles are most prevalent in June-August and during this period drug use accounts for less than 16% of total annual usage, thus providing additional insurance that insect populations would not be unfavorably impacted. Since drug is excreted for only a finite period after treatment, any pasture with recently treated cattle will contain only a limited number of fecal pats containing significant drug residues. Such pats are a threat to beetles for only a day or two after they are voided because beetles visit only fresh pats.

c. Vertebrate Wildlife

Exposure of terrestrial vertebrate wildlife to doramectin is likely to be incidental through occasional dietary intake. Such incidental dietary exposure is not expected to affect these non-target organisms. An acute LD_{50} of > 2000 mg/kg body weight for doramectin administered to adult bobwhite quail indicates very low toxicity. Similarly, when avermectin B_1 (abamectin) was assessed for acute toxicity toward bobwhite quail, the LD_{50} following a single oral dose was > 2000 mg/kg; the dietary LD_{50} of abamectin presented in-feed to bobwhite quail for 5 days was 3102 ppm (Wislocki et al, 1989). Mallard ducks were more sensitive to abamectin than the bobwhite quail, with an acute oral LD_{50} of 85 mg/kg body weight and a dietary LD_{50} of 383 ppm. A chronic study in the mallard duck showed that 12 ppm abamectin in the diet administered for 18 weeks had no effect on reproductive success and caused no overt signs of toxicity (Merck & Co., 1990). Using the LD_{50} and NOEC values for abamectin in the mallard, Merck and Co. developed exposure scenarios for Ivomec® pour-on to evaluate whether dietary exposure from incidental intake of hair from the backs of treated cattle by magpies or secondary consumption of magpies or carrion of treated cattle by raptors might present a potential hazard to these species. Assuming the same worst case assumptions as for ivermectin, i.e. hair is 12% of a 20 g daily

food intake for a 200 g magpie, and assuming that 100% of the maximum doramectin residue of 755 ng/g at the site of pour-on application in hide and hair is in the hair only, daily dietary intake of doramectin would be 0.09 ppm if hair only from the site of application was consumed. Although chronic feeding studies with mallard duck or magpie have not been conducted for doramectin, this concentration is 130 times below the chronic NOEL established for abamectin in the former species. Similarly, intake of an amount of drug residue equal to the acute oral LD_{50} would be extremely unlikely. For example, bobwhite quail with a body weight of 0.2 kg could consume more than 400 mg of doramectin, or the entire dose from 2-3 cattle, without adverse effects. A mallard duck of average body weight 1.5-2.0 kg could consume the entire 150 mg dose from a single steer before exposure would approach that of the acute LD_{50} of abamectin for this species. Therefore, the drug should not represent a hazard for foraging birds.

2. Aquatic Species

The potential exposure of aquatic organisms to doramectin is expected to be intermittent, since it depends upon rain runoff from cattle feedlot wastes or soil fertilized with cattle manure containing drug residues; and short-lived, since the concentration of doramectin in water would decline as the drug sorbed to suspended particulates and was degraded by photolysis and transformed by microorganisms. The maximum predicted concentration of doramectin in undiluted surface runoff from a cattle feedlot is 3.0 ppt (Section 7.B.1); such runoff is directed to retention facilities and therefore not expected to impact on surface water habitats. The maximum predicted environmental concentration in undiluted runoff from waste-amended soil is 2.9 ppt (Section 7.B.3), although this maximum concentration would be transient due to the susceptibility of doramectin residues in soil to microbial degradation. Runoff from waste-amended soils may enter ponds or streams, where it would be immediately diluted into the receiving water body. Residue levels would be further reduced by the sorption of any free doramectin to organic matter in the receiving water body, as well as by photolysis. Maximum concentrations of less than 1 ppt would be found in the aquatic compartment of water bodies receiving such runoff or receiving residues washed off from hides of treated cattle (Sections 7.B.3 and 7.B.4). Such levels are not expected to have untoward effects on non-target aquatic organisms. For the water flea, *Daphnia magna*, the aquatic species that was most sensitive to doramectin of those tested, the EC_{50} of 100 ppt is more than 100-fold greater than the maximum concentrations that might be found in a surface water body. The desmethyl and 8-α hydroxy analogs of doramectin, the principle excretion and soil biodegradation metabolites, were also evaluated against *Daphnia magna* and were found to be 8 to 11 times less toxic than doramectin (Section 8.B.2). Finally, the doramectin LC_{50} values for bluegill sunfish and rainbow trout of 11 and 5.1 ppb, respectively, are more than 5×10^3 times higher than the maximum predicted aquatic concentration. In summary, exposure of aquatic organisms to doramectin is expected to be intermittent and transient, with only very low

levels likely to be found in surface waters due to the tight binding of doramectin to organic matter, its extremely low water solubility, and its susceptibility to degradation and to photolysis. Therefore, doramectin use is not expected to impact aquatic organisms.

9. USE OF RESOURCES AND ENERGY

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

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

10. MITIGATION MEASURES

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

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

11. ALTERNATIVES TO THE PROPOSED ACTION

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

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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.

Larry R Chappel 8/2/96

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14. REFERENCES

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Appendix a-1

Material Safety Data Sheets

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Emergency Telephone: 203 441-410C

May, 1994
[supercedes Sept. 1991]

MSDS #0132

Doramectin

[UK-67,994]

SECTION I: *PHYSICAL DATA*

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

SECTION II: *FIRE AND EXPLOSION HAZARD*

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

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

SECTION III: *HEALTH HAZARD INFORMATION*

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

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

SECTION IV: *FIRST AID INFORMATION*

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

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

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

SECTION V: *REACTIVITY DATA*

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

SECTION VI: *SPILL OR LEAK PROCEDURE*

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

SECTION VII: *PRECAUTIONARY INFORMATION*

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

issued by: D. P. Brannegan

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

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

May, 1994
[original]

MSDS #204

DECTOMAX® Pour-On

[Doramectin 0.5%, UK-67,994]

SECTION I: *PHYSICAL DATA*

Appearance:	Clear, blue liquid
Composition:	Solution of doramectin, (0.5%), containing 16% cetearyl octanoate in isopropanol. Doramectin is a broad spectrum antiparasitic agent for cattle and swine. The Pour-on formulation contains 5 mg/ml of doramectin. Because the formulation contains isopropanol (isopropyl alcohol), the mixture is classified as a flammable liquid.
Chemical Family:	Avermectin

SECTION II: *FIRE AND EXPLOSION HAZARD*

Doramectin Pour-on has a flash point of 44.6°F and contains a high percentage of isopropyl alcohol. The solution is classified as a flammable liquid. Doramectin Pour-on will burn if involved in a fire. If Doramectin Pour-on is involved in a fire, an appropriate extinguishing medium, including water, may be used.

Doramectin Pour-on should be handled in a manner which prevents exposure to heat sources and open flames.

Standard precautions to minimize static charge build-up should be employed.

Doramectin Pour-on does not present an explosion hazard.

SECTION III: *HEALTH HAZARD DATA*

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

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

The Doramectin Pour-on formulation contains isopropanol (isopropyl alcohol). Isopropyl alcohol is flammable liquid which can cause skin and severe eye invitation. The eight (8) hour time weighted average exposure for isopropyl alcohol is 400 ppm (10th edition of ACGIH tables) and 500 ppm for any 15 minute period (STEL). Normal handling precautions should be used to minimize exposure to this material.

SECTION IV: *FIRST AID INFORMATION*

Ingestion: In the event of ingestion of Doramectin Pour-on, medical attention should be summoned immediately.

Inhalation: In the event of inhalation of Doramectin Pour-on, remove the exposed individual to fresh air, give artificial respiration. If breathing is difficult, give oxygen.

Skin/Eye Contact: Skin contact with Doramectin Pour-on should be immediately washed with water. Contaminated clothing should be removed and the skin flushed with water. Doramectin Pour-on may be a severe eye irritant. Wash all eye contact immediately with water and call for medical attention.

SECTION V: *REACTIVITY DATA*

Doramectin Pour-on is a stable solution. Due to the presence of isopropyl alcohol, Doramectin Pour-on should be kept away from strong oxidizers.

SECTION VI: *SPILL OR LEAK PROCEDURE*

Spills of Doramectin Pour-on should be absorbed by use of appropriate materials. Spills may present a fire hazard due to the presence of isopropyl alcohol. Thus, sources of ignition must be controlled when cleaning up spills.

Spills of Doramectin Pour-on should not be flushed to collection systems which lead to fresh or salt water sources. All wastes from spills of Doramectin Pour-on should be collected for disposal by incineration.

SECTION VII: *PRECAUTIONARY INFORMATION*

Skin contact should be avoided by the use of gloves when handling Doramectin Pour-on. Eye protection is also advisable when handling Doramectin Pour-on.

issued by: D. P. Brannegan

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

Appendix a - 2

Certification of Compliance - Pour-On Solution Manufacturing Site

June 21, 1996

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.

Frank R. LaPietra

Frank R. LaPietra
Director of Operations

Appendix b

Data Summary Charts

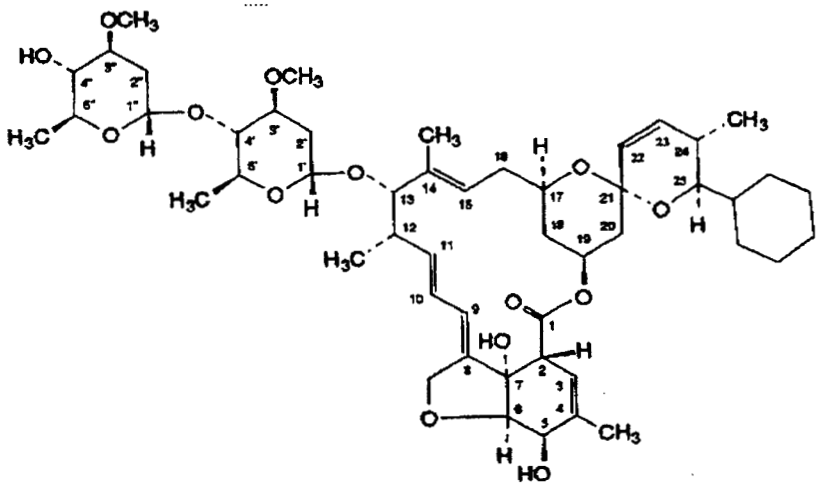
APPENDIX b

DATA SUMMARY CHARTS

PHYSICAL-CHEMICAL AND ENVIRONMENTAL FATE DATA

Generic Name: Doramectin

Structural Formula:



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

Molecular Weight: 899.13

Solubility in Water: 25 ppb

n-Octanol Water Partition Coefficient: 25,787

Vapor Pressure: Non-volatile

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

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

Melting Temperature: 160.5 - 162.2°C

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

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

Photodegradation: Half-life (hours) 4.45

Biodegradation in Soil:

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

ACUTE AND SUBACUTE TOXICITY STUDIES

TERRESTRIAL ORGANISMS

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

ACUTE AND SUBACUTE TOXICITY STUDIES

Bobwhite Quail	Acute Oral LD_{50} > 2000mg/kg
Dung Dwelling Insects	LC_{50}(ppb)
Hornfly	3
Dung beetle	38.2

AQUATIC ORGANISMS

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

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

Appendix c-1

Excretion of Doramectin by Medicated Cattle

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 [^{3}H] 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 were determined by high performance liquid chromatographic analysis of extracted drug after conversion to a fluorescent derivative. The profile of drug and metabolites in feces collected 21 days post dose was characterized by two HPLC systems with detection by radioactivity monitoring. Quantitation of the tritium profiled by HPLC was accomplished by liquid scintillation counting of HPLC eluent fractions.

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 21, 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 755 ng/g and fell to <19 ng/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±10 ng/g) and liver (9±6 ng/g) followed by kidney (2.2±1.6 ng/g) and muscle (0.9±0.5 ng/g). Doramectin was the most abundant residue in all tissues 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 profiled 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	0.477
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	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, castrated 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	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 in 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%

Appendix c-2

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 [^{3}H] 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 [^{3}H] 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.

Appendix c-3

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 mg/kg (1 mL/10 kg) against two species of dung burying Scarabaedae: *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

Appendix c-4

Acute Oral Toxicity (Ld_{50}) Of Doramectin In Bobwhite Quail

Report Summary: ACUTE ORAL TOXICITY (LD_{50}) OF DORAMECTIN IN BOBWHITE QUAIL

Study Number: PFZ 537

Test Species: Bobwhite Quail (*Colinus virginanus*) 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 homogenously 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 LD_{50} value of doramectin for the Bobwhite quail lies in excess of 2000 mg/kg.

Appendix c-5

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 0.5ml 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.

Treatment	Time After Application (hr)	Mean Value (0 - 4) Erythema	Edema
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