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IVOMEC® SR Bolus for Cattle
Environmental Assessment

VOLUME 1 PAGE

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IVOMEC® SR BOLUS FOR CATTLE
Environmental Assessment

1. **Date:** October 15, 1996

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

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

4. **Description of the proposed action:**

A. Requested action:

To approve the marketing of IVOMEC (ivermectin) sustained-release bolus formulation for use in cattle.

B. Need for the action

The IVOMEC SR Bolus formulation is a treatment for the control of endo- and ectoparasites of cattle. It is designed to release ivermectin into the rumen/reticulum of calves at a uniform rate (~12 mg/day) over approximately 135 days. One bolus, given to a calf weighing 100 to 300 kg on the day of administration, will control important internal and external parasites during the grazing season. The cost of parasitism, in terms of morbidity and resultant depression of growth and feed efficiency, has long been recognized as a significant factor in the economical production of both beef and dairy products. The beef and dairy industries suffer intensive economic losses due to both internal and external parasites. These losses have been primarily attributed to reduced rate of gain and feed efficiency due to pathological effects of internal parasites and interruption of feeding habits caused by external parasite infestation and flies. In addition to its effects on endo- and ectoparasites, the ivermectin bolus formulation suppresses larval development of face flies and horn flies in manure of treated animals. Use of this product will reduce the economic loss to the beef and dairy industries resulting from parasitism affecting pastured calves.

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

The drug substance (ivermectin) will be manufactured at the applicant's facilities in Danville, Pennsylvania, Elkton, Virginia and Barceloneta, Puerto Rico. Drug product (IVOMEC SR Bolus) will be manufactured at the applicant's facility in West Point, Pennsylvania and packaged in the applicant's facilities in West Point, Pennsylvania and Haarlem, Holland.

The types of environments present at the locations mentioned above, specific to the vicinity of drug substance (avermectin and ivermectin) manufacturing or drug product (IVOMEC SR Bolus) manufacturing and packaging, are described in the following sections.

i) The type of environment at Elkton, Virginia

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

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

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

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

Water Resources - Separate sanitary, process and storm water sewer systems are maintained by the plant. The sanitary wastes, after solids separation and chlorination, are mixed with the process waste for additional treatment in the plant's waste water treatment facility. Water from the storm water system and non-contact cooling water is mixed with the waste water treatment plant effluent and discharged to the South Fork Shenandoah River through the plant's VPDES outfall. There are no injection wells on the plant's property, and the only surface waters within 1000 feet of the plant is the South Fork of the Shenandoah River. The 100-year flood plain elevation at the plant is approximately 973 feet above mean sea level. One well supplies the plant's potable water needs with an additional well as backup.

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

ii) The type of environment at Danville, Pennsylvania

Location - The Danville plant is located on a 180 acre site in the Susquehanna River Valley approximately 70 miles north of Harrisburg, Pennsylvania. The plant is located adjacent to the south bank of the North Branch of the Susquehanna River. Coordinates of the plant's location are latitude N 40 deg. 57′ and longitude W 76 deg. 38′. The plant is located in the Borough of Riverside. The U.S. Census Bureau listed Danville's 1980 population as 5,200 people.

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

(NAAQS). There are no Class I Visibility Areas within 50 km of the plant. Prevailing winds near the plant are from the west-northwest direction.

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

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

Soils at the Danville Plant have been modified by the construction of buildings, streets, parking lots, and other structures, and are classified as Urban Land soils according to the Soil Conservation Service. The southwestern edge of the Plant is underlain by Wyoming and Basher soils. Wyoming soils are gravely sandy loams with rapid permeability and low to very low available water holding capacity. The soils are medium to extremely acid. Basher soils are deep, moderately well-drained to poorly-drained, dark reddish-brown silt loams. These soils have moderate to moderately slow permeability and moderate to high available water holding capacity. Basher soils are medium to strongly acid.

The surficial soils are underlain by up to 50 feet of un-consolidated, Quaternary-age materials which were deposited by both alluvial and glacial processes. These fluvial deposits (hereafter referred to as "alluvium"), are comprised of sands and gravel, silt and clay. The degree of sorting of these deposits is variable. A large portion of the deposits consists of coarse-grained gravel with cobbles and boulders.

The alluvium is underlain, in ascending stratigraphic order, by the Silurian age Rose Hill, Mifflintown, Bloomsburg, and Wills Creek Formations. The Rose Hill Formation is a light olive-gray shale with interbeds of fine-grained sandstone; the formation is approximately 950 feet thick. The Mifflintown Formation is an interbedded dark-gray shale and medium-gray fossiliferous limestone; the maximum thickness in Montour and Northumberland Counties is 156 feet. The Bloomsburg Formation is a grayish-red and greenish-gray shale, siltstone, and very fine- to coarse-grained sandstone with some calcareous claystone beds. The maximum thickness of the Bloomsburg Formation in this region is 840 feet. The Wills Creek Formation consists of interbedded calcareous shale, argillaceous dolostone and limestone, and calcareous siltstone and is approximately 650 feet thick in the region.

Structurally, the Site lies on the southern limb of the northeast-trending Berwick (Montour) anticline. Strata underlying the Site strike 76 degrees east of north and dip 44 degrees to the south. Three joint sets are recognized in the area: parallel to bedding, perpendicular to bedding, and oblique to bedding.

iii) The type of environment at Barceloneta, Puerto Rico

Location - The Merck Sharp & Dohme Quimica de Puerto Rico, Inc. (MSDQ) facility is located on a 166 acre site in Barceloneta, Puerto Rico. The city of Barceloneta contains a population of 20,000 people and is located 38 miles due west of San Juan and three miles south of the Atlantic Ocean. The MSDQ plant is located at km 56.4 along State Highway 2. Coordinates of the plant's location are latitude N 18 deg. 25′ and longitude W 66 deg. 32′.

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

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

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

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

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

iv) The type of environment at West Point, Pennsylvania

Location - The West Point plant is located on a site (~450 acres) in Upper Gwynedd Township, Montgomery, County, which is approximately 30 miles northwest of Philadelphia. The center of the West Point is located near latitude 40° 12' 54" N and longitude 75° 17' 59" W. Land use surrounding the plant is primarily residential and agricultural with other industrial sites approximately one-half mile away.

Weather/Air Resources - Air quality in this area is in compliance with the Environmental Protection Agency's (EPA) National Ambient Air Quality Standards (NAAQS) of the Clean Air Act for total suspended particulates, sulfure oxides, and nitrogen oxides. This compliance is based on monitoring and reporting by the Pennsylvania Department of Environmental Resources (PA DER) under the requirements of the State Implementation Plan. At this time, Montgomery County does not meet the ozone standard set forth by the NAASQ. The West Point plant lies within the outer zone of the Southeast Pennsylvania air basin. Pennsylvania is part of the EPA Region III and PA DER is responsible for implementing the State Implementation Plan which includes new stationary source permits for manufacturing. Meteorological data for the region is collected at the Philadelphia International Airport. Annual rainfall is approximately 42 inches (107 cm) and the mean ambient monthly temperature varies between 33 and 77°F (0.5-25°C). Predominant winds are from west to southeast.

Water Resources - Potable water is supplied to the plant operations via an on-site storage tank which is supplied by on-site wells and a public water supplier, North Wales Water Authority. North Wales Water Authority operates as many as three public wells within a half-mile of the plant property. The plant potable water quality meets all requirements of the Federal Safe Drinking Water Act and the Pennsylvania Safe Drinking Water Act. Compliance with these standards are also required in applicable Good Manufacturing Practices.

Stormwater drainage is controlled using detention basins which maintain site runoff at levels estimated for undeveloped property and to minimize erosion. This runoff is discharged into either the Towamencin Creek or the Wissahickon Creek.

Any aqueous or organic wastestreams containing ivermectin will be collected and either incinerated on-site or sent off-site for disposal at a permitted waste facility.

Land Resources - The plant is underlain by Triassic age sedimentary rocks, mapped as the Brunswick and Lockatong formations. These formations occur as layered beds of red and very dark gray shale with occasional layers of sandstone. Although these rocks generally have low primary porosities, permeability is maintained and improved by the presence of fractures and joint sets.

The plant site elevation is about 361 feet above mean see level (United States Geologic Survey datum).

v) The type of environment at Haarlem, Holland

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

Weather/Air Resources - Dutch government laws prescribe emission standards for hazardous air pollutants. No significant air pollution generating industries are located in the vicinity. Annual rainfall is 0.754 meter (30 inches). Mean January temperature is 5-8°C (40-45°F). Prevailing wind directions are west and south-west (sea wind) at a windforce of 3 to 8 Beaufort.

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

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

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

The IVOMEC SR Bolus will be used in high value beef (cow/calf operations) and dairy (replacement cattle) calves in pasture environments. In addition, this product will be used in stocker cattle within the allowed weight range (100-300 kg). While cattle are grazed throughout the United States, cow/calf operations and the dairy industry are concentrated in specific geographical areas. Cow/calf operators are generally concentrated in the southeastern and middlewestern states (Anon., 1989) and leading dairy states include California, Florida, Minnesota, Pennsylvania, New York and Wisconsin (Anon, 1989). Stocker operations are an important component in all regions of the United States with the exception of New England and the Middle Atlantic States.

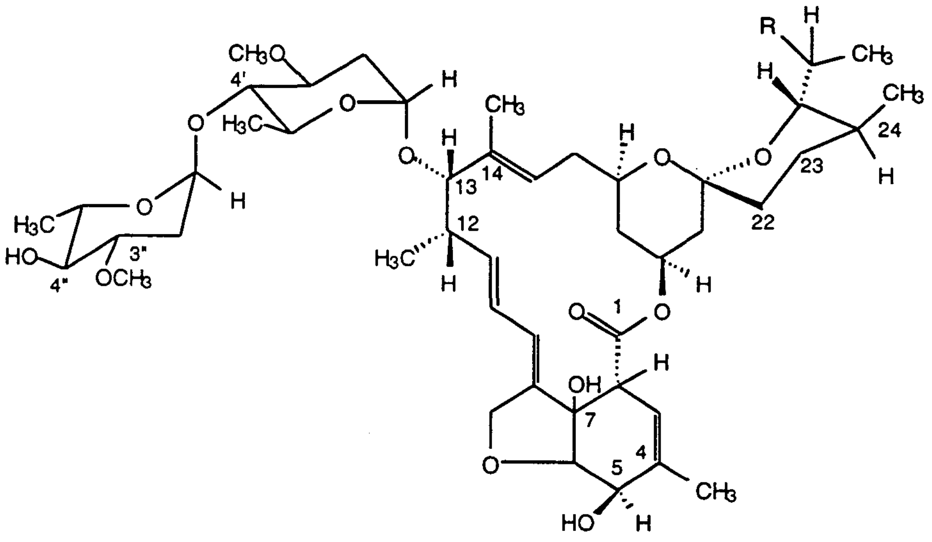
5. Identification of chemical substances that are the subject of the proposed action:

A. IVOMEC SR Bolus for Cattle:

The active ingredient which is the subject of this document:

- Ivermectin (CAS Reg. No. 70288-86-7)
- Chemical name: 5-O-Demethyl-22,23-dihydroavermectin A_{1a} and 5-O-Demethyl 25-de (1-methylpropyl)-22, 23-dihydro-25-(1-methylethyl) avermectin A_{1a}

B. The structure and properties of ivermectin



Empirical Formula	Molecular Weight
(R = C_2H_5) $C_{48}H_{74}0_{14}$	875.10
(R = CH_3) $C_{47}H_{72}0_{14}$	861.07

Ivermectin is produced by fermentation and subsequent chemical hydrogenation and is a mixture of two closely related homologues belonging to a class of compounds known as avermectins.

Ivermectin contains at least 80% of the compound in which R in the above structure is the ethyl group and less than 20% of the compound in which R is the methyl group. It is white to yellowish white crystalline powder and has an ill-defined melting point of about 150°C. The material is optically active and has a specific rotation $[\alpha_D]25°C$ of approximately -19° (C=0.5, CH_3OH).

The ultraviolet absorption spectrum in methanol is characterized by maxima at 237, 245 and 253 nm, with less intense absorption at ~290 and 350 nm. Ivermectin is very insoluble in water: the concentration of a saturated aqueous solution is 4 ppm. Ivermectin is freely soluble in methanol, chloroform, p-dioxane, dimethylformamide and ethyl acetate; soluble in 95% ethanol, diethyl ether, methylene chloride and acetone and aromatic hydrocarbons; and very slightly soluble in aliphatic hydrocarbons. The infrared and nuclear magnetic resonance spectra are consistent with the proposed structures.

Ivermectin has been shown to be stable for at least six months when stored under ambient conditions. Ivermectin is photolabile in solution and as a thin film.

Ivermectin contains at least 95% of the two compounds shown above as determined by UV absorption and liquid chromatography.

Based on radioactivity measurements, the octanol-water partition coefficient for ivermectin is 1651; i.e.,

$$K_D \text{ of } \frac{\text{octanol}}{\text{pH 7 buffer (or water)}} = 1651$$

The present assessment supplements ivermectin data with data generated with avermectin B_1. The structure of avermectin B_1 (AVM) only differs from that of ivermectin (IVM) by a double bond at position 22,23. Ivermectin is produced from avermectin by catalytic reduction of this double bond. Physical properties of ivermectin and avermectin are compared below.

TABLE 1

Comparison of IVM and AVM Physical Properties

Physical Properties	IVM	AVM
Molecular Weight[a]	875	873
Octanol/Water Partition Coef.	1,651	9,900
K_{oc}[b]	12,600-15,700	≥4,000
Aqueous Solubility[c]	4 ppm	8 ppb
E (λmax), Methanol	30,100 (245)	31,850 (243)

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[a] Molecular weight of the B_{1a} component
[b] Different soils used
[c] Different methods used

Both compounds possess low water solubility, high octanol/water partition coefficients and high K_{oc} values. Compounds with K_{oc} values >1000 are immobile in soil.

6. Introduction of substances into the environment:

A. As a result of the manufacture of IVOMEC SR Bolus

The introduction of substances into the environment from manufacturing IVOMEC SR Bolus can occur from drug substance (avermectin and ivermectin) manufacturing facilities and drug product (IVOMEC SR Bolus) manufacturing and packaging facilities.

i) **Elkton, Virginia** - The following summarizes the environmental effects of manufacture of avermectin at the Elkton plant.

Liquid Waste - The manufacturing process generates aqueous waste streams from fermentor vents, fermentor sample funnels, equipment washes and floor drains. All aqueous waste is directly collected via piping or collection sump in a 20,000 gallon tank or directly transferred to either holding tanks or tank trucks. From the collection tank, the waste can be transferred either to an evaporator system to concentrate the liquid prior to shipment off-site or directly to a tank truck. The liquid waste is then sent to the applicant's Danville facility in Pennsylvania for treatment and disposal. The specifics of waste water treatment employed at the Danville facility are described in the section (2) below. On a limited case-by-case basis, liquid wastes that have been determined through process knowledge and detailed analysis to contain less than a threshold concentration of avermectins will be sewered to the site's advanced activated sludge system (wastewater treatment plant).

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

Air Emissions - The fermentation step generates fermentation off-gases that contain typical respiration byproducts, including carbon dioxide

(CO_2). The on-site incinerator emissons consist of typical combustion products.

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

Solid Waste - Burnable, non-hazardous, solid wastes containing "de minimis" amount of avermectin may consist of paper, aluminum, plastic, and drums. Such wastes are incinerated on-site or sent to a permitted incineration facility able to accept such waste streams. Other non-hazardous wastes which cannot be recycled are disposed of at a state licensed landfill. Disposal of non-hazardous solid waste is subject to, and in compliance with, Permit #183 issued under the Virginia Solid Waste Management Regulations. There are no numerical permit limits on solid waste generation and no additional permit conditions are anticipated as a result of the proposed action.

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

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

ii) **Danville, Pennsylvania** - The following summarizes the environmental effects of manufacture of avermectin pure at the Danville plant.

Liquid Waste - The manufacturing process generates two liquid-waste streams: one, a combination of solvent-based waste streams, the other, a combination of aqueous waste streams.

The solvent-based waste streams are generated in the isolation step and in the recovery of solvents used for the isolation. They contain discarded organic compounds in a solution of solvents such as toluene, methanol, ethanol, hexane. The solvent-based waste will be processed so as to recover the major portions of the organic solvents to the extent feasible to minimize any potential release of organic compounds to the environment. Residues from the solvent recovery operations are destroyed by incineration. The incineration process is subject to and in compliance with the Pennsylvania Rules and Regulations for the protection of Environmental Resources, Title 25, Part I, Subpart C, Article I, Land Resources, Chapter 75, Solid Waste Management and Article III, Air Resources and 40 CFR Parts 264 and 265, Standards Applicable to Owners and Operators of Hazardous Waste Treatment, Storage and Disposal Facilities. The incineration process in also subject to, and in compliance with the site's hazardous waste (RCRA) permit #PAD003043353 and Operating Permit #49-301-018

The aqueous-based waste streams consist of spent fermentation broth and wash waters that contain unconsumed fermentation nutrients, unrecovered by-products and traces of avermectins and dissolved solvents such as hexane, methanol, ethanol, and toluene. The aqueous-based streams are treated in an on-site chemical pretreatment unit in a high pressure reactor using caustic designed to destroy residual avermectins. The pretreatment unit operates under Water Quality Management Permit No. 4994201. The effluent from the high pressure reactor is further treated in an onsite two-stage biological waste water treatment plant before being discharged into the Susquehanna River. The final plant effluent is discharged under the requirements of and in compliance with NPDES Permit No. PA 0008419 which is administered by the Pennsylvania Department of Environmental Resources. The amount of avermectin released into the Susquehanna River is below levels of environmental concern.

Air Emissions - The fermentation step generates fermentation off-gases that contain typical respiration byproducts, including carbon dioxide (CO_2). Air emissions generated from the avermectin isolation consist of volatile organic compounds (such as hexane, methanol, ethanol, and toluene) and dust. Volatile organic emissions from the avermectin production process are controlled by condensers and a fume incinerator.

Dust in the process building will be filtered with HEPA-type filters to control the introduction of avermectin and dust into the ambient air with an efficiency greater than 99.9%. Air emissions are in compliance with the regulations of the Pennsylvania Department of Environmental Resources (Title 25, Part I, Subpart C, Article III, Air Resources) and Operating Permit #49-301-032C.

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

Employee Protection - Material Safety Data Sheets (MSDS) are available onsite for all chemicals required by the Occupational Safety & Health Act of 1971 and the Hazards Communication Act of 1985. Employees associated with the manufacturing of avermectin have appropriate MSDS available for their review. The MSDS for Avermectin Broth, Detoxified Avermectin Spent Broth and Avermectin Pure are contained in Section 16 of this assessment. Employee protective clothing, such as gloves, uniforms, and safety shoes, and protective equipment, such as safety glasses, are used during the manufacturing process to assure compliance with the Occupational Safety & Health Act (OSHA) of 1971 and the Hazards Communication Act of 1985. To minimize worker exposure to avermectin, the following monitoring activities are conducted:

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

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

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

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

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

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

iii) Barceloneta, Puerto Rico

The following describes the environmental aspects of converting avermectin into ivermectin and manufacturing and packaging of IVOMEC SR Bolus at the applicants facilities in Barceloneta.

Liquid Waste - The solvent-based waste streams are generated in the chemical processing step. The waste solvents contain discarded organic compounds in a solution of solvents such as ethanol, formamide, toluene, and water. The solvent-based stream is destroyed by incineration. The incineration process is subject to, and in compliance with, the Puerto Rico Environmental Quality Board Regulations for the Disposal of Solid Waste and Regulations for the Control of Atmospheric Pollution and the U. S. Environmental Protection Agency Regulations, 40 CFR Parts 264 and 265. Currently, the solvent incinerator operates under a permit issued by the EQB hazardous Waste Program and under EQB Permit No. PFE-09-12911668-I-III-0 issued by the EQB Air Program. The USEPA hazardous waste identification for the site is PRD090029101.

The aqueous-based waste stream consists of wash waters generated by equipment washings. Holding tanks are provided to contain these washes prior to testing and disposal. Depending on the ivermectin concentration, the holding tank contents will be managed in one of two ways:

1. Contents are tested for ivermectin and recycled through a filter until a specified level is reached, and then are discharged to the chemical sewer.

2. Contents are incinerated.

Effluent from the Barceloneta plant is discharged to the Barceloneta Regional Wastewater Treatment Plant (BRWTP) under Permit #GDA-93-202-045. The BRWTP operates under the requirements of NPDES Permit No. PR 0021237 which is administered by the U.S. Environmental Protection Agency.

Air Emissions - Air emissions generated during the conversion of avermectin to ivermectin consist of volatile organic compounds, (such as ethanol, formamide and toluene) and dust. The emissions of volatile organics are controlled as appropriate by condensers. Air from the process building, formulation area sterile facility is exhausted through HEPA-type filter prior to discharge to the atmosphere to control particulate emissions of ivermectin powder (drug substance). Air emissions are subject to, and in compliance with, the regulations for air emissions of the Puerto Rico Environmental Quality Board Regulations for the Control of Air Emissions. Manufacture of the drug substance is also in compliance with conditions under permit PFE-09-1291-1668-I-II-0.

Solid Waste - Dry solid waste, such as paper, trash, and HEPA-type filters etc., is disposed of in an incinerator which is subject to, and in compliance with, the regulations for air emissions and solid waste disposal of the Puerto Rico Environmental Quality Board (EQB) and permits PFE-09-1291-1668-I-III-0 issued by the EQB Air Program and SI-93-004 issued by the EQB Solid Waste Program.

Employee Protection - Material Safety Data Sheets are available onsite for all chemicals required by the Occupational Safety & Health Act of 1971 and the Hazards Communications Act of 1985. Employees associated with the manufacturing of ivermectin have appropriate MSDS available for their review. The MSDSs for ivermectin, avermectin, and the IVOMEC SR Bolus and materials used in its manufacture, are contained in Section 16 of this assessment. Employee protective clothing, such as gloves, uniforms, and safety shoes, and protective equipment, such as safety glasses, are used during the manufacturing process of ivermectin to assure compliance with the Occupational Safety & Health Act of 1971 and the

Hazards Communication Act of 1985. To minimize worker exposure to avermectin and ivermectin, the following monitoring activities are conducted:

1. At least semi-annual monitoring of dust levels for avermectin and ivermectin where the powder for each, respectively, is handled; and

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

Environmental Exposure - Quantities of substances that enter environmental media (i.e., soil, water, air) as a result of the conversion of avermectin to ivermectin are inconsequential.

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

As per the MSDS for avermectin pure and ivermectin, any solid waste containing either substance is incinerated at a temperature greater than 500°C.

Wastewaters from the conversion of avermectin to ivermectin are collected in a waste storage tank and either:

1. Tested for ivermectin and recycled through a filter until a specified level is reached, and then are discharged to the chemical sewer;

2. Incinerated.

The discarded filters are incinerated onsite at a temperature greater than 500°C. Any residual ivermectin/avermectin remaining in the wastewaters is diluted by approximately one-half million gallons per day of total plant liquid effluent. Further dilution takes place when the total plant effluent is sent to the 6 million gallon per day Barceloneta Regional Wastewater Treatment Plant (BRWTP). Final effluent from the BRWTP is discharged into the Atlantic Ocean where additional mixing and dilution occurs. The trace quantities of ivermectin/avermectin released into the Atlantic Ocean are below levels of environmental concern.

iv) West Point, Pennsylvania

The following describes the environmental aspects of formulating, assembling and packaging of IVOMEC SR Bolus at the West Point facility.

Liquid Emissions -

In the process of formulating, assembling and packaging the IVOMEC SR Bolus, aqueous and organic liquid wastestreams will be generated. All aqueous wastestreams generated during cleaning operations will be collected and either incinerated on-site or sent off-site for disposal at a permitted facility. All organic wastestreams generated during the cleaning operations will be collected and handled as hazardous waste and sent off-site for disposal at a permitted hazardous waste facility. No other liquid waste streams are gnerated in the course of drug product assembly and packaging. Aqueous wastestreams generated from Quality Control Lab Testing procedures will be collected and either incinerated on-site or sewered on-site or sent off-site for disposal at a permitted waste facility.

The allowable residual aqueous emissions from the IVOMEC SR Bolus Quality Control Lab Testing area will be discharged to the Upper Gwynedd Township Waste Water Treatment Authority (UGTA). The current contract with the UGTA limits effluent flow (calculated from a monthly average) to 1.225 million gallons per day; BOD = 250 mg/L (daily maximum); TSS = 300 mg/L; and pH is between 5.5 - 9.0. In addition, the wastewater from the site is subject to and in compliance with the pretreatment standards for existing sources of the Pharmaceutical Manufacturing Category under Title 40 of the Code of Federal Regulations Part 439 (Subcategory D for mixing, compounding and formulation). Approval of the proposed action will not impact the facility's ability to comply with the above stated requirements and no new permit limits are anticipated as a result of the proposed action.

Wastewaster effluent from cleaning the IVOMEC SR Bolus equipment is treated by the UGTA and this effluent is discharged from the UGTA under NPDES Permit Number PA 0023256. This permit is administered by PA DER under the authority of EPA. The wastewater is also regulated by the UGTA and is in compliance with the existing contract and the "Rules and Regulations Governing the Discharge of Sanitary and Industrial Wastewaters into the Public Sewers of the Upper Gwynedd Township Authority". These regulations are based on the requirements of the Federal Clean Water Act and the Pennsylvania Clean Streams Law.

Approval of the proposed action will not impact the facility's ability to comply with the above stated requirements.

<u>**Air Emissions**</u> - Insignificant emissions are expected from the bolus formulation, assembling and packaging operations. Any potential operation which may emit particulate emissions into the environment will be HEPA filtered. Trace quantities of organic solvent used for cleaning are emitted into the air. In addition to HEPA filtration on the air exhausts, the bolus assembly room will operate under negative pressure.

The on-site incineration facility employs necessary operating conditions to ensure compliance with permitted emission levels. As a contingency, off-site incineration will be conducted at a permitted facility.

The air emission controls for the disposal of this product meet the requirements of the Pennsylvania Air Pollution Control Regulations under Title 25 of the Pennsylvania Code, Part I - Department of Environmental Resources (PA DER), Chapters 121, 141.

Particulate emissions are limited to 0.04 grains/dscf by regulation. Approval of the proposed action will not impact the facility's ability to comply with the above stated requirements. No new permit limits are anticipated as a result of the proposed action.

Solid Emissions - Finished product discards and product related residuals, containing traces of residual raw materials or products, will be collected and disposed of by on-site or off-site incineration at a permitted facility. Solid waste generated from these operations include paper waste, cleaning rags, containers, gowns, gloves both contaminated and not contaminated with ivermectin.

Appropriate controls for the disposal of unused market packages are utilized as part of the site solid waste management program. The waste is incinerated at permitted disposal facilities. Ash generated from the on-site incineration process is disposed of at a permitted facility and is monitored to conform its acceptability with prevailing solid waste regulations.

Solid waste management at the West Point plant requires conformance with conditions set forth in Permits 400459 and 400674 issued by PA DER and Permit PAD002387926 issued by both EPA and PA DER. These requirements assure comprehensive control for management of waste throughout the plant including returned market packages. The requirements of the Pennsylvania Code, Title 25, Part I - Department of Environmental Resources, Chapter 75, are the primary regulations and are subject to the requirements of the Federal Resource Conservation and Recovery Act, the Federal Hazardous and Solid Waste Amendments, and the Pennsylvania Solid Waste Management Act.

Approval of the proposed action will not impact the facility's ability to comply with the above state requirements. The facility is not currently limited by the amount of process wastes generated although efforts will be made to minimize the amount of solid wastes generated.

Employee Protection - Material Safety Data Sheets are available on-site for all chemicals required by the Occupational Safety & Health Act of 1971, the Hazard Communication Act of 1985 and Title 29 Code of the Federal Regulations Part 1910.1200. Employees associated with the manufacture and packaging of drug substance have appropriate MSDSs available for their review. Employee protective clothing, such as gloves, uniforms, and safety glasses are used during the manufacturing process to assure compliance with the Occupational Safety & Health Act of 1971 and the Hazard Communication Act of 1985 and Title 29 Code of Federal Regulations, Subpart I.

Environmental Exposure - Quantities of substances that enter the environmental media (i.e., soil, water and air) as a result of the manufacture and packaging of the IVOMEC SR Bolus are inconsequential. HEPA-type filters control the potential operations which may introduce ivermectin dust into the ambient air with an efficiency greater than 99.9%. Spent filters exposed to drug product are drummed for on-site incineration or off-site incineration at a permitted facility.

Returned goods of the IVOMEC SR Bolus are incinerated on-site or off-site, if necessary, at permitted facilities. Return goods that must be destroyed are incinerated at temperatures greater than 500°C.

v) Haarlem, Holland

The following describes the environmental aspects of manufacturing and packaging the drug product (IVOMEC SR Bolus) at the MSD AGVET plant in Haarlem.

Liquid Waste - No aqueous waste streams containing ivermectin are generated in the formulation of the drug product (IVOMEC SR Bolus). Small quantities of organic solvents, such as n-heptane and methanol, from equipment cleaning and wipedowns are generated. Waste organic solvents are placed in drums and sent to the Rotterdam incinerator. The disposition of organic solvents is in compliance with the Hazardous Waste Act and the Waste Act.

Air Emissions - Air-borne particulates and dust are controlled by HEPA-type filters. Any air emissions from the plant are regulated by, and in compliance with, the State Rules and Regulations Act with regard to environmental pollution. These regulations are administered by the Haarlem Department of Environmental Control.

Solid Waste - Solid waste resulting from production of the drug product, such as HEPA-type filters, will be combined with other plant trash and transferred via closed vehicle to the Rotterdam incinerator. The MSD facility obtains permission and a permit for each truckload of solid waste from production of the IVOMEC SR Bolus as is currently obtained for other products sent to the Rotterdam incinerator.

Employee Protection - Material Safety Data Sheets (MSDS) are available for all chemicals required by the Dutch Safety Law (Arbo Law) and the Dutch Safety Rules for Industry and Workshops. Employees associated with the formulation of IVOMEC SR Bolus have appropriate MSDS available for their review. The MSDSs for avermectin, ivermectin, the IVOMEC SR Bolus and materials used in its manufacture, are contained in Section 16 of this assessment. As additional worker protection, monthly swab tests are performed for ivermectin on equipment, floors, and production bottles in the production area.

The manufacturing is regulated by, and in compliance, with the Dutch Safety Law (Arbo Law) and the Dutch Safety Rules for Industry and Workshops. The manufacturing is also regulated, and in compliance with, the "Wet Milieubelheer" which includes: the Air Pollution Act; the Noise Abatement Act; the Wastewater Regulations; the Hazardous Waste Act; the Waste Act; and the Waste Regulation.

Environmental Exposure - Quantities of substances that enter environmental media (i.e., soil, water and air) as a result of the formulation and packaging of the IVOMEC SR Bolus are inconsequential. HEPA-type Filters control the introduction of ivermectin dust into the ambient air with an efficiency greater than 99.9%.

Finished return goods of the IVOMEC SR Bolus are returned to the MSD facility. Return goods that must be destroyed are incinerated at the Rotterdam incinerator at a temperature greater than 500°C.

B. As a result of the use of IVOMEC® (ivermectin) SR Bolus for Cattle

i) Dosing and excretion

a. Management of the target population and associated pastures

The IVOMEC SR (Sustained-Release) Bolus is designed to release ivermectin into the rumen/reticulum of calves at a uniform rate of ~12 mg/day over approximately 135 days. One bolus is given to each calf weighing approximately 100 to 300 kg at the time of treatment. These animals will be in their first season of grazing. Prophylaxis against newly ingested nematode larvae is the primary efficacy target; hence, most target-weight calves will be treated once, at the time they are turned out to pasture.

Off-label use of the IVOMEC SR Bolus is not expected to occur, to any significant degree, for the following reasons. Firstly, it is important to point out that this is a fixed dose formulation of ivermectin. The fixed dose of ivermectin supports efficacy claims as per the label for animals in the weight range of 100-300 kg body weight at the time of administration. The only manner in which the dose may be varied is to administer multiple boluses. While it may be considered that animals above the target weight range may receive multiple boluses in order to achieve equivalent efficacy, the projected resale cost of this product will prohibit this practice from occurring. Other dosage forms of ivermectin and other anthelmintics available for use in heavier cattle are less expensive and more convenient than the IVOMEC SR Bolus and can effectively control parasites. Secondly, some off-label use may occur in stocker cattle by administering a single bolus to animals that slightly exceed the target weight range. However, the number of cattle treated in this manner is expected to be low. If however, one projected that as many as 20% of stocker cattle in the weight range 300-364 kg (660-800 lb.) were treated with a bolus off-label, the impact on exposure estimates in the risk assessment contained herein would, for any given month, be trivial (<1%).

Cattle targeted for the ivermectin bolus will be of high unit value. Such animals likely will be replacement stock in well-managed operations. As such, all target-weight cattle managed as a unit (i.e., pastured together) will be given a bolus; cows on the same pasture may be treated at the same time with an anthelmintic (e.g., IVOMEC Injection). Cattle being raised under less intensive production requirements likely would not be given an ivermectin bolus, largely because of cost and the inability of the owner to perceive productivity benefits.

Stocking rates vary widely, but cattle given the ivermectin bolus likely will be managed under intense grazing systems. Throughout the southeastern states, a cow-calf unit commonly requires three to six acres under continuous grazing conditions, but one unit on one to two acres can be achieved. Where calves have been weaned and no cows are on the same pasture, one stocker per two to three acres is average, with concentrations as high as two stocker cattle per acre under some circumstances. These high stocking rates can only be achieved where the pastures are well managed. Two University researchers (G.B. Garner, U. of Missouri, and F.M. Rouquette, Texas A&M University, letters in Sec. 16)** have elaborated upon these general observations, and their estimations of stocking rates are consistent with those reported in the literature (Bagley *et al.*, 1987; Hoveland, 1986, Sec. 14). Under arid range conditions, stocking rates decline accordingly (Pitts and Bryant, 1987, Sec. 14).

High stocking rates are only achieved when pastures are intensively managed to maximize forage production. General principles applicable to Georgia are summarized by Johnson (1987, Sec. 14). Principles and practices in various other states and localities are summarized in Proceedings of Forage and Grassland Conference (1988, Sec. 14). Intensive forage production requires fertile, tillable land, fertilization (including dispersion of dung pats to utilize them as fertilizer) and possibly irrigation. Rotational grazing systems are common, employing sets of pastures in which cattle are allowed sufficient time to graze the herbage to a predetermined height before being moved to the next pasture. After the cattle are rotated off, the pasture is often mowed to remove seed heads, control weeds, and allow for uniform regrowth of the grasses present.

Maximum stocking rates result in greater deposition of fecal pats per unit area than in less intensively managed systems. This does not necessarily result in greater accumulation of dung. Numerous factors interact in the process of fecal pat breakdown and dispersion. These include trampling and scattering by animals, the effects of weather, the action of dung fauna, and pasture management practices (Johnson, 1987 and Putman, 1983, Sec. 14). Pastures are usually mowed to maximize herbage regrowth and eliminate tufts of uneaten grass which grow in the immediate proximity of fecal pats. Mowing contributes to dung pat degradation. In addition, harrows or other mechanical devices (such as dragged chain and chain-link fence with weights) are sometimes utilized to break up the pats. Such pasture management practices are associated with high stocking rates and high unit value calves, those animals targeted for the IVOMEC SR Bolus.

b. Introduction of drug residues in pastures

This assessment is based upon the projected seasonal treatment of pastured cattle with anthelmintics. Information supporting this section was provided to the CVM in a detailed confidential report (Title: Seasonal Patterns of Anthelmintic Use in the United States). Information from this report has been incorporated into this Environmental Assessment. Regional specialists in the United States, have confirmed the accuracy of the information contained in this report. To assess anthelmintic usage in cattle by season, the United States was partitioned into regions that could be rationalized based upon the nature of the cattle industry, seasonal availability of pasture and husbandry practices. The cattle industry varies from the northern dairy states, where there are important dairy and cow/calf operations, to the southeast, which is primarily cow/calf, to the western range where cow/calf operations are managed on arid pasture with stocking rates often as low as one cow/calf per 50 acres. In these arid areas, parasitological challenge is less of a concern than in areas of lush grasses.

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

Region	States in Region
Upper Southeast	AR, DE, KY, MD, MO, NC, TN, VA, WV
Lower Southeast	AL, FL, GA, LA, MS, SC
South Central	KS, OK, TX
Southwest	AZ, CO, NM, NV, UT
Pacific States	CA, OR, WA
Hawaii	HI

Big Sky	ID, MT, WY
Plains	NE, ND, SD
Northern Dairy	IA, IL, IN, MI, MN, NY, OH, PA, WI
New England	CT, MA, ME, NH, NJ, RI, VT

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

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

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

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

Dairy cows are not included among pastured cattle for purposes of this assessment. The avermectin endectocides are not approved for use in lactating dairy cows. The addition of dairy cows to the analysis could decrease the percentage of pastured animals that are treated with anthelmintics. This decrease may be significant for those regions or states where dairy cows are pastured. Conversely, in those regions or states where dairy cows are maintained in confinement, the impact on this assessment would be negligible. Dairy calves are reared in confinement until they are weaned. Thereafter, they enter other categories in the USDA statistics, i.e., calves <500 pounds, or dairy replacements. Consequently, diary calves are not considered among pastured cattle for purposed of this assessment.

Classes of cattle that are considered in this assessment include beef cows, beef replacements, beef calves, milk replacements, other heifers >500 pounds, steers >500 pounds, bulls >500 pounds and calves <500 pounds. To estimate total number of pastured cattle by class, USDA statistics for classes of feedlot cattle (other heifers >500 pounds, steers >500 pounds and calves <500 pounds) were subtracted from the USDA statistics for the total numbers of cattle in the corresponding classes.

c. Scenarios for use

Scenarios have been developed to address the treatment of cattle in the various regions of the U.S. with anthelmintics. The percentage and timing of treatment in each region are based upon **estimated actual** usage (Table 2). The estimated actual represents the experts' assessments regarding the percent of cattle actually being treated with anthelmintics. For injectable and pour-on formulations of anthelmintics, the excretion pattern is less than a month so that months of treatment and the period during which residues are found in feces are approximately the same. However, for the ivermectin bolus formulation, the period of antiparasitic activity is prolonged as is the period of residue excretion in dung. Thus, the relevant information is the percentage of cattle on pasture that are excreting significant anthelmintic residues in the feces. Therefore, the **estimated actual** table has been expanded (Table 3) to reflect potential use of the bolus. In this scenario, cattle receiving a bolus are considered to be excreting ivermectin residues for five consecutive months while cattle treated with injectable or pour-on formulations are considered to be excreting anthelmintic residues for one month, the month of treatment. Also, it can be assumed, based on the information presented in Section 8A.xii.6, that residues in dung pads excreted up to approximately two to three weeks after subcutaneous or pour-on treatments with ivermectin and approximately 5 months post administration of the IVOMEC® SR Bolus will completely inhibit development of dung beetle larvae.

Use of the IVOMEC® SR Bolus would be restricted to only those cattle which weigh 100 to 300 kg at the time of dosing in the spring or summer (or late fall/early winter in the Pacific Coastal region only). Cattle outside of this weight range and time period, if treated, would receive other anthelmintics. These scenarios are conservative, in that they assume that ivermectin is the only anthelmintic used and that all animals in the 100 to 300 kg weight range treated with an anthelmintic in the spring/summer (or late fall/early winter in the Pacific Coastal region only) would be treated with an expensive bolus. This is a gross overstatement of the number of animals likely to be treated with a bolus and is clearly a worst case. Consider that of the range cattle dewormed in 1993-1994, only 67% were treated with an avermectin-based anthelmintic. Further projected sales of the IVOMEC SR Bolus are within the range of 1-1.5 million per year in the US which equates to a range of 4-6% of the eligible cattle (100-300 kg/bw) treated with a bolus.

To determine the numbers of cattle in each region which would be in the weight range to receive a bolus, USDA numbers and treatments for each class of cattle per region were calculated as follows:

Table 2. ESTIMATED ACTUAL ANTHELMINTIC USE BY REGION: PERCENT OF PASTURED CATTLE TREATED

	JAN	FEB	MARCH	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
Upper Southeast			4	21	1		15	4		13	9	
Lower Southeast		3	15	13			13		3	15	7	
South Central			5	9					13	21	9	
Southwest				7	13					4	4	1
New England			6	12	9	3				33	41	8
Hawaii	16	2	8	7	1			3	11	2		2
Big Sky		0		3	3					17	7	
Plains (North Central)		1	2	3	8	1			5	18	14	3
Northern Dairy			4	24	12	1	3	1		5	8	
Pacific Eastern				9	2							
Pacific Coastal	9											37

Table 3. ESTIMATED ACTUAL PERCENTAGES OF PASTURED CATTLE CRETING ANTHELMINTICS BY REGION AND MONTH

	JAN	FEB	MARCH	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
Upper Southeast			4	23	17	16	26	16	1	12	8	
Lower Southeast		3	16	18	11	11	22	6	3	13	7	
South Central			4	9	4	4	4	4	12	20	8	
Southwest				6	16	11	11	11	7	3	3	1
New England			6	15	19	18	17	14	7	33	40	8
Hawaii	16	4	8	11	10	8	7	7	11	2		2
Big Sky	0			3	4	3	3	3	1	14	7	
Plains		1	2	3	9	6	5	5	9	18	13	3
Northern Dairy			4	27	28	23	25	22	7	5	8	
Pacific Eastern				9	5	4	4	4	1			
Pacific Coastal	21	15	15	15	3							37

- Numbers for beef cows, beef calves (spring and fall) and bulls were not adjusted.

- Numbers of beef replacements, dairy replacements, other heifers, steers >500 lbs and calves <500 lbs were partitioned to reflect the fraction of the animals in the weight range to receive a bolus (Eller, 1994. Sec. 14.). USDA statistics for beef and dairy replacements include animals between 1 and 23 months of age; half were considered to be less than 1 year and in the 100 - 300 kg weight range. Other heifers and steers >500 lbs are included in the stocker cattle classification with an upper weight range of 800 lbs. Assuming an equal distribution within the 500 - 800 lbs (227 - 364 kg) weight range, 53% of these cattle would be in the 100 - 300 kg weight range. For calves, assuming an equal distribution within a 34 - 227 kg weight range, 66% would be in the 100 - 300 kg weight range.

- Fall-born beef calves would be dosed with a bolus at weaning. By the next fall, they would have moved into other categories, i.e., beef replacements >1 year or stockers.

- Spring-born beef calves would be also dosed at weaning, but not with a bolus at this time. At this time of year, cattle are moving into winter housing or will be over wintered on pasture with supplemental feeds. In either case, parasite challenge is low. The objective of this fall treatment is to remove acquired parasite burdens to maximize nutrient utilization at a time that cattle are being fed expensive feed concentrates. Thereafter, calves would have moved into other categories, i.e., beef replacements >1 year or stockers.

- Beef replacements <1 year would be dosed only in the fall but not with a bolus. The rationale for not treating with a bolus in the fall is as described in the preceding paragraph.

- Beef replacements >1 year would be dosed in the spring with a bolus. They would not be dosed again in the fall.

- Dairy replacements which are not candidates for a bolus would be dosed in the fall and again in the spring with other anthelmintics. Dairy replacements which are candidates for the bolus would be dosed in the spring with a bolus. They would not be dosed again in the fall.

- Beef cows and bulls are too heavy for bolus treatments.

Percentages of the cattle in various regions of the U. S. excreting anthelmintic residues and reflecting the use of the bolus based upon the **estimated actual** usage data are presented in Table 3. The percentages in Table 3 are based on the assumptions that all cattle in the 100-300 kg weight range that would be treated with an anthelmintic at the appropriate season will be given a bolus and that avermectin-based anthelmintics constitute 100% of the cattle anthelmintic market. Two points need to be made here. First, only 4-6% of eligible cattle, which is equivalent to 1-1.5% of the total cattle on pasture, would receive a bolus. Second, avermectin-based anthelmintics constitute only about 67% of the anthelmintic market. Therefore, in reality, the percentages of cattle on pasture excreting anthelmintics will be smaller than indicated in Table 3 and percentages for those excreting avermectin-based anthelmintics will be even smaller. The overall assessment of any impact of anthelmintic residues in cattle dung upon dung beetle populations is based on **the estimated actual** usage of anthelmintics, which will represent the large majority of users. It is possible, however, that within a limited area of a region a cattle manager would treat all eligible cattle at the maximum recommended level based on husbandry practices and the seasonality of parasite infectivity without regard for cost of product or labor. The extent to which treatment of all eligible cattle with anthelmintics will be practiced is based upon decisions of individual cattle managers. Thus, such a high level of use will very likely only be scattered throughout a region, used by a few but not all cattle managers, in some locales and not in others. Such a locale would be an island of higher usage in a sea of the lower, more-**realistic estimated actual** usage. The percentages of the cattle excreting anthelmintic residues can be compared with the activity patterns for dung beetles in those regions for which such activity data are available.

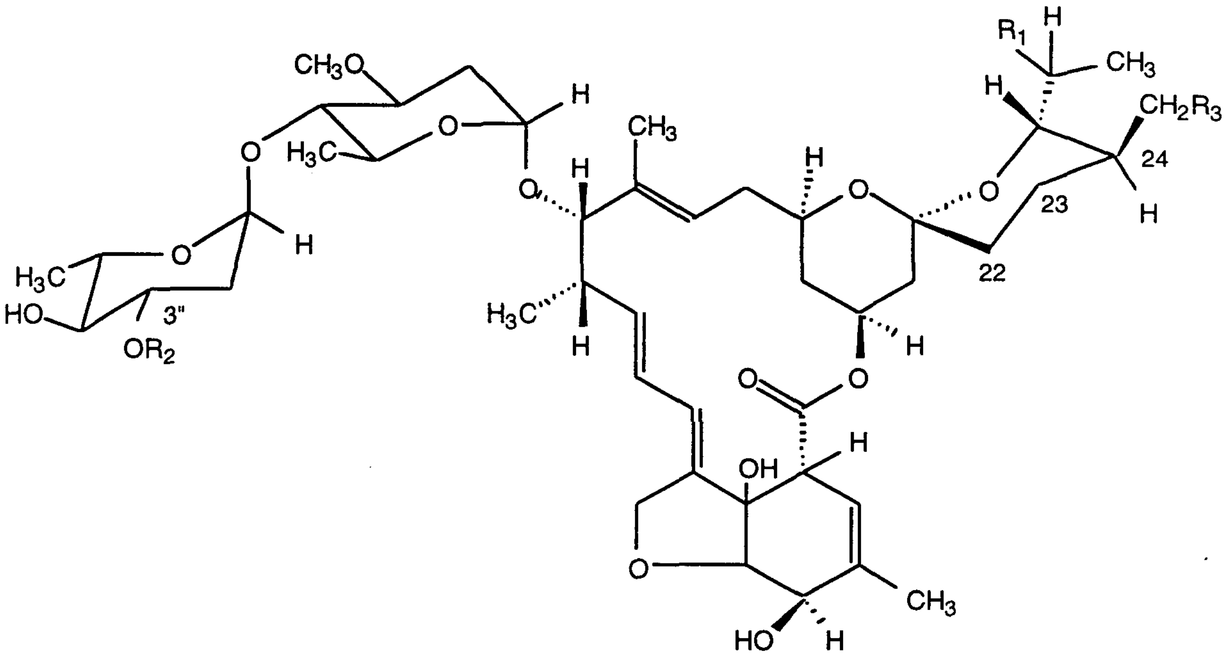
ii) Metabolism

Ivermectin accounts for only 40-50% of the residue in cattle feces following subcutaneous injection of the drug. For calves dosed with ivermectin via the IVOMEC SR Bolus, ivermectin accounts for not less than 75% of the residue in feces. Two metabolites, 24-hydroxymethyl ivermectin and 3″-O-desmethyl ivermectin (Figure 1), account for not more than 18 and 5%, respectively (Narasimhan *et al.*, 1990b, Sec. 16). Indirect evidence suggests that the 24-hydroxymethyl ivermectins are less toxic to *Daphnia* than is ivermectin. The percolate (leachate) from columns prepared from soil and feces from cattle dosed sc with ivermectin is far less toxic toward *Daphnia* than is ivermectin (Halley *et al.*, 1989, Sec. 14). This leachate contained no detectable ivermectin, but the presence of more-polar, drug-related species was demonstrated. The reverse phase HPLC of these species indicates they are more polar than the monosaccharide or aglycone of ivermectin, and have elution characteristics similar to those of the 24-hydroxymethyl metabolites of ivermectin (Halley *et al.*, 1989, Sec. 14). Of the total amount of residue excreted by the animals in the above scenarios, it can thus be assumed that parent drug accounts for not less than 75% of the fecal residue following administration via the IVOMEC SR Bolus. Studies conducted in vivo and in vitro demonstrated the absence of a significant change in ivermectin metabolism in calves treated with the IVOMEC SR Bolus (Narasimhan *et al.*, 1990a, Sec. 16).

iii) Drug residue in excreta

It is projected (Figure 2) that ivermectin-related residues in fecal pats from the 200-kg calves will range from approximately 1000 ppb at the beginning of the treatment period to about 700 ppb just prior to bolus shutdown, whereas with pats from cattle dosed subcutaneously with ivermectin the residue will peak at approximately 600 ppb a day or two post dose and drop exponentially to zero within three to four weeks.

Strong and Wall (1988, 1994, Sec. 14) estimated steady state fecal concentration of approximately 400 and 500 ppb of ivermectin-related residue in the feces of 200-kg calves which received boluses designed to deliver 8 or 12.7 mg/day, respectively.



	R1	R2	R3
H_2B_{1a}	CH_2CH_3	CH_3	H
H_2B_{1b}	CH_3	CH_3	H
3”-0-Desmethyl- H_2B_{1a}	CH_2CH_3	H	H
3”-0-Desmethyl- H_2B_{1b}	CH_3	H	H
24-Hydroxymethyl- H_2B_{1a}	CH_2CH_3	CH_3	OH
24-Hydroxymethyl- H_2B_{1b}	CH_3	CH_3	OH

Figure 1. Structures of ivermectin H_2B_{1a} and H_2B_{1b} and its two fecal metabolites.

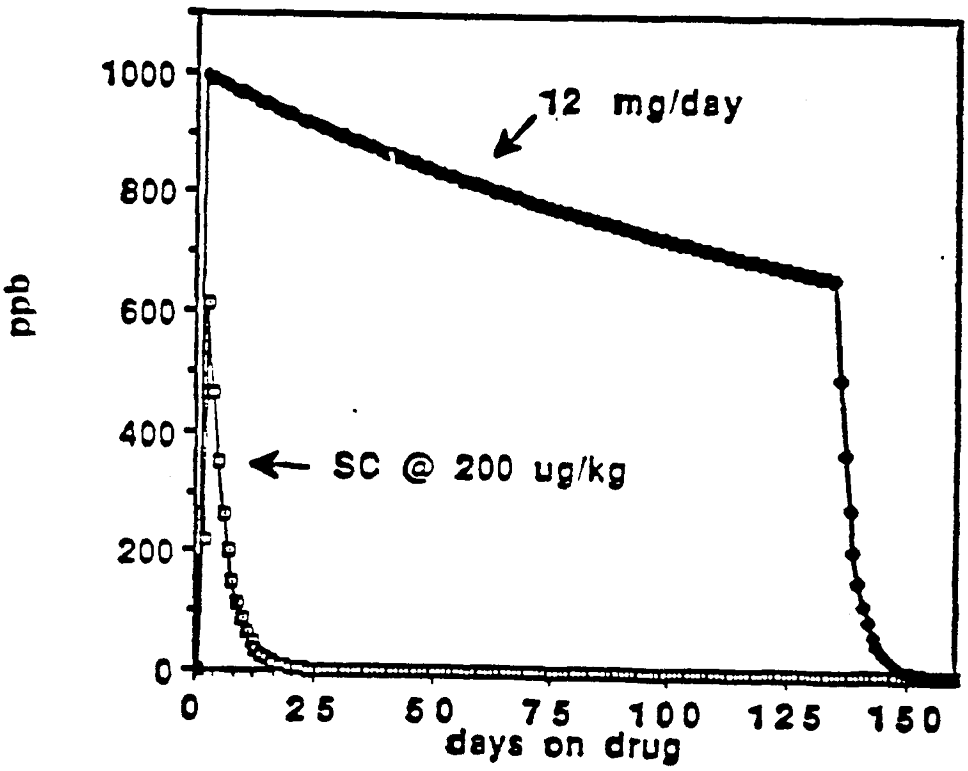


Figure 2. Projected total residue levels in the feces of a 600-kg cow dosed subcutaneously with 200 μg/kg of ivermectin and a 200-kg calf given an IVOMEC SR Bolus designed to deliver 12 mg/day of ivermectin.

Residue levels in dried dung pads or in fresh pads but expressed on a dry weight basis would be approximately 6-8 times higher, assuming 85-88% moisture in the pads (Barth, 1993, Sec. 14).

7. Fate in the environment

Information on the stability of ivermectin in soil and in aqueous extracts of steer, swine and sheep feces and on its soil translocation was presented in the Environmental Assessment of NADA 135-008, IVOMEC® (ivermectin) Injection for Swine (Sec. 16). The present assessment includes additional information on the environmental fate of ivermectin and supporting information on the environmental fate of abamectin, which differs from ivermectin only in that abamectin bears a double bond at position 22,23. Ivermectin is produced from abamectin by catalytic reduction of this double bond .

A. Key fate studies

i) Photodegradation

In a study of the photolysis of ivermectin H_2B_{1a}, Halley (1990, Sec. 16)* used a high-pressure xenon arc lamp to simulate sunlight. Based on the degradation of ivermectin under these conditions, it was calculated that ivermectin would photodegrade near the surface of open, flat bodies of water under clear skies in summer and winter sunlight with half-lives of 12 and 39 hours, respectively. This rapid photodegradation in water should effect swift elimination of ivermectin from the aquatic environment. Based upon data from a preliminary study, ivermectin undergoes photodegradation as a thin, dry film on glass with an estimated half-life of about 3 hours in summer sunlight (Yeager and Halley, 1988, Sec. 16). Avermectin B_{1a} possesses an absorption maximum [E, λ max (methanol) of 31,850, at 243 nm] similar to that of ivermectin (Sec. 5). Additionally, both possess low intensity, long wavelength absorption at approximately 290 and 350 nm. Avermectin B_{1a} photodegrades on soil TLC plates with a half-life of 21 hours (Ku and Jacob, 1983a, Sec. 16). Rapid photodegradation is consistent with the rapid loss of avermectin B_{1a} from the surface of cotton leaves (Bull *et al.*, 1984, Sec.14)**. Radiobalance data indicated that slightly more than one-half of applied radioactivity remained on the leaves at 2 days post-treatment, but only one-third of the recovered radioactivity was starting compound. A nonpolar photodegradation product of avermectin B_{1a} has been identified as the $\Delta^{8,9}$-isomer (Ku and Jacob, 1983a, Sec. 16).

ii) Mobility in soil

Compounds possessing K_{oc} values greater than 1000 are tightly bound to the soil organic matter, and such compounds can be considered to be immobile in soil. As ivermectin has K_{oc} values of 12,600 and 15,700 with Iowa clay loam and Missouri silty clay loam soils, respectively, this drug has been classified as tightly bound to soil and hence immobile (Halley, 1985, Sec. 16). Avermectin B_{1a} was found to be immobile on soil TLC plates (six soil types) with water as the developing solvent (Ku and Jacob, 1983b, Sec. 16; Gruber *et al.*, 1990, Sec. 14), demonstrating the tight binding to soil of this close structural relative of ivermectin.

Consequently, the possibility of translocation of ivermectin through soil from one site to another in the environment is remote. When ivermectin was partitioned between water and Iowa soil, a soil to water distribution of 333 was found, predicting that 99.7% of the drug would be bound, with only 0.3% in solution (Halley, 1985, Sec. 16).

iii) Fate in feedlot runoff

The environmental fate of ivermectin in cattle feedlots was evaluated following a request by the FDA (Nessel *et al.*, 1989, Sec. 14). This study (carried out in the month of June) was designed to determine the potential for ivermectin runoff from a cattle feedlot following treatment of five steers (about 365 kg each) with ivermectin (200 µg/kg) via subcutaneous injection. Surface and subsurface water samples from the dirt feedlot pen (20 x 50 ft) were collected over a 28-day period following dosing and assayed for ivermectin using toxicity toward *Daphnia* which has a limit of detection for ivermectin of 10 ppt. The water samples were also analyzed by HPLC for the H_2B_{1a} component of ivermectin. No ivermectin-related toxicity was observed, nor was any (10 ppt or greater) ivermectin found in the water samples by HPLC. Essentially all of the subcutaneously administered dose, a total of 365 mg for five steers, or 73 mg per steer, would have excreted (approximately 50% as ivermectin, 50% as

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* Supporting information has been summarized and compiled in Sec. 16.

** Literature cited, Sec. 14.

metabolites) during this study into an area of only 1000 sq ft (365 µg/sq ft). Nevertheless, the runoff water showed no ivermectin-related toxicity toward *Daphnia*; further, HPLC analysis demonstrated that H_2B_{1a} (major component of ivermectin) concentrations were below 10 ppt, the assay detection limit.

iv) Aerobic degradation in soil

Ivermectin degrades rapidly outdoors in soil/feces mixtures during the summer (half-life of 7 to 14 days) to more-polar compounds, and this would preclude accumulation of ivermectin in soil (NADA 135-008, Sec. 16). The rate of degradation of ivermectin in soil/feces mixture is reduced in winter (half-life of 91-217 days). As the dung pats degrade, residual ivermectin will be exposed to sunlight and hence undergo photodegradation.

Laboratory studies (Bull *et al.*, 1984, Sec. 14) have shown that under aerobic conditions in soil, [^{3}H]avermectin B_{1a} degrades to at least thirteen radioactive products; half-lives for the drug (at 1 ppm) in Lufkin fine sandy loam, Houston clay and coarse sand soils are 14-28, 28-56, and 56 days, respectively. The major degradation product is an approximately 1:2.5 equilibrium mixture of 8α-hydroxyavermectin B_{1a} (an acetal) and the corresponding ring-opened aldehyde. At all treatment levels in Lufkin fine sandy loam, 90% degradation of [^{3}H]avermectin B_{1a} occurs within 168 days of exposure. Avermectin B_{1a} is strongly absorbed by ditch-bottom sludge (Vonk and van den Hoven, 1985, Sec. 16) and other soil types and is immobile (Ku and Jacob, 1983b, Sec. 16; Gruber *et al.*, 1990, Sec. 14). It would follow that ivermectin will be affected in a similar manner.

v) Uptake by vegetation

Low levels (≤0.1 ppm) of radioactivity were found in the leaves and stems of cotton seedlings grown in Lufkin fine sandy loam containing 10 ppm of [^{3}H]avermectin B_{1a}; some radioactivity (≥3 ppm) was found on the seedling roots, but whether it was absorbed or adsorbed was not determined (Bull *et al.*, 1984, Sec. 14). Little radioactivity from labeled avermectin B_{1a} or its degradates was taken into the vascular system of the cotton seedlings. This low level of uptake is consistent with the observed lack of phytotoxicity for a number of other plant species grown in soil containing avermectin B_1 (NADA 135-008, Sec. 16). The observed lack of pronounced systemic insecticidal activity for ivermectin and avermectin B_1 in plants also indicates little or no uptake of these compounds by plants (NADA 135-008, Sec. 16).

The slight uptake by cotton seedlings of radioactivity from soil containing [^{3}H]avermectin B_{1a} suggests that, if soil were to contain the close structural analog ivermectin, uptake of the latter by plants grown in the soil would also be minor (Bull *et al.*, 1984, Sec. 14). These authors also reported no uptake of radioactivity by grass from a plot treated with [^{14}C]avermectin B_{1a} ant bait formulation, nor is there any pesticidal activity of avermectin applied systematically (soil). These observations support the view that avermectin is not taken up from soil by plants.

Relatively low radioactive residues were found in crops (sorghum, lettuce, carrots and turnips) grown in three types of soil to which [^{14}C]avermectin B_{1a} had been applied 3 to 12 times at 0.025 to 0.030 lb/acre/application (Moye and coworkers, 1987, Sec. 14). Radioassay of the crops indicated a maximum total residue of 14 ppb. As only 4.4% of the total radioactive residue in a lettuce leaf was extractable with acetone, it is clear that most of the residual radioactivity is either chemically different from avermectin B_{1a} or present in a strongly bound form (probably incorporated into the vegetable matter as endogenous small molecules resulting from degradation of the avermectin B_{1a}).

B. <u>Fate scenarios for translocation of ivermectin in water through soil from dung pats deposited near bodies of water</u>

The high K_{oc} values for ivermectin demonstrate that this compound binds tightly to organic matter in soil, and likely also to organic matter in dung pats. One can predict that, if a dung pat were dispersed in water, less than 1% of the fecal ivermectin would partition into the water. Hence, very little of the ivermectin in a dung pat would be expected to partition into water in contact with dung in a pasture. This is surely a reasonable assumption, as only the outer surface and not the entire dung pat would be in contact with water. Further, if a hard outer crust formed on the surface of the pat, as is the case in summer, entrance of rainwater into the pat would be hindered (Marsh and Campling, 1970; Dickinson *et al.*, 1981, Sec. 14). The calculated TLC R_f for ivermectin in soil, based upon its K_{oc} value of 12,600, is 0.003 to 0.004 (Halley, 1985, Sec. 16). This is consistent with the immobility of avermectin B_1 on soil TLC plates with water as a solvent (Ku and Jacob, 1983b, Sec. 16; Gruber *et al.*, 1990, Sec. 14). Because of the tight binding of ivermectin to soil (soil to water distribution of 333, indicating greater than 99% binding) and its low water solubility of 4 ppm, only an insignificant fraction of the ivermectin initially present in water in contact with dung pats would be expected to move with water flowing away from a pat through soil and ultimately into a body of water. This insignificant amount would be further depleted from water (in addition to the initial 99%) as the flow of ivermectin-containing water percolated through additional soil, resulting in continuous depletion of ivermectin from solution. Although achievement of equilibrium (99% bound) does not occur instantaneously, the binding process must be very rapid, based on the immobility of avermectin B_1 on soil TLC plates (Ku and Jacob, 1983b, Sec. 16; Gruber *et al.*, 1990, Sec. 14). If it were not, the avermectin B_1 would have been carried by the water solvent up the soil TLC plate, rather than remaining at the origin. By analogy, ivermectin (calculated soil TLC R_f of 0.003-0.004) in contact with soil would not be expected to be readily transported by water across soil particle surfaces.

Consider an initial concentration of ivermectin in dung of 1000 ppb. This value reflects the estimated concentration anticipated from use in a 200-kg calf (Table 4), but ignores the fact that up to 25% of the residue will not be ivermectin (Narasimhan *et al.*, 1990b, Sec. 16). It can be reasonably estimated, assuming 90% inaccessibility of the interior of the pat to water, and binding (99%) to organic matter, that the concentration of unbound ivermectin residue in water that is in direct contact with the dung pat would be 1 ppb (i.e., 1000 x 0.1 x 0.01).

TABLE 4
EFFECT OF SOIL BINDING UPON IVERMECTIN CONCENTRATIONS ARISING FROM DUNG PATS CONTAINING DRUG RESIDUES

Initial Conc. in Pat	Conc. in Water in Contact with Dung Pat[a]	Conc. entering Pond Following Movement Through Soil[b]	Conc. in Pond Following Binding to Soil Sediment[c]
1000 ppb	1 ppb	0.1 ppt	0.001 ppt[d]

[a] Assumes 0.1% leached from dung pat (only 10% of pat accessible to water, and 1% of drug not bound to fecal matter)
[b] Assumes two losses of 99%.
[c] Assumes a third loss of 99%.
[d] Scenario assumes no dilution by rainwater or by water in pond, and that all the residue is ivermectin.

The ivermectin residue concentration in water en route to a nearby body of water (e.g., pond) would be decreased by multiples of 99% because of adsorption to soil. Just two adsorption/desorption equilibrations would reduce the above concentration, by a factor of 10,000, to 0.1 ppt. A greater distance between the dung pat and the body of water would require more extensive movement, through soil, of water carrying dissolved unbound ivermectin, resulting in further binding and greater reduction in available ivermectin. This scenario ignores the slow release of ivermectin from dung pats, resulting from the slow degradation of pats. During this time period, ivermectin would be undergoing decomposition. Even if traces of ivermectin were to reach a body of water, as it entered, up to 99% of the drug would be bound by suspended soil particulates and sediment (to give an estimated concentration of 0.001 ppt). These concentration changes are summarized in Table 4, and do not include dilution effects (which would surely pertain, both on a pasture surface during a rainstorm, and especially as the ivermectin-containing water from the pasture entered the body of water). Further, the unbound ivermectin in the pond would undergo rapid photodegradation with calculated summertime and wintertime half-lives of approximately 12 and 39 hours, respectively (Halley, 1990, Sec. 16), and the initial concentrations would decrease in 4 days by factors of ~16 and ~2 during summer and winter, respectively (Figure 3).

The above scenario is supported by results from the cattle feedlot runoff study [Sec. (7)(A)(iii)]. The 365 mg of ivermectin-related compounds excreted per 1000 sq ft (15899 mg/acre, or 365 µg/sq ft) in the cattle feedlot runoff study can be compared with the 3440 mg of ivermectin-related compounds excreted per acre (79 µg/sq ft) when two calves receiving ivermectin via the bolus route are pastured on one acre. In the cattle feedlot study, less than 10 ppt of ivermectin was present in the water (HPLC assay). Therefore, in the pastured calves situation, less than 2 ppt of ivermectin- related compounds would be expected in runoff water (prior to its flow across land toward a body of water). Recall that the tight binding to soil of the excreted ivermectin greatly attenuated the effective ivermectin concentration in the cattle feedlot runoff water. This is consistent with reduced toxicity of ivermectin (Ostlind and Cifelli, 1980, Sec. 16) and avermectin B_1 (Forbis, A.D., 1989, Sec. 16) toward *Daphnia* as a result of tight binding (99%) to soil [Sec. (7)(A)(ii)]. Consequently, the possibility of transport of ivermectin from dung pats in a pasture by water runoff is remote.

C. Fate scenarios for direct introduction of ivermectin residue into bodies of water

The worst-case scenario for introduction of ivermectin into a body of water would result from calves defecating into a pond or stream. A farm pond one acre in area with an average depth of 4 ft contains 174,000 cu ft or 4.9 x 10^6 liters of water. With the IVOMEC SR Bolus the daily fecal output of the drug from 50 calves [12 mg of residue per steer per day, assuming 100% (in reality at least 75%) ivermectin] is 600 mg of the drug. Most of the ivermectin would enter the water "prebound" to organic matter in the feces. Then, at least 99% of the available ivermectin would be bound to suspended soil, sediment and organic matter in the pond. This would result in a concentration of 0.012 ppt,

$$\frac{600 \text{ mg}}{100 \times 100 \times 4.9 \times 10^6 \text{ L}}$$

This concentration would be further reduced by photodegradation.

Further, it is highly unlikely that each of the animals would deposit its entire daily dung output into the pond, as cattle defecate 10-12 times per day (Marsh and Campling, 1970, Sec. 14).

With respect to direct deposition of ivermectin residue-containing dung into a slowly moving stream, consider 50 calves (12 mg ivermectin residue per steer per day fecal output) standing in a 2-ft wide by 2-ft deep stream flowing at 1 mile/h (1.6 km/h = 26.7 m/min). If 10% (one out of ten defecation events, a high proportion) of the calves' daily fecal output (0.1 x 600 mg) of ivermectin - 60 mg - were to be introduced into the stream during a 10-minute period, the ivermectin would be diluted by 130,830 L of water (10 min x 26.7 m/min x 0.7 m x 0.7 m x 1000 L/m^3) flowing past the calves. Assuming that 99% of the ivermectin were prebound to fecal matter, and 99% of the unbound ivermectin then bound to suspended soil, sediment and organic matter in the stream, the concentration of ivermectin in the stream would be 60 mg x 0.01 x 0.01 (free fraction)/ 1.3 x 10^5 L = 0.046 ppt. This scenario assumes that all 50 calves defecate during the same 10-minute period, a highly unlikely event based on defecation rates of 10-12 per day. Faster flowing streams would have greater dilution volumes and therefore lower ivermectin levels.

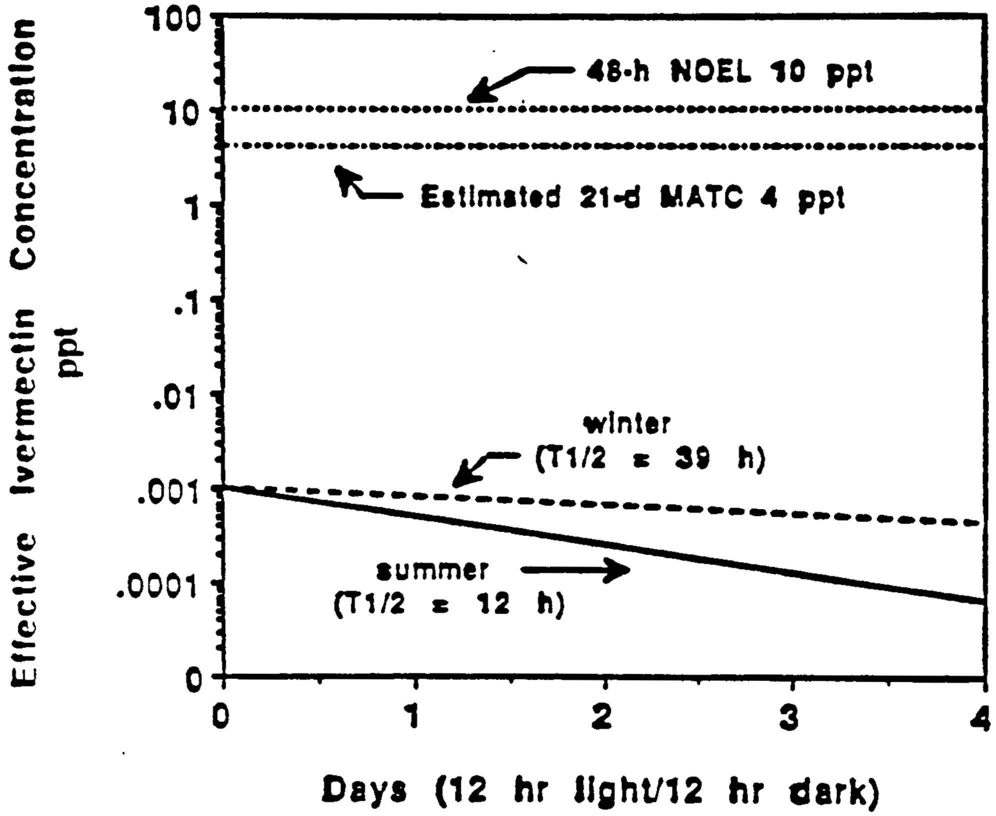

Figure 3. Comparison of ivermectin 48-h NOEL and estimated 21-d MATC for *Daphnia* with effective ivermectin concentration in pond as impacted by photodegradation. Scenario involves introduction of ivermectin from dung pats deposited near bodies of water.

D. Summary of ivermectin fate studies and scenarios

The impact of the tight soil binding and degradation characteristics of ivermectin upon the estimated concentration of the drug entering the environment are addressed below.

Ivermectin in contact with soil is unlikely to undergo translocation sufficient to lead to concentrations even approaching 10 ppt in a nearby body of water. This conclusion is supported by results of the cattle feedlot runoff study [Sec. (7)(A)(iii)]. Runoff water collected directly from the feedlot (365 µg ivermectin-related residue per sq ft), and not at a distance from it, was not toxic to *Daphnia* and was shown by HPLC analysis to contain <10 ppt of the drug. In a pasture with calves treated with an IVOMEC SR Bolus, the expected level of excreted ivermectin-related residue would be only 79 µg/sq ft, approximately 20-25% that in the cattle feedlot. This assumes all pats are available at the same time, even though they will be deposited over a 135-day period, and that no degradation of ivermectin occurs within this period of time. Also, only a minimal amount (surface only) of the pats in a pasture will be exposed to rainfall (hence, ivermectin is less available for leaching) and any leached ivermectin will have to move across significant distances to reach a body of water. It thus appears very reasonable to conclude that runoff water from a pasture would contain far less than 2 ppt ivermectin-related residue.

Given the tight binding of ivermectin to organic matter and soil [Sec. (7)(A)(ii)], which greatly reduces its effective concentration, significant transport of ivermectin residues from pastures to bodies of water in the vicinity is highly unlikely. The tight binding of ivermectin to soil sediment and organic matter in a body of water will very significantly reduce the effective aqueous concentration of this drug in a pond or stream. Both oxidative degradation in soil under aerobic conditions and photodegradation (especially in water) will diminish the environmental concentration of ivermectin. Based on the discussion of photodegradation, soil binding and soil metabolism [Sections (7)(A)(i), (ii) and (iv), respectively], it can be reasonably predicted that ivermectin present in the environment would not be expected to undergo significant movement or translocation, and would not accumulate. Given its environmental fate characteristics, ivermectin will be readily eliminated from the aquatic and terrestrial environment.

8. Environmental effects of released substances

A. Environmental effects studies

i) Toxicity towards *Daphnia*

Daphnia, the freshwater aquatic species found to be most sensitive to ivermectin (48-hr $LC_{50,}$ 48-hr NOEL and estimated 21-day MATC of 25, ~10 and 4 ppt, respectively), will be used for aquatic hazard assessment purposes. The effects of ivermectin, avermectin and related compounds upon a number of aquatic species (including *Daphnia*), as determined in laboratory tests, were reported in previously submitted environmental assessments, and are summarized in Table 5.

TABLE 5
EFFECT OF IVERMECTIN (AVERMECTIN B_1) AND RELATED COMPOUNDS UPON AQUATIC SPECIES

COMPOUND	SPECIES	EFFECT	REFERENCES
Ivermectin	Daphnia	48-Hours LC_{50} 25 ppt	Halley, et. al., 1989 Sec. 14
Ivermectin (H_2B_{1a}) Monosacharide	Daphnia	48-Hours LC_{50} 400 ppt	Halley, et. al., 1989 Sec. 14
Ivermectin (H_2B_{1a}) Aglycone	Daphnia	48-Hours LC_{50} >1700 ppt[a]	Halley, et. al., 1989 Sec. 14
Ivermectin	Daphnia	48-Hours NOEL ~10 ppt	Halley, et. al., 1989 Sec. 14
Feces from Ivermectin-Dose Steer/Soil Column Percolates[b]	Daphnia	48-Hours LC_{50} >>3,200 ppt[c]	Halley, et. al., 1989 Sec. 14
Ivermectin	Bluegill Sunfish	96-Hours LC_{50} 5.3 ppb	NADA 135-008, Sec. 16
Ivermectin	Rainbow Trout	96-Hours LC_{50} 3.3 ppb	NADA 135-008, Sec. 16
Avermectin B_1	Daphnia	48-Hours LC_{50} 340 ppt	Surprenant and LaBlanc, 1981, Sec. 16
Avermectin B_{1a}	Bluegill Sunfish	Estimated Label Threshold 6.7 ppb NOEL 2.3 ppb (Dynamic 7-Day Toxicity Study)	Forbis, 1983, Sec. 16
Avermectin B_1	Carp	96-Hours LC_{50} 42 ppb	Douglas & Pell, 1985, Sec. 16
Avermectin B_1	Channel Catfish	96-Hours LC_{50} 24 ppb	McAlister et. al., 1985, Sec. 16
Avermectin B_1	Mysid Shrimp	96-Hours LC_{50} 22 ppb	Surprenant, D.C., 1988a, Sec 16
Avermectin B_1	Sheepshead Minnow	96-Hours LC_{50} 15 ppb	Ward, 1985, Sec. 16

TABLE 5 (Continued)
EFFECT OF IVERMECTIN (AVERMECTIN B_1) AND RELATED COMPOUNDS UPON AQUATIC SPECIES

COMPOUND	SPECIES	EFFECT	REFERENCES
Avermectin B_1	Oyster	48-Hours EC_{50} 430 ppb	Ward, 1985, Sec. 16
Avermectin B_1	Rainbow Trout	96-Hours LC_{50} 3.6 ppb	Sousa, J.V. 1981, Sec. 16
Avermectin B_1	Bluegill Sunfish	96-Hours LC_{50} 9.6 ppb	Wilson, 1981, Sec. 16
D8,9Avermectin B_{1a} (photochemical degradation product of avermectin B_{1a})	Daphnia	48-Hours LC_{50} 14 ppb	Forbis, et. al., 1985a, Sec. 16
8a Hydroxyavermectin B_{1a} (aerobic soil degradation product of avermectin B_{1a})	Daphnia	48-Hours LC_{50} 26 ppb	Forbis et. al., 1985b, Sec. 16
Avermectin B_1	Daphnia (Life Cycle)	21-day MATC 0.03-0.09 ppb ACR[d] 6.5	Surprenant, D.C. 1984, Sec. 16
Ivermectin	Daphnia (Life Cycle)	Estimated MATC 4 ppt	Calculated Value[e]
Avermectin B_1	Mysid Shrimp (Life Cycle)	28-day MATC 0.0035-0.0095ppb ACR 3.6	Surpenant, D.C., 1988b, Sec. 16
Avermectin B_1	Rainbow Trout (ELS)	MATC 0.52-0.96 ppb ACR 4.6	McAlister, W.A., 1986.
Avermectin B_1	Duckweed	14-day EC_{50} 3900 ppb	Hollister, 1981a, Sec. 16
Avermectin B_1	Selenastrum capricornutum	9-day EC_{50} 100,000 ppb	Hollister, 1981b, Sec. 16
Ivermectin	Chlorella pyrenoidosa	Maximum Growth Rate, No Effect at 10,000 ppb	Halley, et. al., 1989, Sec. 14

[a] LC_{50} could not be determined accurately as the highest concentration of the aglycone studied was 17,000 ppt.

[b] Feces from steers (see footnote[c]) dosed with radiolabeled ivermectin was mixed with soil and applied to the tops of soil columns. Water was allowed to percolate through the columns and tested.

[c] Because the low concentrations and low toxicities of ivermectin-related compounds in feces/soil column percolates limited the extent of testing, sufficient data could not be collected to calculate toxicities accurately.

[d] ACR = Acute to Chronic Ratio; LC_{50}/MATC (Maximum Acceptable Toxicant Concentration).

[e] An estimated MATC for ivermectin was calculated from the 21-day MATC for avermectin (30 to 90 ppt; geometric mean of 52 ppt) and the ratio of the ivermectin and avermectin 48-hr LC_{50} values for *Daphnia* (25 and 340 ppt, respectively): X/52 = 25/340; X = 4 ppt.

[f] Early life stage, 60-day study.

Ivermectin and avermectin B_1 show comparable aquatic toxicity; however, ivermectin is more toxic to daphnids than is avermectin B_1. The concentrations at which toxicities are observed in these tests should be regarded as "worst-case" and not be directly compared to estimated exposure concentrations in Sec. (6)(B)(i)(b) without consideration of the environmental fate of ivermectin discussed in Sec. 7. Ivermectin and avermectin B_1 show comparable mammalian toxicity (Lankas and Gordon, 1989, Sec. 14).

ii) Toxicity toward fish

Fish are at least 100-fold less sensitive to the toxicity of ivermectin than are *Daphnia*. The ivermectin 96-hr LC_{50} values (Table 5) for rainbow trout and bluegill sunfish are 3.3 and 5.3 ppb, respectively. In general, the acute toxicity of avermectin toward fish [e.g., LC_{50} values of 3.6 and 9.6 ppb for rainbow trout (Sousa, 1981, Sec. 16) and bluegill sunfish (Wilson, 1981, Sec. 16), respectively] is approximately the same as that exhibited by ivermectin.

iii) Bioconcentration in sunfish

The bioconcentration of $[^3H]$avermectin B_{1a} by the bluegill sunfish is modest and occurs gradually (Forbis and Franklin, 1983, Sec. 16). In water containing 0.099 µg of test compound per liter (0.099 ppb) the daily bioconcentration factor for whole fish was only 19 to 69, with a tissue uptake concentration for whole fish of 1.9 to 6.8 ppb; accumulation ceased by about day ten. A 95 percent clearance of radioactivity for whole fish was found for a 14-day depuration period; the whole-fish concentration dropped from 6.8 to 0.32 ppb (day 14). This bioconcentration value of less than 100 and the rapid depuration are favorable, as they demonstrate that avermectin B_{1a} (and hence ivermectin) in fish does not concentrate and is not retained.

iv) Toxicity toward other aquatic species

The toxicity of ivermectin and abamectin toward other aquatic species is also presented in Table 5. Ivermectin has a moderate effect upon the growth characteristics of Chlorella pyrenoidosa, a fresh water unicellular, non-motile chlorophyte, at the relatively high concentrations of 1 to 10 ppm (Halley *et al.*, 1989, Sec. 14). Avermectin B_1 exhibits 14- and 9-day EC_{50} values of 3,900 and 100,000 ppb, respectively, with duckweed (Hollister, 1981a, Sec. 16) and a freshwater algae Selenastrum capricornutum (Hollister, 1981b, Sec. 16).

v) Phytotoxicity

The low phytotoxicity toward six plant species (cucumber, lettuce, soybean, perennial ryegrass, tomato and wheat) has been demonstrated with ivermectin in both a seed germination and root elongation study (Feutz and Stuerman, 1995a, Section 16) and a seedling growth study (Feutz and Stuerman, 1995b, Section 16). The results (NOEC Values) from the studies are presented below (Table 6). All NOEC values were based on mean measured concentrations.

TABLE 6
RESULTS FROM THE SEED GERMINATION AND ROOT ELONGATION PHYTOTOXICITY STUDY WITH IVERMECTIN

SPECIES	NOEC, ppm	
	GERMINATION	ROOT ELONGATION
Cucumber	≥ 980	98
Lettuce	≥ 980	≥ 980
Soybean	≥ 930	≥ 930
Perennial Ryegrass	≥ 980	98
Tomato	≥ 980	≥ 980
Wheat	≥ 930	≥ 930

RESULTS FROM THE SEEDLING GROWTH PHYTOTOXICITY STUDY WITH IVERMECTIN IN SAND

SPECIES	NOEC, ppm		
	SHOOT LENGTH	SHOOT WEIGHT	ROOT WEIGHT
Cucumber	0.68	0.68	≥ 790
Lettuce	6.9	0.68	≥ 790
Soybean	≥ 790	6.9	≥ 790
Tomato	0.68	0.68	0.68
Wheat	6.9	0.68	0.56

In addition, a seedling growth study was conducted with perennial ryegrass in sand and sandy loam soil (Feutz and Stuerman, 1995b, Section 16). The low phytotoxicity of ivermectin to perennial ryegrass was further reduced by approximately 2000-fold, as measured by the NOEC for shoot weight (the most sensitive parameter) in sandy loam soil relative to that in sand. The results are reported in Table 7.

TABLE 7
RESULTS FROM THE SEEDLING GROWTH PHYTOTOXICITY STUDY FOR PERENNIAL RYEGRASS WITH IVERMECTIN IN SAND AND SANDY LOAM

GROWTH MEDIUM	NOEC, ppm		
	SHOOT LENGTH	SHOOT WEIGHT	ROOT WEIGHT
Sand	7.7	0.57	≥ 780
Sandy Loam Soil	≥1100	≥1100	≥1100

vi) Toxicity toward avians

The acute toxicity of avermectin B_1 (MK-936), when administered as a single oral dose, was determined for the bobwhite quail (Beavers, Jaber and Faulcon, 1983a, Sec. 16) and mallard duck (Beavers and Fink, 1981, Sec. 16). Although no ivermectin avian toxicity data are available for these two species, avermectin B_1 and ivermectin are very close structurally (differing by only a double bond), and data on the former compound should be directly applicable to the latter. LD_{50} values with avermectin were >2000 mg/kg and 85 mg/kg for the quail and duck, respectively (see Table 8); at all dosage levels the mallards regurgitated immediately after dosing.

TABLE 8
AVIAN SAFETY OF AVERMECTIN B_1 (MK-936) IN BOBWHITE QUAIL AND MALLARD DUCK

	BOBWHITE QUAIL	MALLARD DUCK
Acute, Oral LD_{50}[a]	>2000 mg/kg	85 mg/kg[b]
Subacute Eight-Day Dietary LC_{50}[c]	3102 ppm	383 ppm

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[a] Single oral dose, birds observed for 14 days.
[b] Regurgitation observed at each dosing level tested.
[c] Birds exposed to drug in feed for 5 days, then maintained on avermectin B_1-free diet for 3 days.

With respect to sublethal effects, at the lowest dosage level employed in the mallard duck LD_{50} test (10.0 mg/kg), slight lethargy and loss of coordination occurred immediately following dosing and lasted through day one. At 17.8 mg/kg, prostrate posture and lower limb rigidity were evident, but all birds appeared normal by day two. These results are similar to those observed by Schepkens *et al.* (1985, Sec. 14), who treated pigeons with ivermectin to eliminate parasites; no adverse reactions followed oral dosing at 4.3 mg/kg, whereas at the higher dose rates of 17.4-20.3 mg/kg some impairment of equilibrium and vision was noted. The subacute LC_{50} values for avermectin B_1, when administered via the feed in an eight-day dietary study, were found to be 3102 ppm for the bobwhite quail and 383 ppm for the mallard duck (Beavers, Jaber and Faulcon, 1983b and c, respectively, Sec. 16; Table 8). At the lowest concentration studied (162 ppm avermectin) in the mallard, lethargy, reduced reaction to external stimuli, wing droop, loss of coordination and lower limb weakness were observed as sublethal effects within three hours exposure to the avermectin-containing diet. These effects lasted only during the on-drug phase of the study, and all birds appeared normal 24 hours following their return to the basal diet.

A definitive 18-week avian reproduction study was performed in male and female mallard ducks exposed to avermectin B_1 at levels of 3, 6, and 12 ppm in the diet for approximately 10 weeks prior to egg laying and continuing through the period (Beavers, Jaber and Hinken, 1987a, Sec. 16). The mallard duck was chosen as the test species as it is at least one order of magnitude more sensitive to the toxicity of avermectin B_1 than is the bobwhite quail. The birds showed no treatment-related mortality, overt signs of toxicity or effects upon body weight or feed consumption. No statistically significant differences, compared to control birds, were noted in the number of eggs laid or in the number of hatchlings from live 3-week embryos. Thus, chronic exposure of mallard ducks to avermectin B_1 at concentrations of 3, 6, and 12 ppm in the feed did not affect overall reproductive success. This demonstrates a no observable effect level (NOEL) of 12 ppm. It was observed in a range-finding study (Beavers, Jaber and Hinken, 1987b, Sec. 16) that, at a concentration of 64 ppm of avermectin B_1 in the diet (6-week feeding), mallard ducks laid fewer eggs and the hatchability of the eggs laid was reduced. No other signs of sublethal toxic effects were observed, however, even at this high level. The NOEL is 64 ppm for all except reproductive effects.

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

vii) Effect on nitrification

Avermectin B_1 has no effect upon nitrification in humic sandy or loam soils at up to 0.4 mg/kg soil, or 0.4 ppm (Barug and van Agteren, 1985, Sec. 16). There was no effect upon nitrification or respiration (Halley *et al.*, 1989, Sec. 14) for soil containing 30 ppb (the highest concentration tested) of ivermectin and metabolites in feces from subcutaneously dosed (300 μg/kg) steers.

viii) Antibacterial and antifungal activities

Avermectins do not have significant antibacterial activity except at extremely high concentrations (Burg and Stapley, 1989, Sec. 14). Onishi and Miller (1985, Sec. 14) reported that avermectin B_{1a} lacks detectable antifungal activity at 400 ppm. Using standard antibacterial and antifungal screens, ivermectin has been shown to have no antibacterial or antifungal effects at concentrations as high as 2000 ppm (Halley *et al.*, 1989, Sec. 14).

ix) Effect on methanogenesis

Avermectin B_1 was found to impair the total gas production and the methane production of anaerobic methane-forming bacteria above a concentration of 1000 mg/L or 1000 ppm, the NOEC (Hanstveit *et al.*, 1985, Sec. 16). The EC_{50} for total gas production was determined (by extrapolation) to be >>3200 mg/L; a significant inhibition of methane production rate could not be detected.

x) Earthworm toxicity

The LC_{50} earthworm toxicity for ivermectin is 315 mg/kg soil (315 ppm) and the corresponding 28-d NOEL is 12 ppm (Halley et al., 1989, Sec. 14).

Gunn and Sadd (1994, Sec. 14) studied the effect of a commercial formulation of ivermectin, added directly to soil, on the earthworm Eisenia fetida. They reported a 14-day LC_{50} value of 15.8 mg/kg on a dry-soil weight basis, a NOEL based on weight change of 4 mg/kg and a NOEL based on cocoon formation of 2 mg/kg. They assumed 1) that the emulsifiers and stabilizers in the formulation had no effect on the observed toxicities and 2) that these emulsifiers and stabilizers would be present in the feces of treated animals. Since the emulsifiers and stabilizers are present at much higher concentrations than the ivermectin in the tested formulation, the observed toxicities might have been due, in part, to the emulsifiers and stabilizers or to a synergy (altered solubility or enhanced uptake) between the compounds present. The emulsifiers and stabilizers are much more water soluble than ivermectin and would likely be eliminated in the urine or metabolized before being eliminated. Therefore, neither assumption is valid and the results of this study are of little relevance.

xi) Toxicity toward insects

Both ivermectin and avermectin are toxic toward a wide variety of agricultural pests including the Mexican bean beetle, Southern army worm, aphids and mites. The effect of ivermectin upon animal ectoparasites including flies, fleas, lice, ticks and mites has also been determined (Fisher and Mrozik, 1984, Sec. 14). Review articles by Strong and Brown (1987, Sec. 14) and Dybas (1989, Sec. 14) discuss the avermectins in insect control.

Drug residues in the manure of ivermectin-treated animals can affect insects, including pests, associated with fecal pats. In general, the toxicity of ivermectin in dung toward insects is species dependent, and larvae of flies and beetles are more sensitive to ivermectin in dung than are adult insects. This view is supported by results published by a number of investigators. For example, Miller *et al.* (1981, Sec. 14) reported that drug residues in the feces (day 9 of treatment) from steers given ivermectin daily at 5 µg/kg (oral capsules) were lethal to all horn fly and face fly larvae, but only to approximately 60% of stable fly larvae. Even at a dose rate of 1 µg/kg/day, all horn fly larvae were killed by the manure. These authors observed that a single subcutaneous dose of ivermectin at 0.2 mg/kg prevented development (>93% mortality) of horn flies in steer manure collected for up to four weeks post dose, whereas with stable flies over the same period, mortality averaged less than 40%. This observation was confirmed by Schmidt (1983, Sec. 14), who reported that adult horn flies failed to emerge from manure produced by cattle on day 1 to 28 following treatment with ivermectin (0.2 mg/kg <u>im</u> injection). In contrast, adult stable flies emerged from all manure samples. Control of manure-breeding horn flies and face flies is a desirable characteristic of the ivermectin bolus, as these pests cause significant economic losses to cattlemen.

With respect to non-Diptera species, Schmidt (1983, Sec. 14) found that emergence of several insects (e.g., sphaerocerids and sepsids) from manure containing ivermectin residues was greatly reduced; however, the manure did not kill all dung-dwelling insect species, for the populations of both gnats and staphylinids in dung from cattle were found to be unrelated to treatment.

xii) Effects of ivermectin upon dung beetles

The life cycle of nearly all species of dung beetles (Coleoptera; Scarabaeidae) is intimately associated with dung. Characteristics of these dung-associated insects which are most responsible for the successful utilization of dung for feeding and reproduction include mobility, and feeding and reproduction patterns. These will be discussed in relation to the sensitivity of the dung beetles to ivermectin in dung and the usage pattern of the IVOMEC SR Bolus in cattle. Information supporting this section was provided to the CVM in a detailed confidential report (title: Hazard Assessment of the Effects of IVOMEC SR Bolus Use in Pastured Cattle on Dung Beetles (Coleoptera: Scarabaeidae) and information from this report has been incorporated into this section of the Environmental Assessment. Recognized experts have agreed to the contents and conclusions of the report.

a) Mobility of dung beetles

Dung beetles are highly mobile. Mobility is crucial for insects which use fresh dung to feed and reproduce. Hanski (1990, Sec. 14) described this habitat as "patchy and ephemeral". Dung is distributed discretely throughout the range of the animal which produces it, and each dung pad may only be attractive and suitable for dung beetles for a limited period of time. Thus, it is essential that dung beetles are able to move readily from pad to pad and from pasture to pasture in pursuit of dung in suitable condition for feeding and/or ovipositing.

The evolutionary success of dung beetles, given the nature of the macro-and micro-environments in which they live, is evidence for their robustness and ability to adapt to the many and varied perturbations which might temporarily affect them. From an evolutionary perspective, some dung beetles evolved to follow migratory sources of dung and became associated with large grazing mammals such as the species which now inhabit the grasslands and plains of Africa. Mammals and dung fauna experienced cycles of expansion and contraction during their evolution, as the herds of mammals and beetles migrated across available regions (Cambefort, 1991, Sec. 14). Grazing cattle are highly mobile and generally travel several miles per day (Havstad *et al.*, 1986; Hepworth *et al.*, 1991; Funston *et al.*, 1991; Brandyberry *et al.*, 1991; Hart *et al.*, 1993, Sec. 14), with proximity to water supplies controlling to a great extent the distances traversed (Wilkinson *et al.*, 1989; Hart *et al.*, 1991; Pinchak *et al.*, 1991; Seman *et al.*, 1991, Sec. 14). As forage is consumed the cattle move from cropped to fresh areas within a pasture. This movement results in the deposition of dung pads across wide expanses of pastures.

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

Dung beetles have been called "proficient" (Bornemissza, 1976, Sec. 14), "strong and swift" (Heinrich and Bartholomew, 1979, Sec. 14) and "excellent" (Halffter and Edmonds, 1982, Sec. 14) fliers, with speeds of 5-6 m/sec (Halffter and Mathews, 1966, Sec. 14) and 30 km/hour (Heinrich and Bartholomew, 1979, Sec. 14). Further, migration of adult dung beetles over long distances has been well documented (Waterhouse, 1974; Bornemissza, 1976; Blume and Aga, 1978b; Fincher *et al.*, 1983; Kohlmann, 1991, Sec. 14). Some adult beetles have flown up to 30 km across seas to colonize off-shore islands (Waterhouse, 1974; Bornemissza, 1976, Sec. 14), demonstrating their ability to make long-distance flights. In Australia, *O. gazella* colonized areas several hundred kilometers from their release site within two years with spread rates of up to 80 km per season (Waterhouse, 1974; Bornemissza, 1976, Sec. 14). Blume and Aga (1978b, Sec. 14) reported that *O. gazella* released in Kleberg County, Texas in 1972 spread 32 km in 1974 and an additional 32 km in 1975. According to Fincher *et al.* (1983, Sec. 14)

O. taurus, a common European dung beetle which entered the U.S. in approximately 1970 or 1971 in the vicinity of Pensacola, Florida, dispersed 300 km across southern Georgia into South Carolina in about 13 months or less during the mid 1970s. These observations support the conclusion that dung beetles move readily, even through cattle-producing areas in which adequate food is available locally. High mobility of these insects and their strong dispersal instincts insure movement of dung beetles among pastures and across regions.

With respect to movements over smaller distances and shorter periods of time (e.g., hours), two papers are relevant. Eschle *et al.* (1973, Sec. 14) studied the suppression of horn fly populations using cattle at a site on a West Texas ranch. No cattle had been on the ranch for one year. The herd of cattle nearest to the study site was 1.5 miles away, but numerous deer were present. Dung pads from the cattle involved in the study were often partially or wholly destroyed by dung beetles (mainly *Canthon* spp.) and raccoons. As cattle had been absent from the ranch for a year, the dung beetles attracted to the pads either migrated from at least 1.5 miles away and/or had been sustained by the fecal excreta of the deer. The former is in line with the available evidence that dung beetles are highly mobile and are attracted to dung from considerable distances. If the dung beetles which destroyed the cattle pads were from a local population, it follows that these beetles are not restricted to using cattle dung for food and reproduction. Further support concerning the mobility of dung beetles comes from work by Hanski (1980, Sec. 14) who discussed "long-distance" movements (0.5 up to at least 1.5 km) observed in England for *Aphodius* spp. To this author, long-distance movements are within an "ecological range" (an area of some tens of square kilometers), whereas within a "behavioural range" (the area of a pasture, i.e., about one hectare) movements are assumed to represent facultative migration between dung pads.

Hanski (1990, 1991, Sec. 14) reported that mature female dung beetles may leave a dung pad if it is too crowded or otherwise unsuitable and seek out a more suitable pad for the laying of eggs, with the result that the new generations of beetles will arise across a wide area.

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

b) Use of dung for feeding and reproduction

Dung beetles which use cattle dung on open pastures in the U.S. are dung generalists. The large majority of these dung beetles use dung of mammals as the source of food for both adults and larvae. Hence, their reproductive success depends upon the availability of this excrement. *Aphodius* species are the dominant dung beetles in northern temperate regions (Hanski, 1991, Sec. 14). Cervenka and Moon (1991, Sec. 14) recorded 11 species of *Aphodius* in Minnesota, compared to 3 species of *Onthophagus* dung beetles and one *Geotrupes*. Kessler and associates (1974, Sec. 14) reported that seven species of *Aphodius* and two of *Onthophagus* were among the most prevalent species of dung beetles in both cattle and sheep manure in east-central South Dakota. Of the 26 *Aphodius* species listed by Blume (1985, Sec. 14) as being associated with bovine dung in American pastures (north of Mexico), most are dung generalists, rather than bovine dung specialists, which also utilize dung of other domestic mammals, especially horses and sheep (Hanski, 1991, Sec. 14). Among the ten species of *Aphodius* which are European imports and which prefer open pastures and bovine dung (Gordon, 1983, Sec. 14), four were observed in both cattle and sheep manure in east-central South Dakota (Kessler *et al.*, 1974, Sec. 14). In coastal California areas, the introduced dung beetle *Aph. fimetarius* inhabits the small, soft dung pads (non-pellets) typical of deer and sheep from about February through April (Anderson, J. R., personal communication). In eastern Washington and northwestern Idaho, several *Aphodius* spp. were found in cow, horse and sheep dung (Coffey, 1957, Sec. 14). Native American dung beetles which are found in open pastures and bovine dung, such as *Onthophagus* spp. (mostly *O. hecate*) and *Phanaeus vindex*, are attracted in greater numbers to swine feces than to cattle feces even on open pasture and are also attracted to dung from other sources, including opossum, fox, human, rat, raccoon, horse and sheep (Fincher *et al.*, 1970, Sec. 14). *O. hecate,* one of the most widely distributed and most common of North American species, utilizes droppings from dogs, rabbits and woodrats in addition to the sources cited above (Howden and Cartwright, 1963, Sec. 14). Various species of native ball-rolling dung beetles, including *Canthon pilularius* (L.), *C. vigilans* and *C. chalcites* (Halderman), utilize either cattle or horse/mule dung while *Boreocanthon praticola* (LeConte) utilizes cattle and prairie dog dung (Gordon and Cartwright, 1974, Sec. 14). The dung of deer, elk, moose and sheep, mostly excreted as small, hard pellets, can serve as a restricted seasonal resource for such introduced dung beetles as *Aph. fimetarius* (Anderson, J. R., personal communication). The intake of fresh green forage, along with early spring worm infections, results in these animals producing soft, mushy dung pads that resemble miniature cattle dung pads; such dung pads are used by *Aph. fimetarius*. Thus, dung beetle species commonly found in cattle dung and open pastures in the U.S. tend to be dung generalists and will utilize dung from other species besides cattle.

Deer dung can also be used by some species of native *Aphodius* species inhabiting the forested areas of the eastern U.S. (Gordon, 1975; Hanski, 1991, Sec. 14). In contrast to the situation in Europe, large-scale deforestation in the U.S. did not take place until the westward expansion began in the late eighteenth century. Native American peoples, who did not have any domesticated mammals except dogs, did increase the sizes of old fields and of early successional forests, and this led to increases in the deer population.

Conditions until the fairly recent past thus favored forest-dwelling *Aphodius* species specializing in deer dung, and these native *Aphodius* dung beetles have not colonized the recent pasture ecosystems, probably because of their adaptations to forest habitats (Hanski, 1991, Sec. 14).

Gordon (1983, Sec. 14) reported that about 210 species of *Aphodius* are described for North America north of Mexico. Of these, 17 species of eastern *Aphodius* are associated with deer dung (in an obligate fashion or strong preference) and the obligate deer dung species will not use bovine dung. About 60 species of *Aphodius* are associated with dung in rodent or tortoise burrows. Eight native generalist species are not known to have dung preferences (other than rarely using deer droppings); their main sources of dung do not include cattle. In the U.S., *Aphodius* species of European origin are mostly generalists, preferring open pastures and bovine dung. In contrast, the native species tend to occupy non-pasture areas and utilize dung of native wildlife rather than that of recent arrivals, i.e., cattle. It is unlikely, then, that native American *Aphodius* dung beetles will be much exposed to ivermectin residues in dung.

Little is known concerning the native insect fauna associated with bison dung on the Great Plains, although there is evidence that three dung beetle species became extinct long ago (Hanski, 1991, Sec. 14). In this part of the U.S., as pointed out by Gordon in Hanski (1991, Sec. 14), the climatic conditions, i.e., low precipitation and humidity, leading to rapid desiccation of pads, do not favor dung beetles which use bovine dung.

Blume (1985, Sec. 14) listed 450 species of insects in the U. S. and Canada associated with bovine droppings on pasture; none of these is listed (U.S. Fish and Wildlife Service, 1990, Sec. 14) as endangered or threatened (Blume, R. R., personal communication).

Matthews (1965, Sec. 14) indicated that six species of canthonines are the only known representatives of scarabaeine dung beetles in Puerto Rico. The introduction of dung beetles (including *C. pilularius*, the "tumblebug" commonly found in the U.S.) was attempted in connection with horn fly control, but none of the beetles were ever seen following release. Puerto Rican canthonines are not found in open (un-wooded) areas, nor in cattle pastures. These forest-dwelling dung beetles apparently are not exploiting the cattle dung now present in Puerto Rico, and "cow dung remains virtually untouched in Puerto Rico" (Matthews, 1965, Sec. 14). Hence, it is unlikely that dung beetles in Puerto Rico will be exposed to ivermectin residues.

According to Nealis (1977, Sec. 14) and Lumaret *et al.* (1992, Sec. 14), most species of dung beetles use a wide variety of fecal matter. Fincher *et al.* (1970, Sec. 14) reported that *Onthophagus* species (and other dung beetles) are attracted to feces from a wide variety of mammals in addition to cattle, including horses, sheep, and especially swine. Kirk and Ridsdill-Smith (1986, Sec. 14) reported that numerous species of dung beetles, candidates for introduction into southwestern Australia for fly control in cattle dung pads, including *Onthophagus* species such as *O. taurus* (also found throughout much of the southeastern U.S.), are attracted to sheep, goat, horse and mule dung as well as cattle dung. Although *Phanaeus* spp. of dung beetles are most commonly found on cattle dung pads, they are also strongly attracted to the feces of swine, horses and humans (Stewart, 1967; Blume and Aga, 1978a, Sec. 14). *Canthon* species (also of the subfamily Scarabaeinae) of dung beetles including *C. pilularius* (the "tumblebug" commonly found in the eastern half of the U.S., which rolls dung away for burial and use for food and reproduction) utilize cow, horse and sheep dung (Fincher *et al.*, 1970; Gordon and Cartwright, 1974, Sec. 14). According to Gordon and Cartwright (1974, Sec. 14), in general most of the *Canthon* species exhibit a wide geographical distribution and are often present in high abundance. The success of these beetles may lie in the fact that most of the *Canthonini* species are not restricted to one type of dung and in the absence of preferred food they will accept a reasonable substitute (Gordon and Cartwright, 1974, Sec. 14). Supporting the premise that many species of dung beetles are dung generalists, Halffter and Matthews (1966; and references cited therein, Sec. 14) report that coprophagous Scarabaeinae are less concerned about the kind of excrement they utilize than about where it occurs (e.g., pasture or woodland). Stewart (1967, Sec. 14) has commented that, "The fact that most citations to feces attraction of dung beetles in the literature refer to cattle droppings may reflect only the particular investigator's interest or a more or less monofaunal locale where observations were made."

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

c) Dung beetle activity and reproduction

Cycles of temperature and precipitation strongly influence the activity of dung beetles at a given site. Dung beetles will seek out and utilize fresh dung for reproductive purposes as long as the environmental conditions (e.g., temperature and moisture content of pads and soil) are conducive to such activity. Oviposition is generally not a one-time effort for Scarabaeinae, but rather occurs with spatial and temporal variation over the lifetime of a mature adult female (Halffter and Edmonds, 1982, Sec. 14); this can be several months or more for a female *O. gazella*. In the southern U.S., beetles such as *O. gazella* can reproduce more or less continuously from spring through summer and into the autumn. A period of feeding and sexual maturation of several weeks to a month or more, subsequent to the emergence of the new generation of adults and prior to their reproducing, is necessary and common for many teneral adults of Scarabaeinae species. This period, known as Reifungsfrass, results in a delayed period of ovipositing (Halffter and Edmonds, 1982, Sec. 14). However, some of these Scarabaeinae species, e.g., *O. gazella*, begin reproductive activity soon after emergence. According to Halffter and Edmonds (1982, Sec. 14), under optimum conditions the life cycle (egg to egg) of *O. gazella* may be completed in 30 days.

Fecundity of dung beetles is, to a first approximation, inversely proportional to the efforts adults of a species expend in the preparation of nests. Most species of Scarabaeinae, which put their maximum reproductive effort in nesting behavior, have generally low fecundity. Fairly high levels of fecundity are found, however, with some Scarabaeinae species, several of which have been introduced into the U.S. and Australia for dung control programs (Halffter and Edmonds, 1982, Sec. 14). For example, adult female

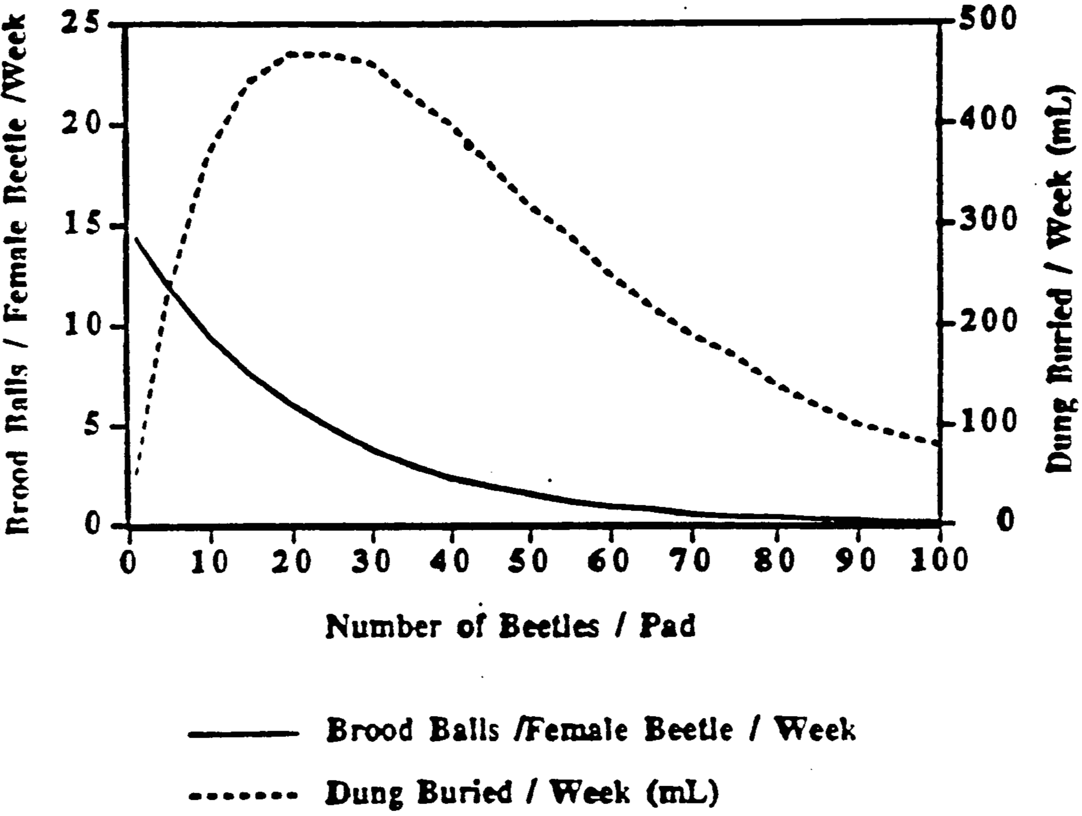
O. gazella (one of the species introduced into Australia and the southern U.S.) can produce up to 200 eggs in a lifetime (Bornemissza, 1976; Blume and Aga, 1975; Halffter and Edmonds, 1982; Sommer *et al.*, 1993b, Sec. 14). Most species of Scarabaeinae (which put their maximum reproductive investment in nesting behavior), however, have generally low fecundity. *Aphodius* species (dominant dung beetles in north temperate regions such as the U.S.) possess high fecundity, as their maximum reproductive output is invested in egg production and not nesting (Halffter and Edmonds, 1982; Hanski, 1991, Sec. 14).

Aph. fimetarius accounted for a major portion of the biomass in cattle dung pads studied by Merritt (1974, Sec. 14) and Merritt and Anderson (1977, Sec. 14) in the western foothills of the California Sierra Nevada Mountains at a research site about 97 km north of Sacramento. Teneral adults begin to emerge in April and reach peak numbers from May to mid-June when they feed in fresh dung pads. Following this, they burrow into the soil and undergo a 5-7 month period of aestivation until the fall rains begin. At that time (October, November) the adults emerge, inhabit fresh dung pads (causing a second population peak), and feed, mate and oviposit. Nulliparous females continue to appear in small numbers into December, and declining numbers of older, parous females continue to feed and oviposit until early spring. The newly laid eggs hatch and larvae spend the winter and early spring undergoing slow development, with pupation occurring in March/April and a new generation of teneral adults emerges from April to mid-June (Anderson, J. R., personal communication). Seasonal activity (e.g., inhabitation, oviposition and larval development) and the number of generations per year for *Aphodius* species will likely vary considerably depending upon geographical region (including latitude and elevation) and species.

A behavior characteristic of dung beetles that aids in maintaining their population level is density-dependent reproduction. Overpopulation and underpopulation are kept in check by a change in the number of eggs laid per female in pads (Moon, R. D., personal communication). Thus, in a crowded pad there will tend to be fewer eggs laid per female than in a less-crowded one (Holter, 1979a, Sec. 14). Ridsdill-Smith *et al.* (1982, Sec. 14) have reported that brood ball production per female with *O. binodis* is inversely related to the number of beetles per pad (Figure 4). Intraspecific competition among an excessively large population of larvae in a pad will result in a diminished number of larvae developing to a large size. However, if undercrowding or lowered density of ovipositing beetles occurs because of a reduced population, the number of eggs laid per pad per female can increase. Such an increase can compensate for the decrease in the number of adult beetles per pad (Holter, 1979a, Sec. 14; Anderson, J. R., personal communication). This phenomenon, plus the fact that females lay multiple clutches of eggs (Anderson, J. R., personal communication), may allow the population of the next generation to reach a level approaching normal, and will serve to maintain the population of dung beetles in areas that may, for whatever reason, have had a lowered density of adults. Also, based on data reported by Holter (1979a, Sec. 14), fewer eggs per pad does not necessarily result in a proportional decrease in the number of large developing larvae per pad. This effect presumably results from an increased opportunity for development of larvae in less-densely populated pads, as there would be diminished competition for food and habitat in the pads among the larval progeny of the fewer females (Anderson, J. R., personal communication). With respect to their observations on dung beetles which produce brood balls, Ridsdill-Smith and associates (1982, Sec. 14) state that "the ecological implications of the results are that maximum rate of increase of dung beetle populations will occur when low densitites are present in the pad."

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

Figure 4
Production of Brood Balls per Female *O. binodis* per Week as a Function of Actual Beetle Density in that Week. Based on Figure 2 in Ridsdill-Smith *et al.* (1982, Sec. 14).



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

In the U.S. dung beetles play, at most, a minor role in the degradation of cattle dung pads. Dung beetles can be classified into three distinct groups according to their habits of food manipulation: the "tumble-bugs" (or telecoprids), which form feces into balls and roll them away for burial; the dung-burying beetles or paracoprids, which bury feces under or beside the deposit; and dung feeders or endocoprids, which feed on dung and nest inside the dung deposit (Bornemissza, 1976; Fincher, 1981, Sec. 14). Cervenka and Moon (1991, Sec. 14) concluded that dung feeders such as *Aphodius* spp. and other large beetles failed to achieve sufficient densities to disrupt cattle dung pads during May through October in Minnesota or to affect survival and size of large dung-feeding Diptera, which included *Haematobia irritans*. Fincher *et al.* (1986, Sec. 14) also found that although many species of scarabs, including dung-burying species of *Onthophagus* and *Phanaeus*, ball rollers from the genera of *Canthon* and *Boreocanthon*, and dung feeders from the genera of *Aphodius* and *Ataenius,* were present on open pastures from March through November in east-central Texas, their populations were not great enough to bury a significant amount of dung. In that study, maximum dung burial usually occurred in August, which coincided with yearly population peaks of the main species of dung-burying beetles. In two studies which examined the contribution of dung beetles to dung degradation, the conclusion was that dung beetles in temperate climates directly contribute little to overall degradation. Putman (1983, Sec. 14) estimated that dung beetles in the U.K. in autumn contributed up to 13% to pad degradation while Holter (1979b, Sec. 14) estimated dung beetle larvae (mostly *Aph. rufipes)* were responsible for 14-20% of dung disappearance from August to October in Denmark. Holter (1979b, Sec. 14) suggested that mechanisms regulating the population density of *Aphodius* larvae in dung pads might have evolved to protect the larvae from loss of both their food and habitat. The development of *Aph. rufipes* larvae takes 5-8 weeks in dung, so that rapid breakdown of the pad by the larvae might lead to fatal exposure to predators or desiccation. The minor role beetles play in the degradation of cattle dung pads in the U.S. led to Federal (Blume and Aga, 1978b; Fincher *et al.*, 1983, Sec. 14) and state (Anderson and Loomis, 1978, Sec. 14) applied research programs focused on the introduction of exotic dung beetles, largely as part of programs to control populations of pestiferous flies. A few exotic beetles have become established in some southern states and in California, but these programs for beetle importation have been largely discontinued and resources have been shifted to other pestiferous-fly control programs (Roncalli, 1989, Sec. 14; Blume, R. R., personal communication). Exotic dung beetles established

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in the U.S. are capable of dispersing or burying considerable portions (>50%) of cattle dung in those regions where they have become established, but only when optimum soil and climatic conditions coincide with times of peak activity of adult beetles (Blume, R. R., personal communication).

Merritt and Anderson (1977, Sec. 14) found that pads treated with insecticide to exclude insects degraded approximately as fast as naturally dropped pads in the fall in northern California. In natural pads at that site, *Aph. fimetarius* created a grass-like material by larval ingestion of and larval growth within the pad and by adults foraging in new pads; these activities aided dung pad breakdown. However, Merritt and Anderson (1977, Sec. 14) concluded that pasture management systems and seasonality had greater effects on pad degradation than did insects in that region. Stevenson and Dindal (1987, Sec. 14), on the other hand, found that adult *Aphodius* spp. increased pad degradation in microcosms containing artificial cattle dung pads in a glasshouse in upstate New York, but did not increase drying or oxidation of the dung.

Relative populations of dung beetles differ by regions in the U.S. In general, beetles which do not bury dung, such as *Aphodius* spp. and *Ataenius* spp., comprise the most numerous dung beetles in cattle dung pads in the northern sections of the U.S. (Cervenka and Moon, 1991; Kessler *et al.*, 1974 and Merritt and Anderson, 1977, Sec. 14), while beetles which bury dung, such as *Onthophagus* spp. and *Phanaeus* spp., and ball rollers, such as *Canthon* spp. and *Boreocanthon* spp., predominate in the southern sections of the U.S. (Fincher *et al.*, 1986; Nealis, 1977 and Stewart, 1967, Sec. 14). In states such as Missouri and Nebraska, members of no one genus predominate, and *Aphodius* spp., *Ataenius* spp. and *Onthophagus* spp. are well represented (Peitzmeier *et al.*, 1992; Wingo *et al.*, 1974, Sec. 14).

Time of year also influences the importance of dung beetles in degrading dung or removing it from pastures. Fincher (1981, Sec. 14) estimated that 82-88% of artificially deposited 2.5-kg cow pads were buried within 1 week in Texas during the months of July through September, which coincided with the peak activity of *O. gazella*, an exotic, introduced species, while only 0-4% of feces deposited March through June were buried after 1 month. Between 75-90% of the feces deposited during the winter were still present 9 months later.

Thus, differing beetle populations, food-manipulation habits and seasonally dependent population densities affect the contribution of dung beetles in degrading dung or in removing it from pasture surfaces in the U.S., but, overall, dung beetles play, at most, a minor role in dung pad degradation in the U.S.

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

Dung beetle species associated with cattle dung and open pastures are widespread across the U.S. Readers are referred to Blume (1985, Sec. 14) for details of the distribution of dung beetles by species. Because of this widespread distribution, a localized elimination of beetles, for whatever reason, would not threaten the survival of a species. The tumble-bugs, which include the genera *Canthon* and *Boreocanthon*, and the dung-burying beetles, which include the genus *Onthophagus*, are distributed throughout much of the U.S. (Blume, 1985, Sec. 14). Some *Onthophagus* species, e.g., *O. hecate*, are distributed as far west as the Rocky Mountains and north into Canada, but the introduced species *O. gazella* had spread only throughout the south and into California by 1985 (Blume, 1985, Sec. 14). There are two species of the dung-burying beetle *Phanaeus* which are widely distributed in the U.S. (Blume and Aga, 1978a, Sec. 14). *P. difformis* is found primarily in and around Texas, Oklahoma and Kansas, while *P. vindex* occurs from the Atlantic coast to the Rocky Mountains and as far north as the Ohio and Missouri River valleys and northeast to Cape Cod (Blume and Aga, 1978a, Sec. 14). The dung feeders, which include *Aphodius*, the dominant genus in the north temperate zone (Hanski, 1991, Sec. 14), and *Ataenius,* are distributed widely across the U.S. *Aph. fimetarius*, a European species, has been reported in virtually all states (Blume, 1985, Sec. 14). It is the most abundant species in cattle droppings in northern California (Merritt and Anderson, 1977, Sec. 14) and one of the most numerous species in Minnesota (Cervenka and Moon, 1991, Sec. 14). *Aph. fimetarius* is also the dominant dung beetle, in terms of numbers of adults per pad, in central Texas during the late fall, November and December, and early spring, March and April (Blume, R. R., personal communication).

Figure 5 shows the distribution of some of the major genera of dung beetles associated with cattle dung on pastures in the U.S., based on data from Blume (1985, Sec. 14) and on the observation and collection of *Aph. fimetarius* in northwestern and north-central Nevada, in counties along the California border (Anderson, J. R., personal communication). States which are not shaded do not necessarily indicate the absence of dung beetles, but rather, a lack of published sightings in that state (Blume, R. R., personal communication). Comparing the species of dung beetles which are found in cattle dung pads in open pastures (Cervenka and Moon, 1991; Hanski, 1991;

Kessler *et al.*, 1974; Merrit and Anderson, 1977; Fincher *et al.*, 1986; Nealis, 1977; Stewart, 1967 and Wingo *et al.*, 1974, Sec. 14) with their distributional records (Blume, 1985; Hanski, 1991, Sec. 14), it is clear that these species are widespread across the U.S. Because of this widespread distribution, a localized elimination of beetles, for whatever reason, would not threaten the survival of the species.

f) Effect of ivermectin residues on dung beetles

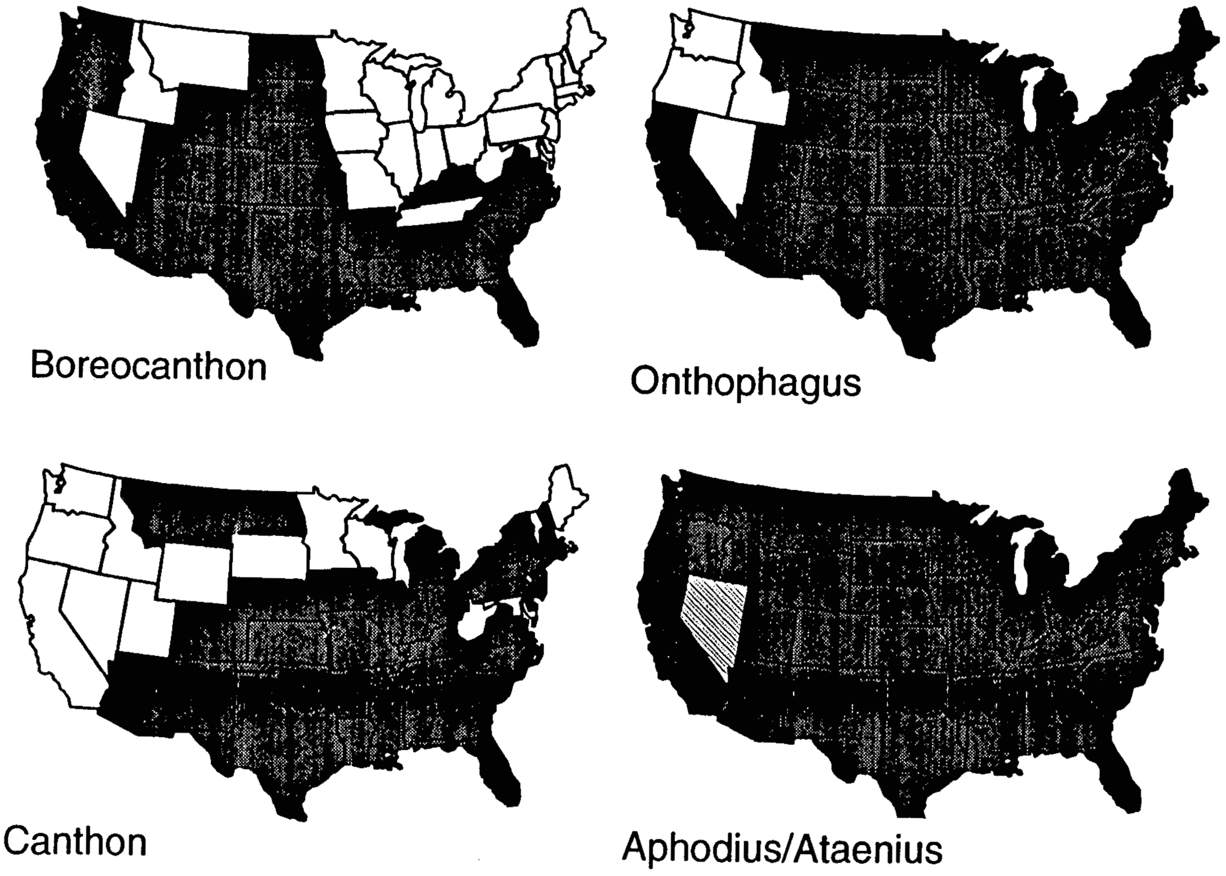
Drug residues in the manure of ivermectin-treated cattle can affect dung beetles. The impact on dung beetles reported most frequently in studies on the effect of ivermectin residues in cattle dung is inhibition of larval development/adult emergence (Roncalli, 1989; Strong and Brown, 1987; Strong, 1993, Sec. 14). Studies reporting effects of ivermectin residues on dung beetles are outlined below.

(1) Bolus formulation

A study to ascertain the effect of ivermectin in dung of cattle treated with a bolus (~12 mg/day for approximately 120 days) upon dung fauna was carried out in Lauterbach, Germany (Barth *et al.*, 1993, Sec. 14). One-quarter segments of pads and the underlying soil (8 cm deep) were collected 3, 7, 14 and 28 days post deposition and examined for adult and larval (immature) dung beetles, Diptera larvae and nematodes. Subject pads were deposited 21/22, 70 and 119 days after initiation of treatment. Compared to dung pads from control calves, those from the ivermectin-treated calves contained fewer *Coleoptera* and Diptera larvae, but no treatment-related effects were observed in numbers of adult *Coleoptera* (Table 9).

FIGURE 5

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



Based on Data from Blume (1985) ■ and Anderson, J.R. (personal communication) ▨

In a trial conducted in Missouri (Wallace *et al.*, 1991, Sec. 14), dung pads from control calves and those receiving ivermectin (~12 mg/day) via a bolus designed to deliver drug for 90 days were examined for Diptera larvae and adult insects. The percent of dung pads containing fly larvae was treatment-related (Figure 6). Excluding day 7 post dose, none of the pads from calves given a bolus contained fly larvae until 112 days after the bolus was administered (three weeks after the designed shut off point of the bolus). These results demonstrate that ivermectin residues effectively control emergence of flies from dung pads, and verify the functionality of boluses used in this trial. Failure to observe larvae in dung pads dropped by either control or treated calves on day 56 may be explained by the fact that it rained on this day. Flies exhibit reduced activity during inclement weather, and it follows that egg laying on fresh dung pads would have been curtailed on day 56. In contrast to the treatment-related effect with fly larvae, the percentage of dung pads containing adult insects (including dung beetles) appears not to be treatment-related (Figure 7). Dung beetle activity was comparable for pads from treated and control calves. Insect tunneling was observed in all pads.

Wall and Strong (1987, Sec. 14) reported that ivermectin residues in the feces of calves (200 kg) receiving drug (40 mcg/kg/day) via an experimental ruminal bolus had an insecticidal effect upon the dung pad insect community (including *Coleoptera* and Diptera). Fresh dung, 0 - 12 hours old, was collected 11 - 17 days after treatment of the cattle and from control cattle. Artificial, 2-kg pads were evenly spaced at 1-m intervals in an enclosure in a dairy pasture. Pads were collected 20, 30, 40, 50, 60, 80 or 100 days later. *Coleoptera*, mostly *Aphodius* spp., were far more abundant in control pads than in pads from treated cattle. Strong and Wall (1988, Sec. 14) later estimated a steady state fecal concentration of approximately 400 ppb of ivermectin-related residue. These authors titrated the toxicity of ivermectin toward dung-breeding insects by adding ivermectin to control manure to achieve a concentration range of 0 to 500 ppb. They found that a concentration of 125 ppb was not toxic to larval Scarabaeidae (mainly *Aphodius* species), but toxicity was present at 250 and 500 ppb. Concentrations of ivermectin at 125 to 500 ppb did not repel dung insects nor affect adult Scarabaeidae during the 6-hour observation period.

(2) Injectable / topical formulation

Treated cattle were dosed subcutaneously with ivermectin at 0.2 mg/kg in the following studies unless otherwise noted.

Onthophagus gazella failed to develop from dung excreted up to 21 days after treatment at 0.3 mg/kg (Roncalli, 1989, Sec. 14). However, viability of adults and production of brood balls were not affected by ivermectin residues, even in dung collected during the first week after dosing.

Onthophagus gazella and *Euoniticellus intermedius* (exotic species introduced into the U.S.) were used by Fincher (1992, Sec. 14) to study the effect of ivermectin residues on the emergence of the dung burying beetles. The capacity of these beetles to reproduce in dung collected at weekly intervals was evaluated under laboratory conditions. The dung was produced by cattle dosed subcutaneously with ivermectin at either 0.02 or 0.2 mg/kg. There was no apparent effect on brood ball production by either species from the two batches of dung. Emergence of *E. intermedius* was inhibited in dung collected from cattle at one week after treatment with 0.2 mg/kg but not later, and *O. gazella* development was inhibited in dung excreted at one and two weeks, but not three weeks, later.

At no time post dose did the dung from the cattle given 0.02 mg/kg cause reduction in the emergence of adults of either beetle species. When confined on control dung, the progeny of those *O. gazella* reared on the 3-week post treatment dung from the higher-dose cattle constructed the same number of brood balls as beetles never exposed to ivermectin.

Sommer and Overgaard Nielsen (1992, Sec. 14) and Sommer *et al.* (1993b, Sec. 14) reported that dung from treated cattle collected at 2 and 7 days post dose was lethal to *O. gazella* larvae, but dung collected on day 17 did not affect larval mortality.

TABLE 9

Pat Weights and Numbers of Dung Insects[a]

Days of Deposition Post Treatment	Age of Pat Days	Pat Wet Weight[b]	Adult Beetles	Immature Beetles	Immature Diptera
GROUP 1: CONTROL					
21/22	3	147.8	25.8	0.5	4.0
21/22	7	117.2	12.6	13.3	103.7
21/22	14	42.6	1.4	16.4	25.2
21/22	28	28.7	0.9	11.0	10.5
70	3	95.3	26.5	8.2	37.7
70	7	98.6	8.7	20.0	49.4
70	14	52.8	2.7	15.8	60.8
70	28	26.0	0.6	25.7	11.7
119	3	223.5	50.4	0.3	7.1
119	7	180.3	45.3	6.5	83.5
119	14	201.7	21.2	50.9	230.6
119	28	126.7	5.6	17.7	47.5
GROUP 2: IVERMECTIN BOLUS					
21/22	3	144.6	25.2	0.3	0.1
21/22	7	77.9	15.2	1.7	0.4
21/22	14	65.1	3.6	3.2	1.0
21/22	28	49.1	0.8	3.4	14.8
70	3	144.4	33.0	3.7	1.2
70	7	139.4	24.7	5.0	2.0
70	14	113.8	6.0	7.1	27.3
70	28	52.0	1.2	5.8	7.8
119	3	161.0	25.2	0.3	2.7
119	7	191.9	56.0	1.7	2.1
119	14	178.5	17.3	9.6	23.4
119	28	89.7	10.1	12.5	13.5

[a] Geometric means of one quarter segments of each of five pats.
[b] Arithmetic means of one quarter segments of each of five pats.

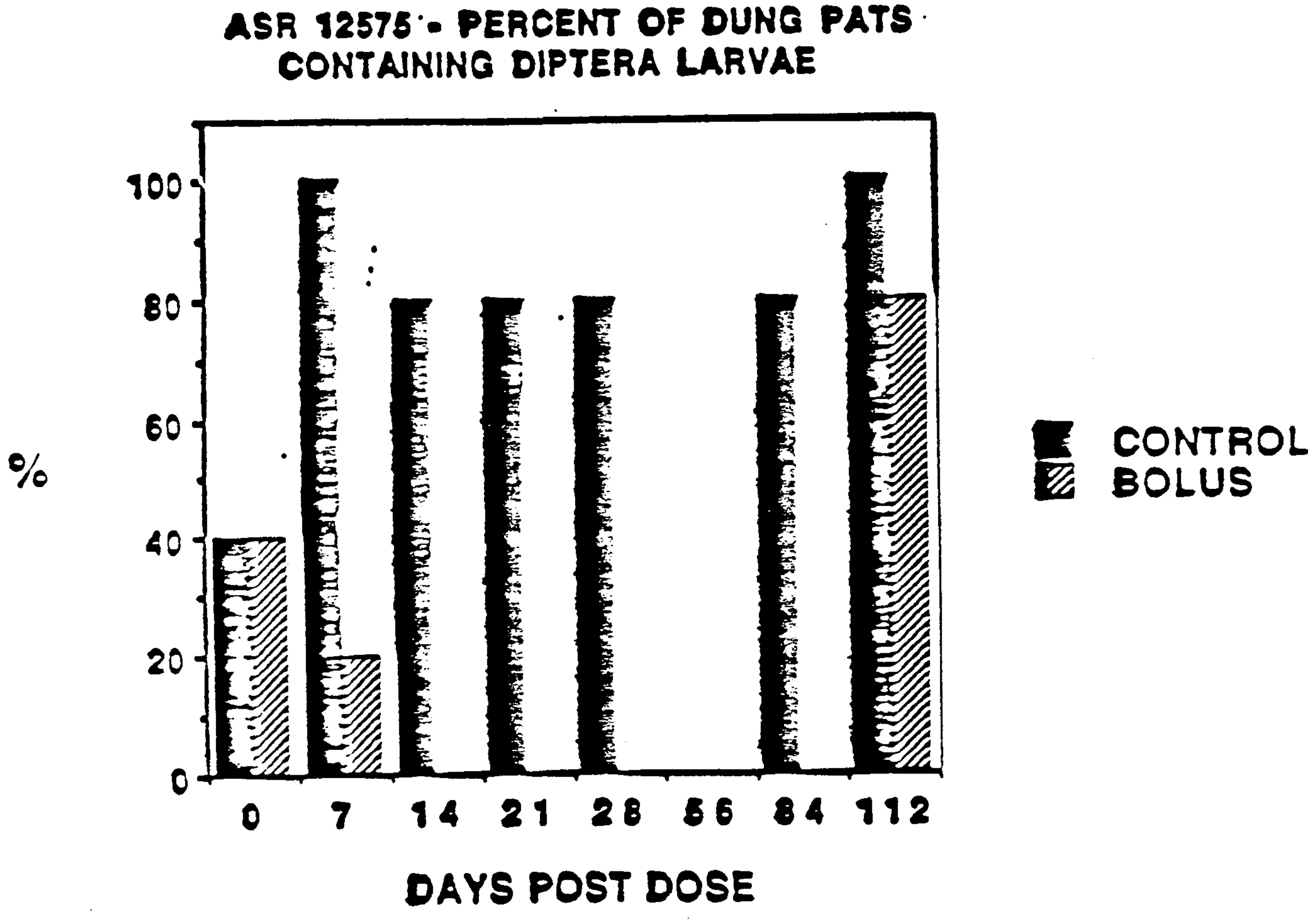


Figure 6. Plot of percent of calf dung pats containing diptera larvae vs day post dose of pat deposition for pats from control calves and calves given an IVOMEC SR Bolus designed to deliver ~12 mg ivermectin daily for ~90 days. Among five pats selected on each sampling day, the number of pats that contained diptera larvae at any time was recorded and percentage of pats with larvae calculated.

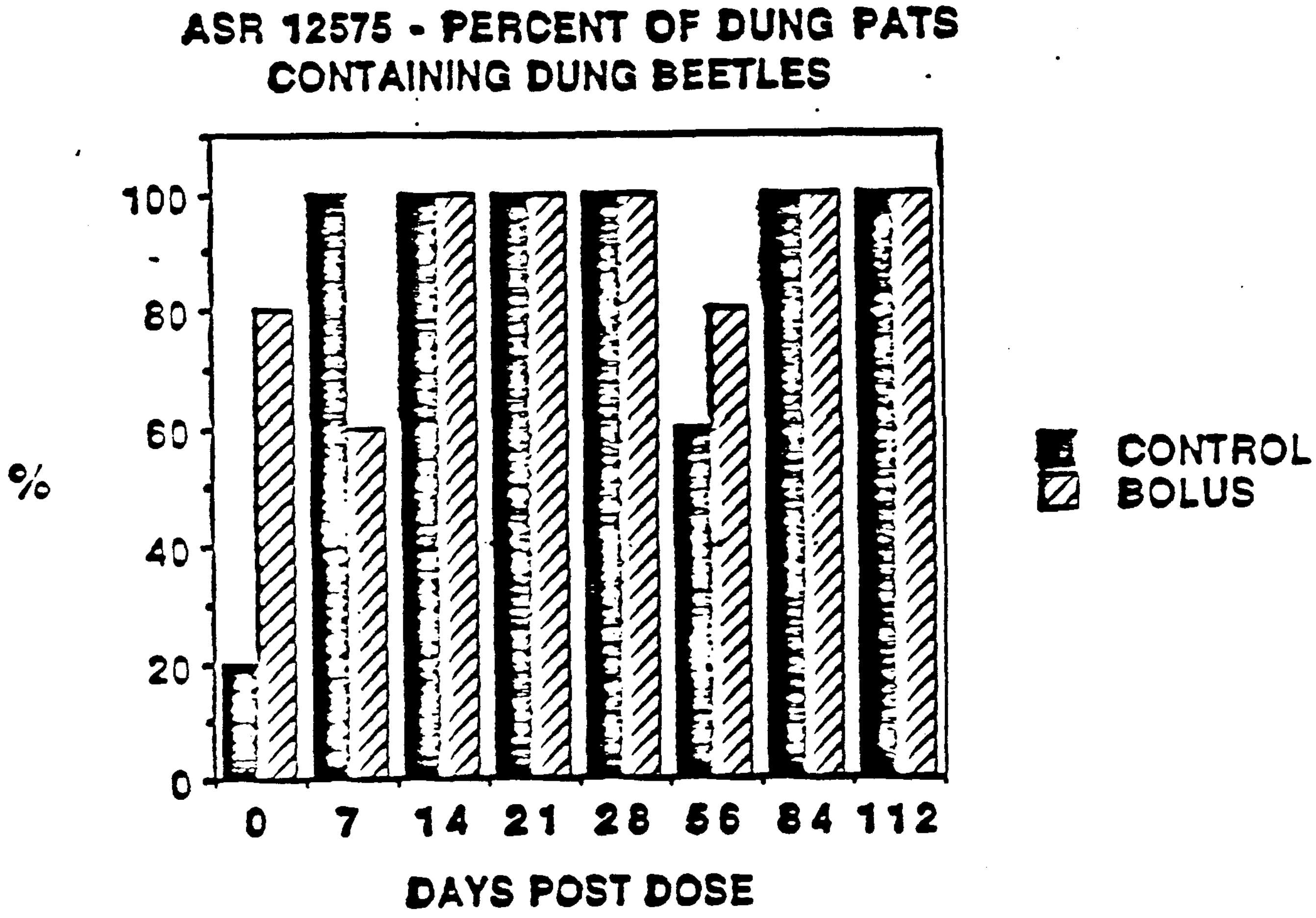


Figure 7. Plot of percent of calf dung pats containing dung beetles vs day post dose of pat deposition for pats from control calves and calves given an IVOMEC SR Bolus designed to deliver ~12 mg ivermectin daily for ~90 days. Among five pats selected on each sampling day, the number of pats that contained dung beetles at any time was recorded and percentage of pats with beetles calculated.

Development of the larval stage of *Aphodius* species of dung beetles (widely found in the U.S.) was inhibited in dung from cattle collected one day after treatment, but dung collected 10, 20 or 30 days post dosing was without effect (Madsen *et al.*, 1990, Sec. 14). Similarly, numbers of *Aphodius* larvae were reduced in dung collected 1-2 days after subcutaneous or topical (0.5 mg/kg) dosing, but not on day 13 or later (Sommer *et al.*, 1992, Sec. 14).

Studying *Diastellopalpus quinquedens*, Sommer *et al.* (1993a,b, Sec. 14) found that the dung-burying capability of this African dung beetle was not affected by the presence of ivermectin residues in the dung (2, 8, 16 days post dose) of cattle. However, there was some reduction in the numbers of developing larvae in brood masses. Twenty-eight percent of the brood masses made from dung excreted 2 days post dose contained live larvae; nearly all of the masses made from dung collected on day 8 and day 16 (90 and 94%, respectively) contained live larvae (compared to 100% for masses made with control dung).

Development of *E. fulvus* larvae was totally inhibited in dung collected 1 day after treatment of steers (Lumaret *et al.*, 1993, Sec. 14). However, in dung collected 10 days post dose only a slight delay in development was observed with no effect in dung collected 29 days post dose. All adult dung beetles fed dung from treated steers survived. Ivermectin did not increase attraction of beetles to dung. However, dung from treated animals was more attractive to beetles on days 5 through 17 post dose. A modification in the gut flora of treated cattle was hypothesized.

Strong and Wall (1994, Sec. 14) investigated effects that ivermectin residues in cattle dung had on colonization, survival and development of insects in June and July in the U.K. Artificial, 2-kg pads were formed from dung collected 2, 7, 14 and 21 days after treatment of the cattle and from control dung. Eight pads from each group were randomly allocated to sites in a field and were protected from birds. On days 7, 14, 21 and 42 following placement, two entire pads from each group were removed, weighed and assayed for invertebrates. Dung beetles were predominately *Aphodius* spp. and numbers of adults were not different between pads from control and treated cattle. This indicates no difference in attraction to pads containing ivermectin residues relative to control pads or toxicity to adults. Larval *Aphodius* spp. were unable to survive in 7-day post dose pads but there were no differences between numbers or dry weights of *Aphodius* spp. larvae in control pads and pads collected 14 days after ivermectin treatment.

When ovipositing *Copris hispanus* females (not found in the U.S.) were fed for 43 days on dung collected from calves three days following intramuscular administration of ivermectin, a reduced rate of oviposition and a lack of survival of immatures were reported (Wardhaugh and Rodriguez-Menendez, 1988, Sec. 14). No adult mortality was observed. Larvae did not survive in brood balls made from dung excreted on days 3 and 8 post dose but survival in dung collected 16 days post dose was approximately equal to that found for controls. Mortality for newly emerged beetles feeding for 43 days on dung from days 2 and 3 post dose was 90%; it decreased to 27% (about twice that of the controls) with dung deposited on day 16, and equal to that of controls (day 0 dung) with dung deposited on day 32 post dose. *C. hispanus* that survived the lengthy exposure to dung collected up to 16 days post dose showed atypical reproductive development. When fed for five weeks on dung collected on days 0, 16 and 32 post dose, there was no mortality of sexually mature *Bubas bubalus* (another dung beetle not found in the U.S.), and there were no suggestions of deleterious effects due to exposure to ivermectin residues in day-32 feces. Following exposure for 32 days to day-32 post dose dung, the population of newly emerged *Onitis belial* exhibited 22% accumulated mortality.

Ivermectin had no effect on the rate of dung beetle colonization in Denmark, Tanzania or Zimbabwe (Holter *et al.*, 1993a,b, Sec. 14). Dung was collected from cattle at intervals from 2 to 30 days after treatment and from control cattle. Powdered ivermectin (source not indicated) was also mixed into some control dung at concentrations from 0.015 to 0.42 ppm on a wet weight basis. Pitfall traps were baited with dung and arriving beetles were counted and identified. A lack of a preference for dung containing ivermectin was observed, consistent with results from studies by Strong and Wall (1988, Sec. 14) and Lumaret *et al.* (1993, Sec. 14).

McCracken and Foster (1993, Sec. 14) used multivariate analysis to examine the effects of ivermectin on invertebrates in artificial 1-kg cattle dung pads in the U.K. The injectable formulation of ivermectin was diluted with water and mixed with control dung to produce levels of 0, 0.5, 1 and 2 mg of ivermectin per kilogram of dung. The pads were placed in stratified random block plots on pastures adjacent to fields containing cattle. Pads, and the soil beneath (4 cm depth), were taken at 15, 30, 45, 60 or 90 days after placement. Placement dates were in May, June, August and September. Initially, there were 60 pads per collection group, but 73 of the original 228 pads were not visible on the day of sampling and another 21 were excluded from further analysis because they contained less than 3 taxa. Data from soil samples from beneath 57 pads were used for analysis.

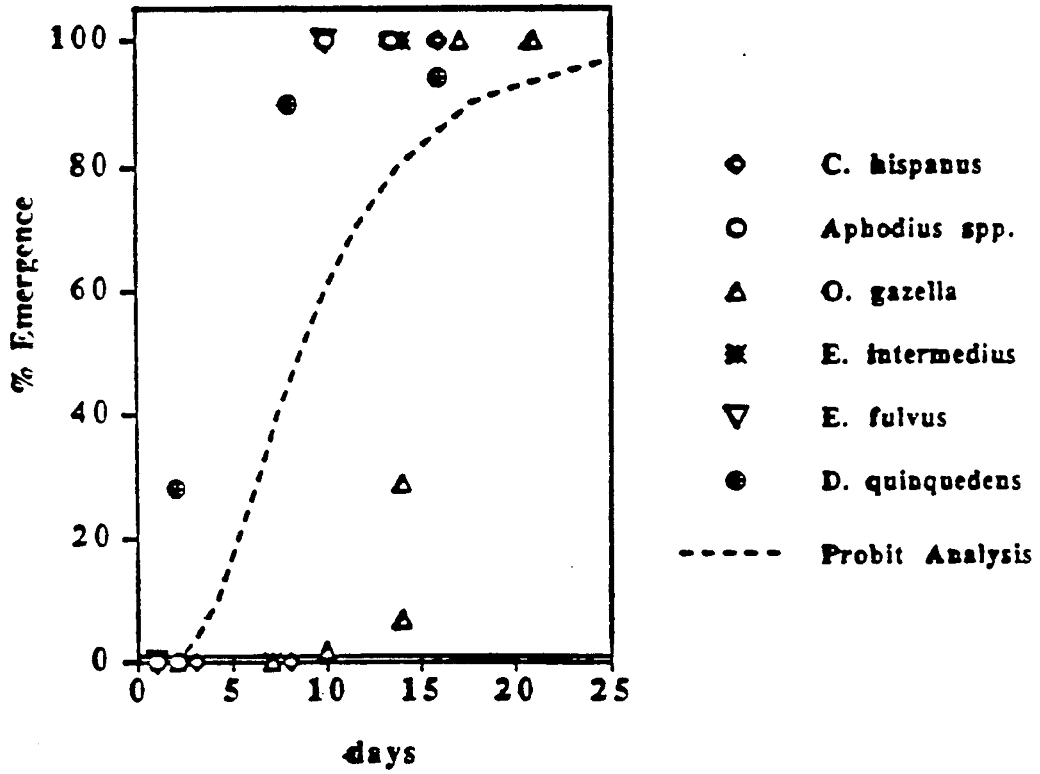
The study concentrated on differences between pads with regard to the numbers and types of Diptera and *Coleoptera* present and the numbers of earthworms. Few differences were detected between the three levels of ivermectin used in the study, so experimental pads were regarded as either treated or controls.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

(3) Summary of effects of ivermectin on dung beetles

The impact on dung beetles reported most frequently in studies on the effect of ivermectin residues in cattle dung is that upon larval development (adult emergence). Further, as larvae are the life stage of dung beetles most sensitive to ivermectin residues, this is a parameter which allows comparison of the sensitivity of various species of dung beetles to ivermectin residues. Little or no impact is found for ivermectin residues in cattle dung excreted 10-21 days after a subcutaneous dose. Probit analysis indicates that 90% emergence can be expected with dung excreted 18 days after a subcutaneous dose (see Figure 8).

Inhibition of development/emergence of larvae of dung beetles commonly found in the U.S., e.g., *Aphodius* spp. and *O. gazella*, does not occur with ivermectin-containing dung excreted 10 to 13-14 or 17 to 21 days post subcutaneous dose, respectively. It is thus a reasonable conclusion that,

Figure 8
Percent of Emergence of Dung Beetles and Probit Analysis of Emergence with Respect to Time of Dung Excretion After Dosing Cattle with Ivermectin



with ivermectin-treated cattle, larval development of most dung beetles found in the continental U.S. will occur normally with dung excreted by subcutaneously or topically dosed cattle three weeks or later post dose (Sommer *et al.*, 1992, Sec. 14), and by bolus-dosed cattle about two or three weeks after shutdown of the bolus. A significant fraction of larvae will emerge from dung excreted a week or so earlier than these times.

g) Use and exposure scenarios

It is clear from the data (Cervenka, 1986; Cervenka and Moon, 1991, Sec. 14) presented in Figure 9 that there are two major peaks of dung beetle activity (mainly *Aphodius* spp.) for Minnesota, a representative northern dairy state. Christensen and Dobson (1977, Sec. 14) reported the presence, in March, of viable *Aph. fimetarius* eggs in overwintered cattle dung pads in Indiana (also a northern dairy state). Larvae and pupae may also overwinter in pads. Thus, the early peak of dung beetle activity (May) in Minnesota likely arises from newly emerged, as well as some overwintering, adults (Moon, R. D., personal communication). As noted in Table 3, the monthly percentages of pastured cattle excreting anthelmintic residues are all below 30%. For May and October, the two months of peak dung beetle activity, the percent of pastured cattle excreting anthelmintic residues is estimated to be 28% and 5%, respectively, hence only a small proportion of fresh dung pads will contain levels of ivermectin residues that might inhibit development of dung beetle larvae. As only a small fraction of cattle will be excreting anthelmintic residues, the non-treated animals and those treated by injection more than one month previously will provide large numbers of residue-free pads.

Thus, the presence of ample amounts of residue-free dung for use by dung beetles is assured, and there will be negligible impact on dung beetle populations.

In east central Texas there is a broad period of dung beetle (mainly *Onthophagus* and *Canthon* spp.) activity, including reproductive activity, from April to September; see Figure 10 (data taken from Fincher *et al.*, 1986, Sec. 14). *O. gazella*, for example, are commonly found throughout these regions and are active from spring through the summer and into the

Figure 9
Comparison of the Estimated Actual Percentages of Pastured Cattle Excreting Anthelmintics in the Northern Dairy States versus Numbers of Dung Beetles by Month in Minnesota

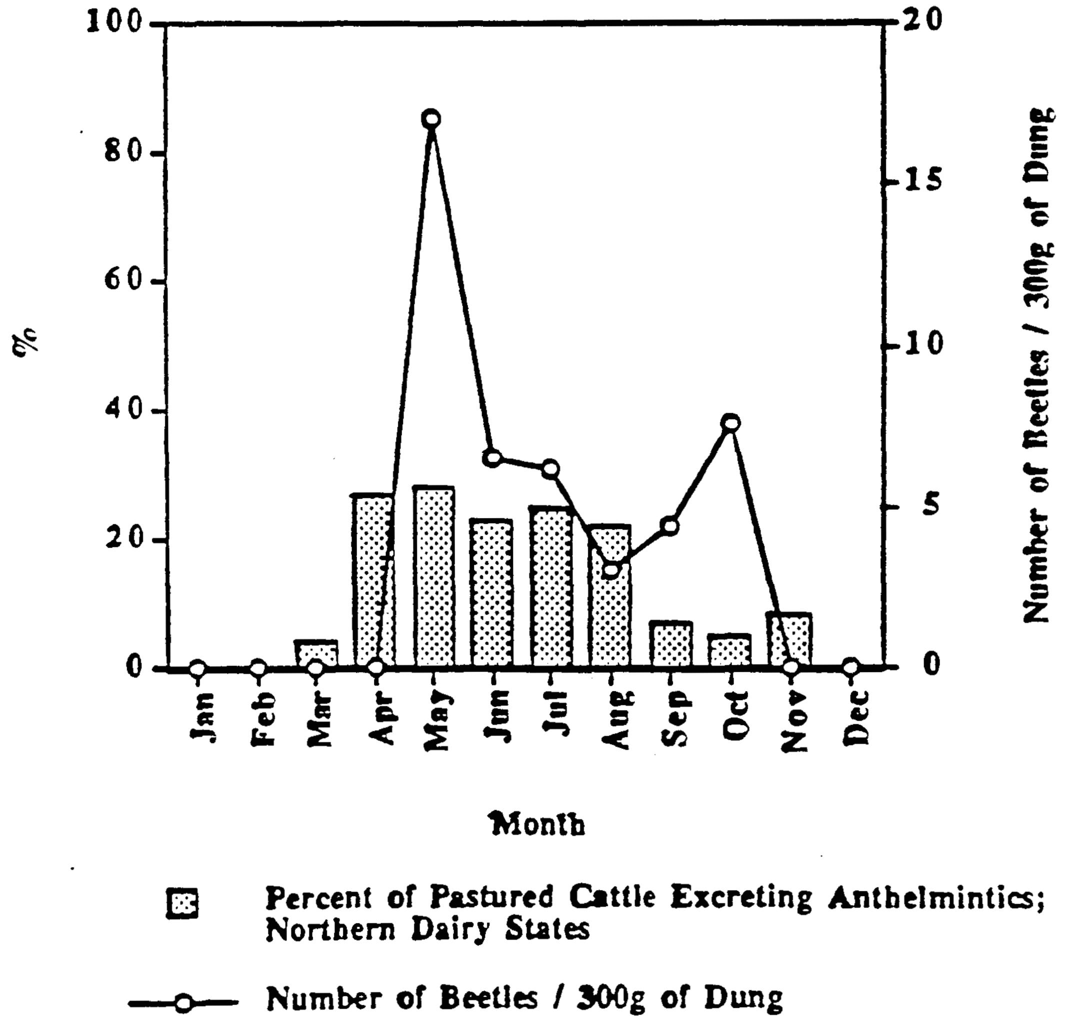
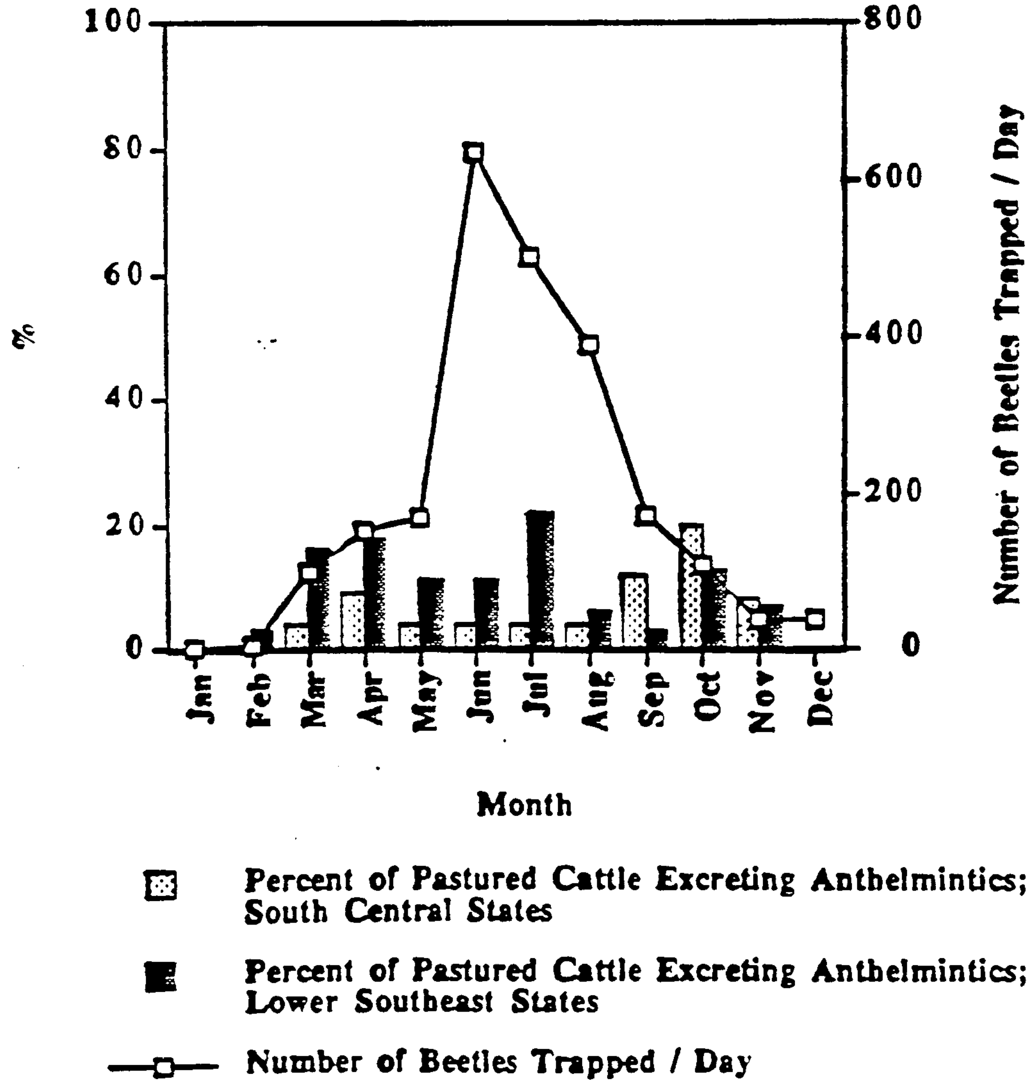


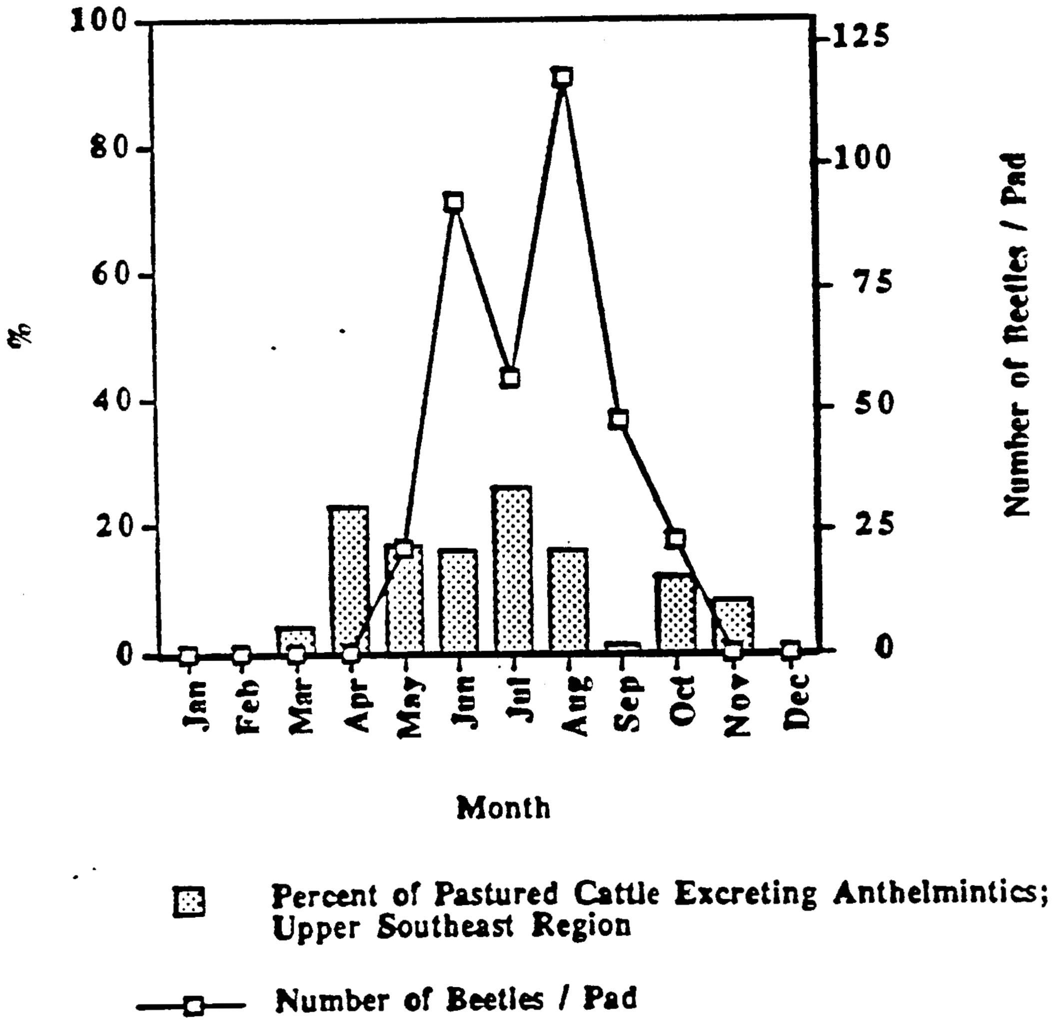
Figure 10
Comparison of the Estimated Actual Percentages of Pastured Cattle Excreting Anthelmintics in the South Central and Lower Southeast Regions versus Numbers of Dung Beetles by Month in Texas



autumn. This period of activity does not coincide with any major time of usage of anthelmintics in the South Central region. Only 4% of all pastured cattle would be excreting anthelmintic residues in feces during the months of greatest dung beetle activity. Indeed, the month of greatest anticipated excretion of anthelmintic residues (October; see Table 3) occurs well beyond the time of major dung beetle activity and involves only about 20% of the pastured cattle for the estimated actual scenario. This pattern of beetle activity should be representative of the South Central and Lower Southeast regions (Blume, R. R., personal communication). A maximum of only 22% of the cattle on pasture would be excreting anthelmintic residues in any month in the Lower Southeast region

The months of major excretion of anthelmintic residues in the Upper Southeast region are April and July, for which the estimated actual excretion of anthelmintic residues by pastured cattle are 23% and 26%, respectively (Table 3). April is two months prior to major beetle activity which was observed in Missouri in June (Wingo *et al.*, 1974, Sec. 14), while July is between the months of greatest activity. There is little excretion of anthelmintic residues in June (16%), August (16%) and September (1%), the other months when beetle activity is high. Thus, the exposure of dung beetles (mainly *Aphodius* spp.) in Missouri to cattle dung containing ivermectin residues will be low (see Figure 11). These results should be applicable to the Upper Southeast Region.

Figure 11
Comparison of the Estimated Actual Percentages of Pastured Cattle Excreting Anthelmintics in the Upper Southeast Region versus Numbers of Dung Beetles by Month in Missouri



In the Pacific Eastern Range, the month of highest estimated actual excretion of anthelmintic residues is April and only 9% of the cattle on pasture would be excreting anthelmintic residues (Table 3). This is at least one month prior to the major peak of dung beetle activity in north central California; the activity represents emergence of mainly *Aphodius* spp., especially *Aph. fimetarius*. These dung beetles in this region (data for the western foothills of the Sierra Nevada Mountains) reproduce during the autumn and winter (second peak of activity) (Merritt, 1974; Merritt and Anderson, 1977, Sec. 14; Anderson, J. R., personal communication), a time of little excretion of anthelmintic residues. The data presented in Figure 12 clearly demonstrate there is very little coincidence of the excretion of anthelmintic residues and dung beetle activity. This assessment should be applicable to the inland areas of the other states of the Pacific Region as well. With respect to the Coastal Pasture region of the Pacific Coast states, seasonal activity of cattle dung beetles in Marin County, CA (a representative area) appears to be close to that described above and shown in Figure 16 for the north central California inland area, with *Aph. fimetarius* females ovipositing from October through February-March, and maximum oviposition in November and December (Anderson, J. R., personal communication). No usage/excretion of anthelmintics occurs in October or November in the Coastal Pasture region; maximal percentages of excretion of anthelmintic residues are in December and January (***estimated actual*** values of 37% and 21% in the Pacific Coastal region for these two months; Table 3). Thus, in one of the two months of maximal oviposition activity, all dung pads will be free of ivermectin residues and hence non-toxic to larval beetles. Even in December, the other peak month for oviposition, less than 40% of the cattle will be excreting anthelmintic residues (only a fraction of those cattle would be treated with the IVOMEC® SR Bolus), and thus the large majority of pads will be free of residue

The estimated actual excretion of anthelmintic residues for the New England region reaches 33% in October and 40% in November (Table 3), but in this and other regions such as the Big Sky (maximum of 14% in October) and Plains (maximum of 18% in October) in which winters are severe, anthelmintics are administered just prior to removal of cattle from pasture. Further, in regions with cold winters there will be little or no dung beetle activity in the late fall or winter months.

The ***estimated actual*** percentages of pastured cattle excreting anthelmintic residues in the Southwest region are low throughout the year. The percentages of pastured cattle excreting anthelmintic are 16% (Table ***3***) or less.

In Hawaii, the greatest percentage of pastured cattle excreting anthelmintic residues in the estimated actual scenario is 16% (Table ***3***) .Most of the anthelmintic treatments in the spring are given to stockers which are treated before they are shipped off of the islands for growing and finishing, and hence treatment of these cattle would not contribute to anthelmintic residues on pastures (Eller, 1994, Sec. 14).

Two behavioral characteristics of dung beetles will facilitate recolonization and compensate for any temporarily reduced populations of adult dung beetles which might result because of reduced emergence of a new generation. One of the characteristics is the mobility of adult dung beetles, which allows them to move readily between locales and recolonize an area which may have a low population density for any reason. Studies by Eschle *et al.* (1973, Sec. 14) and Hanski (1980, Sec. 14) demonstrated that dung beetles will be attracted to dung from at least one mile away, and migration of dung beetles over long distances has been well documented. In-flying dung beetles from other areas and refugia will reproduce using the available non-toxic dung pads excreted by non-treated cattle and those cattle treated subcutaneously or topically with ivermectin or other anthelmintics weeks previously. The second characteristic that will aid in maintaining the dung beetle population is density-dependent reproduction (Holter, 1979a; Ridsdill-Smith *et al.*, 1982, Sec. 14; Anderson, J. R., personal communication). Lowered densities of dung beetles in pads can lead to increased numbers of eggs per pad (Holter, 1979a, Sec. 14) and brood ball production (Ridsdill-Smith *et al.*, 1982, Sec. 14) per female, thus in part compensating for lower numbers of egg-laying females. In addition, even if there are fewer eggs per pad, an enhanced success rate for larval development occurs because of reduced competition among the larvae for food and space (Anderson, J. R., personal communication). Both of these behavioral patterns will serve to maintain the population of dung beetles in a locale where use of avermectin-based anthelmintics might cause a decrease in the number of adults in a succeeding generation.

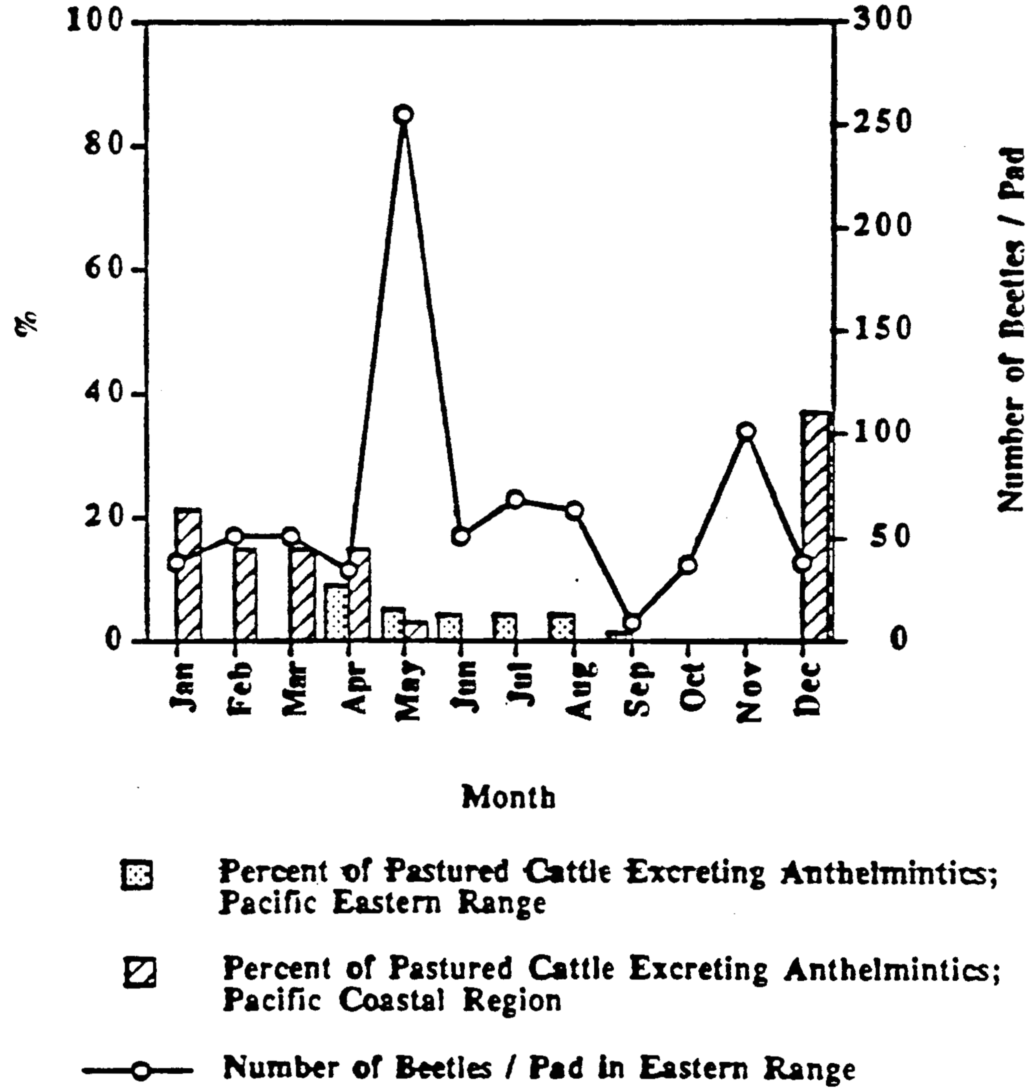
Based on the estimated actual scenarios (Table 3) in the regions where dung beetle activity data are available, 40% or less of the larval dung beetle populations would be exposed in any given month to anthelmintic residues, and only a fraction of the anthelmintic use would be with the IVOMEC® SR Bolus for Cattle. In many cases there is asynchrony between the months of greatest beetle activity and the months with the greatest percentages of cattle excreting anthelmintic residues in the estimated actual scenario (Figures 9 - 12). But even if as much as approximately 40% (the greatest percentage of cattle excreting anthelmintic residues in the ***estimated actual*** scenario, Table 3) of the dung beetle larvae were exposed during the peak month of reproduction/larval development to dung containing anthelmintics, this still would result in negligible long-term impact on dung beetle populations. This is because of the operation of various compensatory mechanisms, as previously discussed. If weather conditions caused a shift in dung beetle activities by a month in any region, the same conditions probably also would cause a corresponding shift in treatment times, since anthelmintic treatments would be governed by appearance of parasites or by time of turnout of cattle onto pasture or removal of cattle from pasture. Hence, asynchrony might be preserved even if weather conditions altered the peak months of dung beetle activity.

Even in a locale in which all of the cattle were treated during a month of major dung beetle reproductive activity, compensatory factors would be expected to attenuate any effects upon populations of dung beetles. Thus, there will be negligible long-term impact upon these populations. **Even the** treatment of all target cattle is, in fact, not observed in practice in any of the regions examined.

Few cattle managers will disregard the economics involved in the amount of labor needed to treat animals with anthelmintics and the cost of these products. Scattered treatments of this nature could occur; however, their impact would be minimal.

The animal husbandry practices which have been identified ensure that there are ample supplies of manure which do not contain ivermectin residues at toxic levels. Mature cattle, which are not candidates for treatment with the IVOMEC® SR Bolus, produce significantly more manure per animal (larger pats) than lower weight cattle. Also, not all manure excreted from cattle treated with anthelmintics, other than the IVOMEC® SR Bolus, will contain anthelmintic residues for the entire month. Thus, the percentages of residue-containing manure and of residue-containing pats will be, in fact, smaller than indicated by the values in Table 3. It is clear that compensatory mechanisms of population dynamics, including mobility and density-dependent reproduction, exist in nature to maintain dung beetle population in cattle pastures.

Figure 12
Comparison of the Estimated Actual Percentage of Pastured Cattle Excreting Anthelmintics in the Pacific Eastern Range and Pacific Coastal Regions versus Numbers of Dung Beetles by Month in California



xiii) Other fauna

In addition to insects, other life forms found in dung pats include earthworms, fungi and bacteria. The LC_{50} of ivermectin in soil for earthworms is 315 ppm, with a NOEL of 12 ppm. This NOEL is above the level of total ivermectin residues expected in freshly excreted (0.7 - 1 ppm, see Sec. 6.B.ii.) or dried dung pads (4.7 to 6.7 ppm, assuming 15% dry matter). It is not surprising that in the Missouri trial (Wallace and Holste, 1989, Sec. 16) the number of earthworms was similar in pats with and without ivermectin residue. Madsen *et al.* (1990, Sec. 14), also found no effect of ivermectin residue in dung pats from drug-treated cattle upon numbers of earthworms. They collected dung from heifers treated subcutaneously at 0.2 mg of ivermectin/kg body weight and prepared 1-kg. pads. Worms were extracted from pads and from the soil underneath and around the pads at various intervals after the pads were placed in a field in Denmark. Total earthworm biomass, total worm numbers and total Lumbricus numbers were not different in and under pads from treated and control cattle. These findings agree with earlier results from Madsen *et al.* (1988, Sec. 14) where artificial, 0.1-kg dung pads, prepared from feces from a single heifer treated subcutaneously with ivermectin 24 hours previously, were prepared and placed into clay pots containing composted garden soil. Similar pots were prepared using feces from heifers treated with other anthelmintics or from non-treated heifers. To each pot was added a mixture of earthworms *Aporrectodea longa* and *A. tuberculata*. The pots were covered although holes allowed access for insects. The pots were placed outdoors in the early summer in Denmark under an open shelter where they were watered frequently. After 98 days, the earthworms were recovered for fresh-weight measurements. No differences in earthworm biomass between treatments was observed. The authors therefore concluded that these worms were not affected by the anthelmintics.

McKeand *et al.* (1988, Sec. 14) observed more sightings of live earthworms in pads from cattle treated with the pour-on formulation of ivermectin than in control pats. Similarly, Wall and Strong (1987, Sec. 14) found slightly more earthworms in and under pads from cattle treated with ivermectin via a sustained-released bolus than in and under pads from control cattle. Wratten *et al.* (1993, Sec. 14) enumerated earthworms in paddocks grazed over three seasons by untreated cattle or cattle treated with ivermectin sustained-release bolus, ivermectin injection or an oxfendazole bolus. They found no evidence of an effect from ivermectin on total numbers of earthworms or on weights of individual earthworms. The collection method for earthworms used by Wratten *et al.* (1993, Sec. 14) has been criticized by Holter *et al.* (1994, Sec. 14), however, Wratten *et al.* (1994, Sec. 14) defended their results since the same methods were used on all paddocks.

Based on all of the literature citations, which show either little or no effect of ivermectin on numbers or weights of earthworms under field or laboratory conditions, and since the concentrations of ivermectin in plowed fields and in excreted pads would be below the NOEL for earthworms, no adverse effect upon earthworms would be expected from residual ivermectin in the dung of cattle receiving the IVOMEC® SR Bolus.

Ivermectin has no antifungal or antibacterial effects at concentrations as high as 2000 ppm (Halley *et al.*, 1989, Sec. 14). Further, avermectin B_{1a} lacks any detectable antifungal activity at 400 ppm (Onishi and Miller, 1985, Sec. 14).

xiv) Dung pat degradation/decomposition

Nearly all of the ivermectin administered to cattle via the IVOMEC SR Bolus ultimately enters the environment via the dung, either as residual drug or metabolites. Dung pats undergo degradation, returning nutrients to the soil, and offer certain insects sites and food necessary for their successful reproduction. Degradation of dung pats is a complicated series of events, involving a wide variety of animate and inanimate forces. The rate of decomposition of dung pats is extremely variable, and depends upon many factors [e.g., climate, season, soil type, faunal inhabitants and microclimate (Halley, *et al.*, 1993; Putman, 1983 and Madsen *et al.*, 1990, Sec. 14)]. A general discussion of dung decomposition and degradation is presented in this section, as an introduction to the assessment of the impact of ivermectin on dung degradation and on certain dung fauna and flora. Dung pat degradation is important not only because it results in recycling of nutrients to the soil, but also because a low rate of degradation can have an adverse economic impact arising from the smothering of new vegetation and inhibiting its growth. Loss of useful forage may result from the phenomenon known as "grazing avoidance", i.e., cattle not eating grass growing in the immediate vicinity of fecal pats.

a. Effects of biological components

Worms, fungi, bacteria and insects (both adult and larval forms) are members of the bovine dung community, all playing roles in the removal and decomposition of dung. During the wet season in the tropics, dung-collecting and dung-burying beetles may degrade an entire dung pad from a large herbivore within 24 hours of deposition (Putman, 1983, Sec. 14);

however, in temperate ecosystems, dung beetles do not play a major role in dung pat removal (See Section 8.A.xii.a.4). Rather, decomposition of dung is primarily a microbiological decomposition process, with the bacteria and fungi of decay serving as major contributors (Marsh and Campling, 1970, Sec. 14). Earthworms also play a key role in the dung degradation process (Putman, 1983; White, 1960, Sec. 14). Dung-breeding insects, including flies, are present in temperate areas and are also included among animals associated with decaying dung. They colonize dung directly, laying their eggs in the dung, upon which the developing larvae feed. Insect larvae and microorganisms, colonizing a dung pat, provide a route for the molecular removal (via metabolism) of organic material from the pat. Tunneling by insects (larval and adult forms) increases aeration of the pat and facilitates deeper penetration of aerobic bacteria and the entrance of fungi into the pat.

b. Effects of physical/mechanical components

Just as there is a biological component to the decomposition and degradation of dung pats, physical and/or mechanical factors also play a key role in pat degradation. Weathering (rain, frost and snow, freezing, thawing, dehydration) and resultant pat cracking is very important in the breakdown of dung pats (Putman, 1983; White, 1960; Bastiman, 1970; Anderson *et al.*, 1984, Sec. 14). Heavy and frequent rains disrupt dung pats, and Dickinson and colleagues (1981, Sec. 14) reported that irrigation of a pasture, to simulate continuously wet weather, promoted the disappearance of cattle dung. In contrast, hot, dry, sunny weather retards pat degradation (Marsh and Campling, 1970; Dickinson *et al.*, 1981, Sec. 14), as the dung quickly develops a hard outer crust retarding entrance of insects; further, activity of earthworms, bacteria and fungi proceed more slowly under dry conditions and in the winter. Growth of new vegetation through cracks in dung pats contributes to further pat degradation. Trampling and scattering of pats by cattle lead to the breakdown of pats (especially on pastures with high stocking rates), as does disturbance by birds (e.g., the Western meadowlark, Sturnetta neglecta) scratching and pecking in dung piles in their search for insects and undigested seeds (Anderson *et al.*, 1984; Marsh and Campling, 1970, Sec. 14).

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

c. Effects of insecticide (lindane) upon pat degradation

Merritt and Anderson (1977, Sec. 14), and Anderson *et al.* (1984, Sec. 14), studied the relationship between cattle feces devoid of insects (created by adding lindane to control feces at the high rate of 282 ppm) and increased dung fouling of pastures. These authors concluded that pat degradation rates are determined more by the season of the year when pats are deposited, and the type of pasture on which they are dropped, than by insect activity. Fastest degradation occurred on cleared but irrigated pasture, and the slowest was observed for non-irrigated pastures with no shade. Increase in time required for degradation was greatest for lindane-containing pats (compared to insecticide-free pats) put outdoors in May and early June (a time of high insect activity); the impact of the lindane upon degradation was least with pats placed on irrigated pastures. Further, little difference in degradation rates was noted during other times of the year between insecticide-treated pats and control pats.

d. Pat weights and surface areas

Anderson *et al.* (1984, Sec. 14) reported that comparative losses in weight between treated and control pats "had little biological or practical meaning" in rangeland pasture. The two important criteria were the pat surface area smothering new growth, and the length of time a pat remains in the pasture. How much less a pat weighed as it aged was not an important criterion. Most cattle dung pats initially contain 75 to 90% water, and significant differences in weight loss can occur among pats with no important effect on the area of ground covered (Anderson *et al.*, 1984, Sec. 14). Weight loss of pats can approach 70 to 80% during a hot, dry spell of a month's duration, resulting entirely from evaporative loss of water (Merritt and Anderson, 1977, Sec. 14).

e. Effects of ivermectin on dung pat degradation

Several studies have investigated the effects of ivermectin on dung pad degradation. Methodologies used in these studies were not consistent; natural and artificially formed pads were used and methods for assessing degradation included measurements of wet weight, dry weight, organic matter content, pad diameter or pad area. For a recent review on the importance of methodology in the interpretation of the factors affecting the degradation of dung and for suggestions on standardizing conditions, see Barth (1993, Sec. 14).

In a report by Schmidt (1993, Sec. 14), there was no apparent impact upon the disintegration on pasture of artificially formed (1.5 kg or less in weight) dung pads produced by cattle which had received ivermectin (0.2 mg/kg via intramuscular injection) compared to the disintegration of pads from control cattle.

Wall and Strong (1987, Sec. 14) also investigated the impact of excreted ivermectin upon fecal pad degradation. Ivermectin was given continuously to 200-kg calves at 0.04 mg/kg/day via ruminal bolus. They concluded that degradation in cattle-free pasture of 2000-g pads, prepared from feces containing ivermectin residues, was prolonged compared to that of pads prepared from control feces. These artificially formed pads were several times the weight of those typically deposited on pastures in trials with cattle. These authors used differences in wet weight of control and experimental (i.e., ivermectin residue-containing) pads with time for a quantitative estimate of the difference in rates of pad decomposition, and speculated that ivermectin treatment could lead to an increase in the amount of pasture land fouled by dung. Results from field studies demonstrate that this speculation is not born out in reality. Since the control pads were "largely degraded within 100 days," the practical significance of a relative difference between small numbers is not clear. Additionally, any differences in moisture content (another important factor for pad area and degradation according to Barth, 1993, Sec. 14) between the control and experimental pads could have lead to the observations (Barth *et al.*, 1993, Sec. 14). The importance of diminution of wet weight by pads, with respect to their degradation and environmental impact, has been discounted by other researchers (Anderson *et al.*, 1984 and Holter, 1979b, Sec. 14).

Schaper and Liebisch (1991, Sec. 14) reported that, compared to dung pads from control cattle, dung pads from cattle that received ivermectin subcutaneously at 0.2 mg/kg did not exhibit delayed degradation. Twenty-one cattle were treated at 3 and 8 weeks after the start of the grazing season in northern Germany. Fresh dung was collected two days after the first treatment and then weekly thereafter. Standardized 1.5-kg artificial pats were deposited in a fenced-in area of pasture along with pats from untreated cattle. The moisture content of pats from both groups were equalized before deposition. Six control cattle grazed on the pasture but outside of the fenced-in area. Pat areas were determined by serial photography at regular intervals over 21 weeks. Schaper and Liebisch also found no differences in numbers of adult or larval dung beetles between treatment groups; however, numbers of diptera and nematodes were reduced in the pats from cattle treated with ivermectin.

McKeand *et al.* (1988, Sec. 14) also found no delay in the degradation of natural pads of cattle treated with the pour-on formulation of ivermectin. Cattle were treated at 3, 8 and 13 weeks after spring turnout in western Scotland. Jacobs *et al.* (1988, Sec. 14) also examined the degradation of natural dung pads from cattle treated with the pour on formulation at 3, 8 and 13 weeks after turnout onto pastures in the UK. They found no feces remaining just before the next grazing season on pastures grazed by treated or control cattle. Rates of degradation of pads were not determined and lungworm infections necessitated treating all control cattle at least once during the trial with parenteral ivermectin.

Madsen *et al.* (1988, Sec. 14) prepared artificial 0.1-kg dung pads from feces from a single heifer treated subcutaneously with ivermectin 24 hours previously and placed the pads into clay pots containing composted garden soil. Similar pots were prepared from feces from heifers treated with other anthelmintics or from non-treated heifers. To each pot was added a mixture of earthworms. The pots were covered although holes allowed access for insects. The pots were placed outdoors in the early summer in Denmark under an open shelter where they were watered frequently. Within a period of 42 to 55 days, all pads, except those from the ivermectin-treated heifer, had disappeared completely. Complete disappearance of the pads containing ivermectin residues was observed by day 98. Thus, in the absence of normal weathering mechanisms and when interactions with some biotic species are prevented, effects of ivermectin on dung-living dipterian larvae might affect dung degradation.

Madsen *et al.* (1990, Sec. 14) also compared the organic matter content of formed pads of 1-kg weight from cattle given ivermectin subcutaneously at 0.2 mg/kg b.w. with that from control animals. As the pads aged in the pasture, the percentage of initial organic matter decreased more slowly in pads excreted by treated animals one or twenty days post dosing than for comparable pads from control animals. Organic matter of pads deposited by treated animals 30 days post dosing decreased at a rate comparable to that of controls. In Denmark, dung degradation, also as measured by percentage of initial pad organic matter and using formed 1-kg pads which were placed on nylon mesh screening and under chicken wire to prevent breakup of the pads by birds, was found by Sommer *et al.* (1992, Sec. 14) to be diminished for dung collected 1 - 2 days post treatment (0.2 mg/kg subcutaneous injection) or up to 13 - 14 days post treatment (0.5 mg/kg topical application) compared to ivermectin-free dung.

Sommer *et al.* (1993, Sec. 14) found no differences, related to treatment of cattle with ivermectin, in the amount of cattle dung buried in fields by afrotropical dung beetles in Zimbabwe. Artificial, 1-kg pads were prepared from dung from control cattle and from cattle treated on 2, 8 or 16 days prior with ivermectin subcutaneously at 0.2 mg/kg body weight. After five days of exposure, most of the residual dung was inextricably mixed with soil; however, the total amounts of non-buried dung organic matter were determined from loss of weight on ignition data.

No significant effects upon feces degradation were observed with respect to use of ivermectin in horses. Ewert *et al.* (1991, Sec. 14) and DiPietro *et al.* (1993, Sec. 14) reported that multiple dewormings with ivermectin did not result in prolonged dung degradation leading to increased pasture fouling as determined by aerial survey mapping. However, Herd *et al.* (1993, Sec. 14) reported that delayed degradation occurred with artificially formed dung pads from horses treated with ivermectin.

Three studies (Wallace and Holste, 1989; Heinze-Mutz and Barth, 1990; Baggott, Wratten and Mead-Briggs, 1990, Sec. 16) were conducted by Merck to determine whether ivermectin in dung of calves treated with an IVOMEC SR Bolus affected dung pat degradation, grazing avoidance or fauna populations. There were no treatment-related effects for dung pat degradation or grazing avoidance. There were, however, treatment-related effects on dung fauna, especially upon insect pests.

Extensive weight loss of pats during hot dry weather is evident from data generated in a study conducted in Missouri (Wallace and Holste, 1989, Sec. 16). Plots showing percentage of initial dung pat wet weight as a function of pat age for pats deposited by calves on 4 different days post dosing (control; IVOMEC SR Bolus) are presented in Figure 13. Data in the figure are means from 5 pats deposited on days 0 (day dosed), 7, 14 and 56 after bolus administration. Overall, these data strongly suggest there is no treatment-related effect upon percent weight loss by the pats.

With respect to pat surface areas remaining to smother new growth, plots of percent of initial pat area vs pat age for pats from control and ivermectin bolus-treated calves (Wallace and Holste, 1989, Sec. 16) are given in Figures 14, 15 and 16. Pats areas employed to generate these three figures were obtained by image analysis of tracings of photographs of dung pats (Memos, VandenHeuvel to Roncalli, 8/30/90 and Zeigler to VandenHeuvel, 8/30/90.) Two pats from each of three different days of pat deposition (dosing day, 14 and 28 days post dose) from both control and bolus-treated calves were photographed periodically for >300 days or more than 10 months. Data presented in Figure 14 for pats deposited on the day of dosing (hence ivermectin residues not in dung) illustrate that ivermectin-free pats show large differences and variations in percent of initial area with time. Data in Figures 15 and 16 demonstrate that there are no treatment-related effects upon reduction of dung pat areas over time. Similar results were found a trial conducted in Lauterbach, West Germany (Heinze-Mutz and Barth, 1990, Sec. 16; Barth, 1993 *et al.*, Sec. 14). The surface areas of fecal pats deposited on days 21/22, 70 and 119 post treatment from control calves and those given an IVOMEC SR Bolus were followed for over eight months. Pertinent data are presented in Table 10.. Degradation of the pats from the IVOMEC SR Bolus-treated calves appeared to be somewhat reduced compared to that for pats from control calves beginning one and one-half to two months post initiation of treatment. However, statistical analysis of these data revealed no difference ($p>0.10$) between treatments in respect to average surface area or change in area over time for dung pats deposited on Days 21/22 or 70. After adjusting for initial differences, control pats deposited on day 119 were slightly larger than ivermectin pats 7 to 49 days after deposition and slightly smaller 63 to 147 days after deposition; the difference was less than 1 cm^2 at 175 days. By 8-9 months both sets of pats were essentially degraded. Further, the decrease in organic matter content of control and ivermectin residue-containing pats was treatment-independent (Table 11). Madsen *et al.* (1990, Sec. 14) suggested that decrease in organic matter of dung pats is an indication of rate of dung pat disappearance. Based upon these results, ivermectin treatment would not be expected to increase pasture fouling and loss of new growth because of smothering.

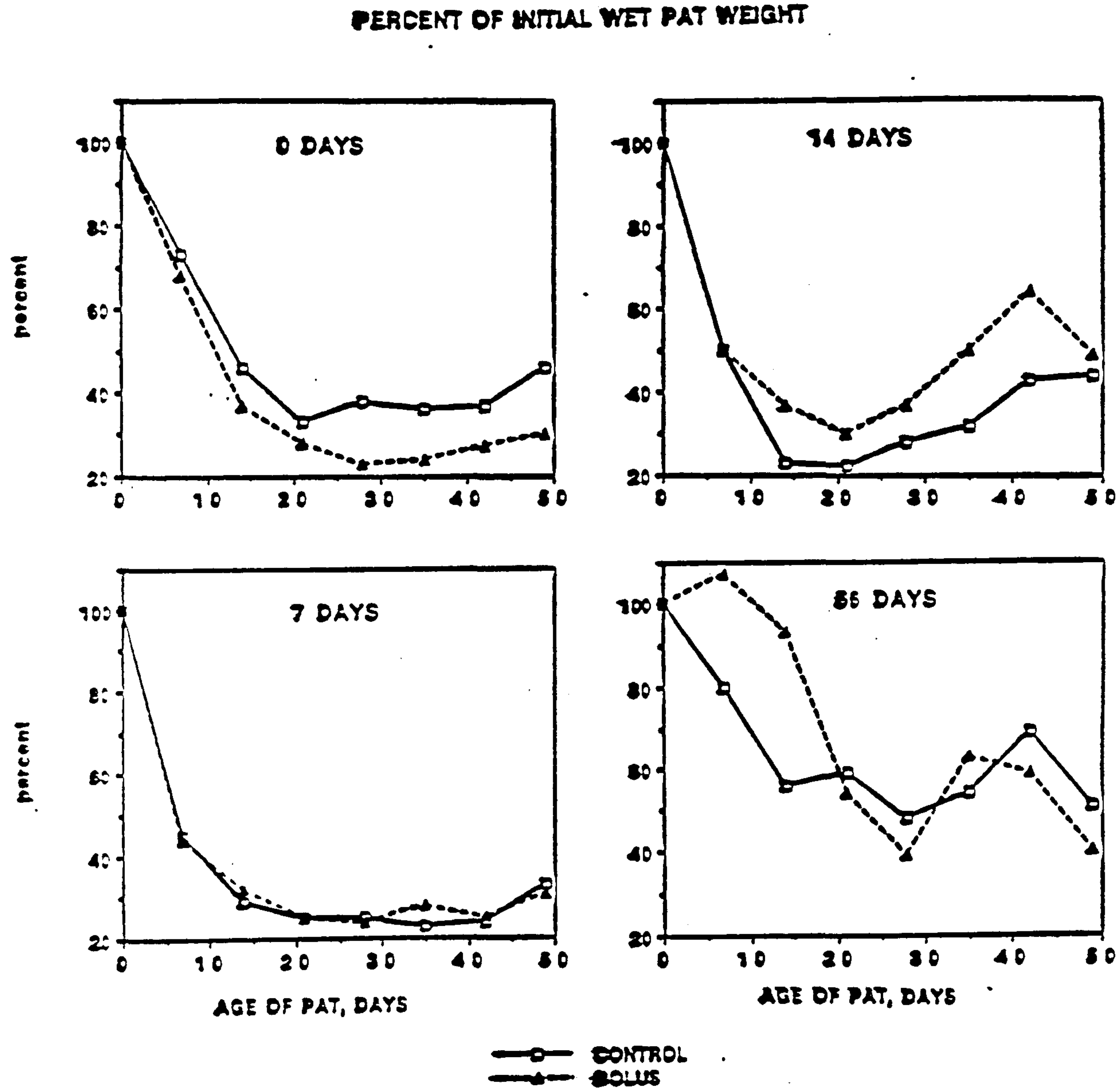


Figure 13. Plots of percent initial wet dung pat weight vs pat age for pats deposited by calves (controls, squares; 90-day IVOMEC SR Bolus, triangles) on days 0, 7, 14 and 56 post dosing. Actual weighings were carried out on one-eighth segments of pats.

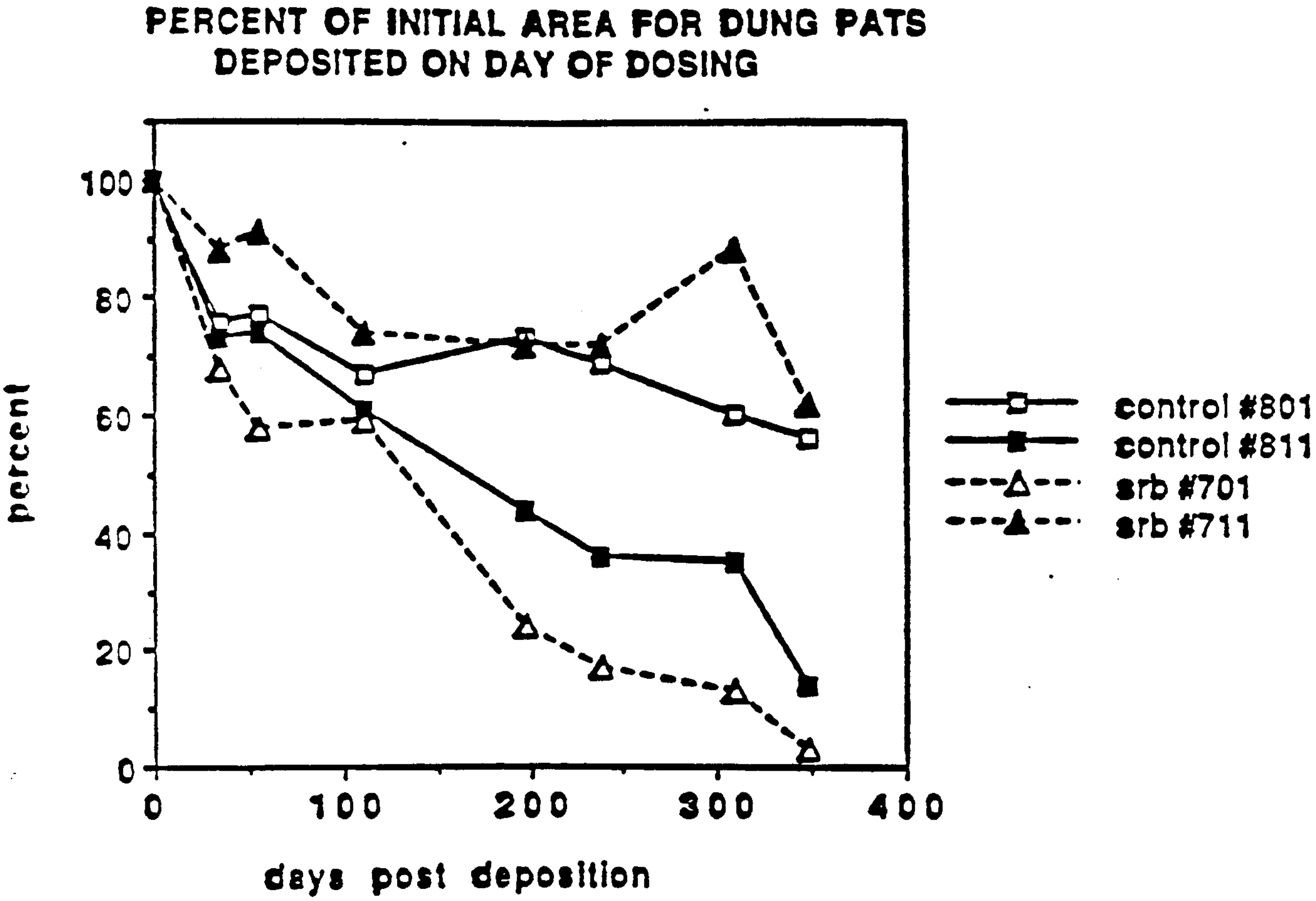


Figure 14. Plots of percent initial dung pat area vs days post deposition for pats deposited on the day of dosing by control calves (#801 and #811) and by calves (#701 and #711) which were each given an IVOMEC SR Bolus (srb).

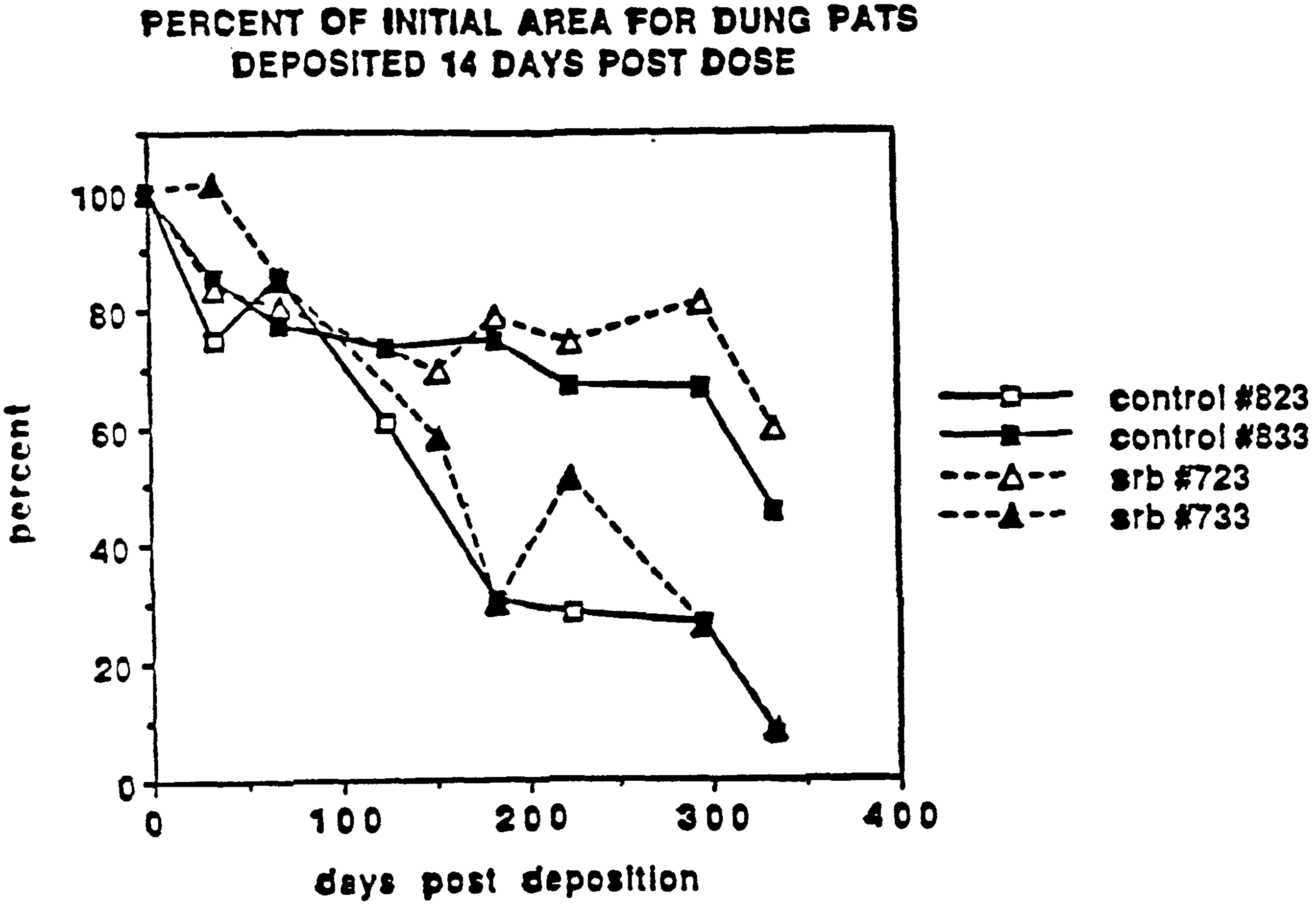


Figure 15. Plots of percent initial dung pat area vs days post deposition for pats deposited on day 14 by control calves (#823 and #833) and by calves (#723 and #733) which were each given an IVOMEC SR Bolus (srb).

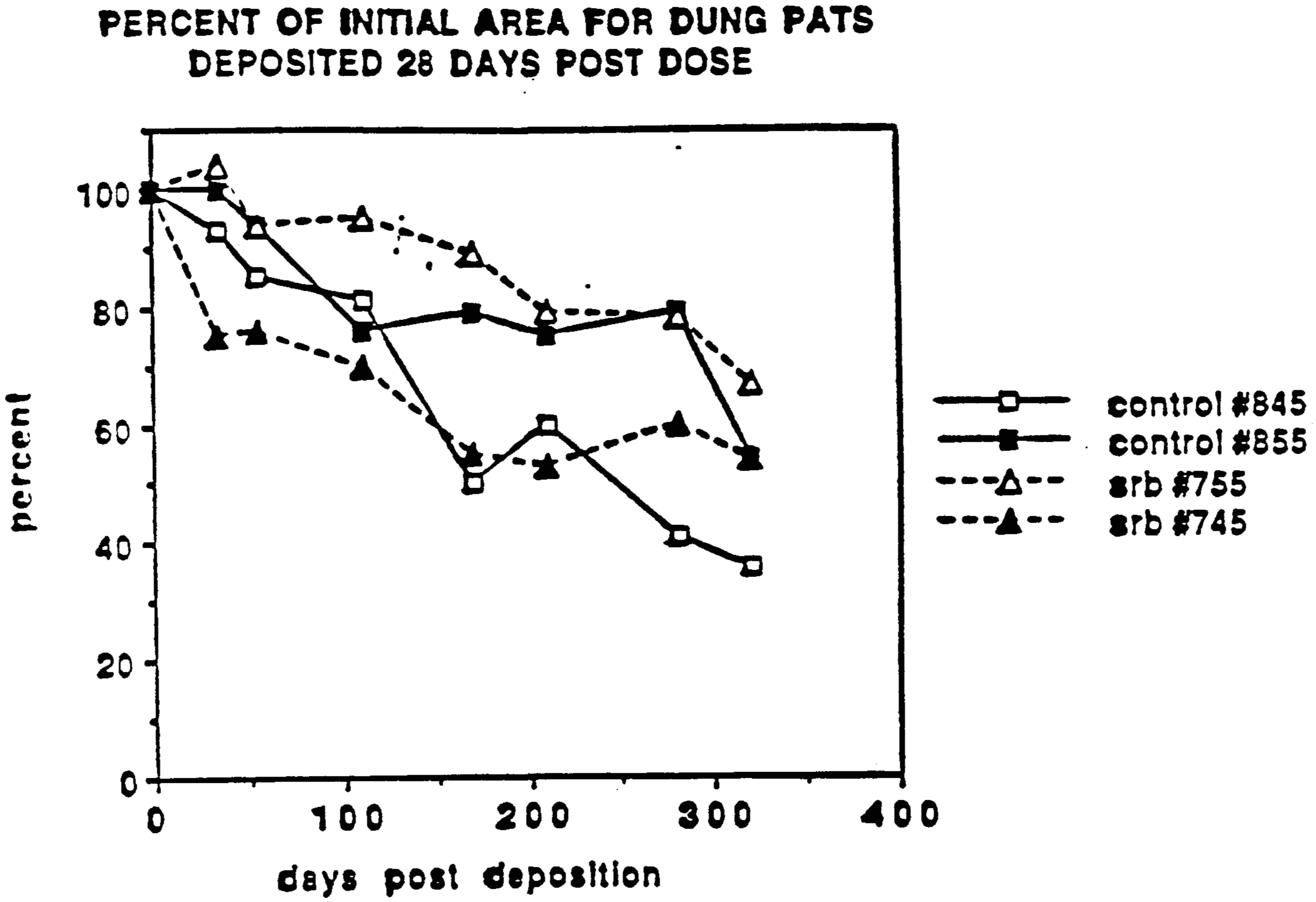


Figure 16. Plots of percent initial dung pat area vs days post deposition for pats deposited on day 28 by control calves (#845 and #855) and by calves (#755 and #745) which were each given an IVOMEC SR Bolus (srb).

Table 10
Percent of Initial Pat Surface Area[a]

Trial Day of Deposit	Days After Deposit (Age of Pat)														
	0	7	14	21	35	49	63	91	119	147	175	203	231	251	259
21/22	June 6								Oct. 3						
Control	100	93	99	99	79	50	17	2	1	<1	<1	<1	<1		
Bolus	100	96	101	99	85	58	37	18	11	3	1	2	2		
70	July 25								Nov. 21						
Control	100	107	100	87	75	36	14	6	5	3	2	2	2		1
Bolus	100	109	105	94	71	59	44	19	6	2	1	<1	0		0
119	Sept. 12								Jan. 9						
Control	100	96	98	86	77	57	27	--[b]	29	21	16	9	0	0	
Bolus	100	99	98	91	82	66	51	--	53	43	35	26	7	<1	

[a] Mean of 10 pats

[b] Measurement of area not possible because of snow and ice crust on pats.

TABLE 11
Organic Matter Content of Pats
(As Percent of Dry Weight[a])

Days of Pat Deposition Post Treatment	DAYS AFTER DEPOSITION (Age of Pat)		
	0	35	63
GROUP 1: CONTROL			
21/22	87.9 / 100[b]	83.6 / 95[b]	72.0 / 82[b]
70	82.6 / 100	72.0 / 87	70.1 / 85
119	82.5 / 100	60.5 / 73	53.5 / 65
GROUP 2: IVERMECTIN BOLUS			
21/22	87.0 / 100	78.5 / 90	75.4 / 87
70	82.5 / 100	74.7 / 91	61.9 / 75
119	82.0 / 100	66.4 / 81	64.6 / 79

[a] Arithmetic mean of 10 pats (grams)
[b] Percentage of initial (0 day) organic matter content

To determine the effect of anthelmintic drugs upon the production and disappearance of cattle dung in pastures, a two-year study (Baggott, Wratten and Mead-Briggs, 1990, Sec. 16;Wratten *et al.*, 1993, Sec. 14) was conducted by scientists from the Agrichemical Evaluation Unit, University of Southampton, at the Merck farm in Hoddesdon, Hertfordshire, UK. Treatments include controls, ivermectin bolus (8 mg/day for approximately 90 days), ivermectin injection (0.2 mg/kg at 3, 8 and 13 weeks) and oxfendazole bolus (750 mg at five intervals of approximately 21 days each). The functionality of the ivermectin bolus is supported by data on fecal EPG counts (Baggott and Pitt, 1990, Sec. 16). There were no treatment-related differences between groups in the rate of dung deposition (weight of dung collected at monthly intervals) and accumulation of dung on the pastures, i.e., no significant difference ($P > 0.05$) in the dry weights of cumulative standing dung.

The rate of decomposition/degradation under natural conditions of dung pats from calves was investigated by locating 40 fresh pats in each paddock in July. At this time, the ivermectin bolus had been operational for two months, hence there was drug residue in the dung. Ten of these natural pats were collected in each paddock immediately following deposition, as were ten each at monthly intervals for three months. The dry weight of each collected pat was determined and a mean value calculated for each paddock at each time point. The collection procedure was repeated with pats deposited in September, at which time the IVOMEC SR Bolus was no longer delivering ivermectin. The results from this experiment (both July and September depositions, ivermectin-containing and ivermectin-free pats, respectively) show that weights of the pats decreased with time, and rate of decrease was not effected by treatment ($P > 0.05$). With respect to the second part of the study (initiated in the Spring of 1989), there were no significant ($P > 0.05$) differences among treatments for dung deposition rates, weight of dung collected at monthly intervals, or rate of decomposition/degradation of natural dung pats.

The importance of weight loss by pats, with respect to their degradation and environmental impact, has been discounted by Anderson *et al.* (1984, Sec. 14). It is, nevertheless, instructive to compare the results from the UK study (Baggott, Wratten and Mead-Briggs, 1990, Sec. 16;Wratten *et al.*, 1993, Sec. 14) using natural dung pats on pastures in which calves were grazing with those from the Wall and Strong (1987, Sec. 14) study. The latter involved cattle-free pastures and pats formed from cattle feces; prolonged degradation of formed pats containing ivermectin residues was observed. In line with the results from the U.K. trial, Schmidt (1983, Sec. 14) reported that manure from cattle given a single injection of ivermectin at 0.2 mg/kg appeared to disintegrate at a rate similar to that of dung from uninjected controls.

Another key component of the U.K. trial (Baggott, Wratten and Mead-Briggs, 1990, Sec. 16) involved taking transects of fields, monitoring the development of grazing avoidance patches, and ascertaining whether the areas of the patches differed among treatment groups. No significant differences ($P > 0.05$) were found among treatments for either year.

In summary, with pads deposited by cattle (or horses) on pasture and allowed to degrade naturally under field conditions, the presence of ivermectin residues, even in feces from cattle which received an IVOMEC® SR Bolus, has no significant effect upon pad degradation. Delays in degradation of artificially formed pads from ivermectin-treated cattle have been reported. It appears that the methodology utilized in the study, in addition to abiotic and biotic factors, can influence the results of dung degradation studies.

xv) Additional pharmacology and toxicity information

An overview of the pharmacology of ivermectin and information on the toxicity of ivermectin to soil microbes, plants, various aquatic organisms, nematodes, arachnids, insects, and annelids, as well as a literature review, can be found in the summary of the Environmental Assessment for IVOMEC (ivermectin) Injection for Swine (NADA 135-008, Sec. 16; Wratten *et al.*, 1993, Sec. 14.). The present Environmental Assessment supplements this with recent information on ivermectin and supporting information on avermectin B_1 as discussed above. Summaries of these reports can be found in the Section 16.

B. Environmental hazard assessment

i) Hazard assessment in aquatic ecosystems

Daphnia is the freshwater aquatic species found to be most sensitive to ivermectin. The 48-hr LC_{50}, 48-hr NOEL and calculated 21-day MATC values for ivermectin toward *Daphnia* are 25, ~10 and 4 ppt, respectively. As indicated in Sec. 7.B., the presence of soil in the test systems reduced the toxicity of ivermectin and avermectin B_1 toward *Daphnia*.

The feedlot runoff study involving subcutaneously dosed steers weighing 365 kg demonstrated that, even with five steers excreting a total of 365 mg of ivermectin-related compounds (73 mg/steer) into an area of only 1000 sq ft, the runoff water showed no acute toxicity toward *Daphnia* and no ivermectin was detected by HPLC. Tight binding to soil of the excreted ivermectin greatly attenuated its toxicity toward this aquatic species. Lack of toxicity should also pertain with a pasture runoff, as the expected amount of ivermectin-related residues introduced in calf excreta as dung pats will be only about 20-25% that for the above-described cattle feedlot (79 μg/sq ft vs 365 μg/sq ft). It can be reasonably concluded that runoff water from a dung pat excreted by a bolus-treated calf on pasture would contain no more than 2 ppt of ivermectin-related residue, below the calculated 21-day MATC value of 4 ppt. It should be noted that all intact dung pats produced during 135 days would not be present in the pasture at any one time since, as new pats are being deposited, old pats are decomposing (and their ivermectin degrading). Further, as dung pats do not come in total contact with water, only a very small fraction of ivermectin residue in a pasture dung pat would be removed from the pat by water, and the ivermectin concentration in this leachate would rapidly diminish as the water moved across the pasture.

Thus, the likelihood of introduction of toxicologically significant amounts of drug-related compounds into the aquatic environment through pasture runoff is remote. Because of tight binding to organic matter in dung, pasture soil and sediment in water, ivermectin concentrations (see Table 4) in a body of water would be 0.001 ppt, far below the 48-h LC_{50}, 48-NOEL and calculated 21-day MATC for *Daphnia*. Metabolites of ivermectin from cattle (tested individually or as feces-soil column percolates) possess much less toxicity toward *Daphnia* than the drug itself (Table 5). Further, photodegradation of the unbound ivermectin in water would rapidly remove the traces of drug from the aquatic environment. This is graphically illustrated in Figure 3. The initial concentration of only 0.001 ppt would drop [by factors of ~2 and ~16 (in winter and summer, respectively) within 4 days] to concentrations even farther below any levels of toxicological concern, even toward *Daphnia*. The photodegradation pathway (plus degradation resulting from aerobic metabolism in soil) increases an already more than adequate margin of safety for the use of ivermectin in a sustained-release bolus for cattle.

With respect to a possible toxic effect toward *Daphnia* of ivermectin residues introduced into a body of water by direct deposition of dung pats (Sec. 7.C.), 50 calves could deposit their entire day's excrement into a one-acre pond (4 ft deep) without the effective ivermectin concentration (0.012 ppt) even approaching the 21-d MATC (4 ppt). If 50 calves were to introduce 10% of their daily excrement into a slowly moving stream during the same ten minute period, the resulting ivermectin concentration, 0.046 ppt, would also be far less than the 21-d MATC.

As ivermectin is toxic toward *Daphnia* at very low concentrations, this Environmental Assessment has focused on this species. It is clear that the environmental fate characteristics of ivermectin make it highly unlikely that environmental concentrations will reach levels toxic to any aquatic species, including *Daphnia*. Data in Table 5 also support the view that ivermectin-related compounds such as its monosaccharide and aglycone, and cattle feces/soil column percolates, which contain ivermectin degradates/metabolites, are much less toxic than the parent compound. Avermectin B_1 is less toxic toward *Daphnia* than is ivermectin, and the known degradation products of avermectin B_{1a} (e.g., the $\Delta^{8,9}$-isomer and the 8α-hydroxy compound) are also much reduced in toxicity toward *Daphnia* compared to their parent compound (Forbis *et al.*, 1985 a and b, respectively, Sec. 16).

Fish are at least 100-fold less sensitive to the toxicity of ivermectin than are *Daphnia*. The ivermectin 96-hr LC_{50} values for rainbow trout and bluegill sunfish are 3.3 and 5.3 ppb, respectively (Table 5). These concentrations are far higher (factors approaching one-hundred thousand) than the estimated concentrations that might occur in ponds as the result of cattle receiving ivermectin via a sustained-release bolus. Other aquatic species (e.g., chlorophytes, duckweed and algae) would also be exposed to ivermectin concentrations far below those that would exhibit an effect on such species.

ii) Hazard assessment in terrestrial ecosystems

a. Plants, earthworms, fungi, bacteria

Given the low concentration of ivermectin residues expected in water in contact with manure from ivermectin-treated cattle (1 ppb), and even lower concentrations following depletion of ivermectin by soil binding (Table 4), no deleterious effects would be expected toward plants, earthworms, fungi or bacteria. For example, the ivermectin LC_{50} toward the earthworm is 315 ppm (28-d NOEL of 12 ppm). The no observed effect concentrations for ivermectin in the seed germination and root elongation study, 98-980 ppm, were all far higher than the levels of total drug and metabolites, approximately 1 ppm, expected in the feces of cattle treated with a bolus. The no observed effect concentrations for ivermectin in sand in the seedling growth study, 0.56 -790 ppm, were approximately equal to or well above levels of drug expected in feces. Use of sandy loam soil instead of sand as the growth medium in the seedling growth study with perennial ryegrass clearly showed that no phytotoxicity would occur to plants in plowed fields, since the no observed effect concentration was >1100 ppm, the highest level tested. Thus, the strong binding to soil organic matter reduced the effective concentration of ivermectin by approximately 2,000-fold, as measured by the NOEC for shoot weight, the most sensitive parameter for ryegrass. Any potential effect the low concentrations of ivermectin in soil might have upon life forms therein would be greatly diminished by its photolytic degradation [Sec. (7)(A)(i)] on, and aerobic metabolism [Sec. (7)(A)(iv)] in, soil.

b. Avians

Birds may eat insects and seeds found in dung pats from calves dosed with the IVOMEC SR Bolus. The assumption is made that the insects consume dung containing 1 ppm ivermectin (from a 200-kg calf, soon after initiation of ivermectin release; see Figure 2). At steady state, the ivermectin content of the insect is 1 ppm, based on a $T_{1/2}$ of approximately 4 h (Bull, 1986, Sec. 14). For a bird consuming only insects from the dung, the maximum dietary ivermectin concentration is 1 ppm, a value well below the NOEL of 12 ppm for abamectin established in a 16-week reproduction study in mallard ducks. This value of 1 ppm is far below the subacute 8-day dietary LC_{50} of 383 ppm for abamectin for the mallard. Therefore, 1 ppm ivermectin in the feces should not result in acute or chronic effects in birds, nor should it cause secondary poisoning to raptors preying on such birds. If a 100-g bird consumed 10 g of insects containing 1 ppm ivermectin on a daily basis, it would ingest 100 μg of ivermectin residue per kg b.w. This results in a daily dietary intake far below any level of concern.

Eagles and other raptors have died from exposure to organophosphorus insecticides as a result of eating carrion arising from dosed cattle (Henny *et al.*, 1987, Sec. 14). This is highly unlikely to occur with the IVOMEC SR Bolus. At steady state, with a ~12 mg/day bolus, the ivermectin residue in cattle liver, the tissue of highest residue, averaged 88 ppb (range 52-120 ppb; Wehner and Skelly, 1990, Sec. 16). As a worst-case scenario, consider a 5-kg eagle consuming 500 g of liver containing 120 ppb ivermectin as its entire day's intake. This is an oral dose of 60 μg of ivermectin, or 0.012 mg/kg body weight (and 0.12 ppm in the diet), far below the avermectin LD_{50} (85 mg/kg) and the avermectin dietary NOEL (12 ppm) for the mallard.

c. Dung Beetle Populations

For the following reasons, a significant long-term impact on populations of dung beetles is not expected from **estimated actual** use of the IVOMEC® SR Bolus for Cattle to treat cattle in a region.

- Anthelmintic use is highly variable within a region and throughout the year.
- High anthelmintic usage rates would be expected to be scattered throughout a region; used by some, but not all, cattle managers.
- Use of the IVOMEC® SR Bolus would be restricted to only those cattle weighing 100-300 kg at the time of dosing in spring or summer (late fall/early winter in Pacific Coastal region).
- Not all eligible cattle will be treated with the IVOMEC® SR Bolus.
- Most dung beetle species which are found on open pastures in the United States are not bovine dung specialists. Native, forest-dwelling species, which are adapted to use deer dung, do not generally feed on cattle dung and would therefore not be routinely exposed to ivermectin residues.
- Usage of anthelmintics in pastured cattle in most regions does not coincide with peak periods of dung beetle reproduction.
- Although ivermectin residues in dung may inhibit larval development, a high percentage of emergence can be expected from dung excreted by cattle treated subcutaneously or topically with ivermectin-based anthelmintics at approximately two to three weeks post dose.
- In regions where treatment and reproduction of beetles may be coincident, the percentage of animals treated is low and sufficient dung would be available for reproduction.
- Mature cattle produce significantly more manure per animal (larger pats) than lower weight cattle that are candidates for the bolus.
- Repopulation of areas with reduced populations is expected to occur because of density-dependent reproduction within

the area and migration of highly mobile dung beetles into the area.
- Ivermectin residues in dung of cattle do not affect numbers of colonizing adult dung beetles.
- Dung beetles play, at most, only a minor role in the U.S. in degradation of cattle dung or in its removal from pastures.

No dung-dependent insects are known to be listed or considered by government authorities as endangered or threatened. Blume (1985) listed 450 species of insects associated with bovine droppings on pasture. None is listed as endangered or threatened (US Fish and Wildlife Service, 1990).

d. Dung pats

Dung-breeding and dung-feeding insect comprise only one of the factors involved in the decomposition and degradation of dung pads (See Section 8.A.xiii). It is very unlikely that any effects on these species will have a major impact upon dung pad degradation. This will be especially true in the temperate areas for which use of the IVOMEC SR Bolus is intended, because bacteria, fungi, earthworms, weathering, trampling, action of birds and foraging animals and pasture management techniques all play very important roles in dung pad disappearance. As discussed in Section 8.A.xii.a.4., dung beetles play, at most, only a minor role in the US in degradation of cattle dung or its removal from pastures. Imported, exotic adult dung beetles, where established in temperate climates, can bury significant amounts of dung, however, most reports indicate that removal of dung from pastures in the US is not an efficient process even during periods of high dung beetle activity. Since 1) the level of ivermectin expected in pads (See Section 6.B.iii) are below those which would affect adult dung beetles. 2) Peak use of anthelmintics in most regions will not coincide with peak periods of dung beetle activity, 3) The percentage of animals excreting anthelmintics will be low even in regions where treatment and beetle activity might be coincident, 4) Not all anthelmintics treatments will be with the IVOMEC SR Bolus or with other formulations of ivermectin, 5) Sufficient dung from untreated cattle and other animals will be available and 6) repopulation of areas with reduced populations would be expected to occur because of density-dependent reproduction within the area and migration of highly mobile dung beetles into the area (See Section 8.A.xii.a.), the role of dung beetles in dung dispersal will not be affected by the use of ivermectin. The highest expected concentrations of ivermectin-related residue in feces from cattle receiving the drug via the SR Bolus (~1000 ppb, Figure 2) are well below those that would be expected to have an effect upon bacteria, fungi, earthworms or birds. Observations based on studies at a Merck farm in Missouri (Wallace and Hoste, 1989, Sect. 16), at the Highfield Farm in Hertfordshire, UK (Baggott *et al.*, 1990, Sec. 16 and Wratten, *et al.*, 1993, Sec. 14) and at Kathrinenhof Farm, Lauterbach, Germany (Heize-Mutz and Barth, 1990, Sec. 16 and Barth, *et al.*, 1993, Sec. 14) indicated no treatment-related impact upon dung pad degradation by ivermectin dose via subcutaneous injection or a sustained-release bolus. Also, increased (relative to control) grazing avoidance by cattle was not found in the Hertforshire trial.

Since ivermectin is not expected to affect the role of dung beetles or other biotic species including earthworms in dung dispersal or its removal from pastures in temperate climates and based upon the results of the field studies, use of the IVOMEC SR Bolus would not be expected to inhibit dung pad degradation and thus not increase pasture fouling or cause loss of new growth because of smothering. Hence, no impact upon pastures would be caused by use of ivermectin.

It is highly improbable that administration of the IVOMEC SR Bolus to cattle will have a detrimental effect on the environment.

- Ivermectin is unlikely to move through the environment (low water solubility, tight binding to organic matter and especially soil).
- Ivermectin degrades readily in the environment (photodegradation, aerobic breakdown by soil microorganisms).
- At concentrations that will be present, ivermectin is not phytotoxic or toxic to aquatic ecosystems and plants, earthworms, fungi, bacteria and avians.
- Ivermectin use is not expected to adversely affect populations of dung beetles.
- Under study conditions when foraging-related mechanisms are prevented, ivermectin residues in dung pats slighted reduced the rate of dung degradation. Grazing avoidance has not been seen in any field trials with ivermectin dosed cattle.

9. Use of resources and energy consumption:

The use of raw materials utilized to manufacture avermectin, ivermectin and the IVOMEC SR Bolus are in ample commercial supply. Approximately 20 percent of the ivermectin produced by the applicant is used to manufacture the IVOMEC SR Bolus.

No effects upon endangered or threatened species and upon property listed or eligible for listing in the National Register of Historic places are anticipated.

10. Mitigation measures:

The measures taken to avoid potential adverse environmental impacts associated with the manufacture of IVOMEC (ivermectin) SR Bolus includes proper disposal of Liquid and Solid Waste as described in Section 6 of this Environmental Assessment.

The following paragraph in the package insert minimizes the potential adverse impacts associated with the use and disposal of IVOMEC SR Bolus:

"Environmental Safety - Studies indicate that when ivermectin comes in contact with the soil, it readily and tightly binds to the soil and becomes inactive over time. Free ivermectin may adversely affect fish and certain water-borne organisms on which they feed. Damaged boluses should be disposed of safely (e.g., by burying at an approved landfills or incinerating). Do not contaminate lakes, streams or ground water."

11. Alternatives to the proposed action:

At this time there are no alternatives to chemotherapeutic agents for the control of the important endo- and ectoparasites of cattle. In addition, fly control has become more difficult as resistance to older products has developed. Compared to the majority of the agents now used, IVOMEC SR Bolus has two important attributes. It has a very broad spectrum and therefore obviates the need for multiple treatments with different agents; and it results in the release into the environment of negligible amounts of active ingredient and metabolites. From an environmental standpoint, IVOMEC SR Bolus poses an environmental risk which is small compared to the alternatives.

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<u>Hazard Assessment of the Effects of Abamectin Use in Pastured Cattle on Dung Beetles (Coleoptera: Scarabaeidae)</u>

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13. Certification:

The undersigned official certifies that the information presented is true, accurate and complete to the best of the knowledge of the prospective contractor or applicant submitting the environmental assessment.

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15. <u>Appendices</u>

I. Key References

The following key references are not generally available in science libraries. They have been provided with the 9/28/90 submission of the Environmental Assessment to NADA 140-988.

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