

FINDING OF NO SIGNIFICANT IMPACT

for

Cydectin[®] Injectable Solution for Cattle

Fort Dodge Animal Health
P.O. Box 5366
Princeton, NJ 08543-5366

For Public Display
(HFA-305)

2005-141-220

FONSI-1

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The Center for Veterinary Medicine has considered the potential environmental impact of this action and has concluded that this action will not have a significant impact on the quality of the human environment and therefore an environmental impact statement will not be prepared.

Fort Dodge Animal Health has submitted a new animal drug application (NADA) for Cydectin[®] Injectable Solution for the treatment and control of internal and external parasites in beef and non-lactating dairy cattle. The product is provided as a single subcutaneous application at a dose level of 0.2mg moxidectin/kg body weight. In support of the application, the drug sponsor has submitted an Environmental Assessment (EA), dated June 2001.

The EA provides information on the environmental chemistry and fate of moxidectin residues, the toxicity of moxidectin to dung insects, terrestrial organisms, avian and aquatic species, and calculations of estimated environmental concentrations. A major section of the EA responds to Agency concerns about potential effects of moxidectin residues on pest and beneficial insect populations in dung.

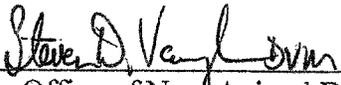
Based on the available information, a FONSI is supported because non-target insect and animal populations are not expected to be adversely impacted by Cydectin[®] Injectable use in beef and non-lactating dairy cattle.

To address concerns over potential toxicity to aquatic organisms from improper disposal of the drug, the following environmental safety statement is placed on drug containers.

Disposal: Do not contaminate water by direct application or by improper disposal of drug containers. Dispose of containers in an approved landfill or by incineration.

We have reviewed the EA and find that it is adequate to determine that significant environmental impacts are not expected from the approval of the NADA for this product.

11-29-02
Date



Director, Office of New Animal Drug Evaluation, HFV-100

Attachment: Environmental Assessment, dated June 2001



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Environmental Assessment – Cydectin (moxidectin) Injectable Solution for Cattle

ENVIRONMENTAL ASSESSMENT

CYDECTIN[®] (moxidectin) Injectable Solution for Cattle

Fort Dodge Animal Health

June 2001

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Environmental Assessment

CYDECTIN[®] (moxidectin) Injectable Solution for Cattle

1. **Date:** June 14, 2001
2. **Name of Applicant:** Fort Dodge Animal Health
3. **Address:** Corporate Administrative Offices:

Fort Dodge Animal Health
9401 Indian Creek Parkway
Overland Park, Kansas 66210

Product Development and Regulatory Affairs Offices:

Fort Dodge Animal Health
Clarksville & QuakerBridge Roads
PO Box 5366
Princeton, New Jersey 08543-5366

4. **Description of the Proposed Action:**

4.1 **Intended Product Use**

A single subcutaneous application at the recommended dose level of 1 mL for each 110 lb (50 kg) body weight (0.2 mg moxidectin/kg body weight) is effective in the therapeutic treatment of cattle infected/infested with the internal and external cattle parasites listed on the product label. CYDECTIN (moxidectin) Injectable Solution for Cattle will be used for beef and non-lactating dairy cattle throughout the United States and is appropriate for all management systems.

4.2 **Need for Product**

Internal and external parasitism cause large production losses to cattle raised in the United States. Serious health consequences and in some cases death, can result if effective antiparasitic therapy is not provided to affected cattle. CYDECTIN (moxidectin) Injectable Solution for Cattle effectively treats and controls a broad spectrum of endo- and ectoparasites.



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4.3 Manufacturing Locations

Two manufacturing facilities of Fort Dodge Animal Health will be employed to produce CYDECTIN (moxidectin) Injectable Solution for Cattle. The manufacturing process begins with the aerobic fermentation of nemadectin (alternatively referred to as LL-F28249- α or F- α), a natural derivative of *Streptomyces cyaneogriseus* ssp. *noncyanogenus*. The nemadectin is then refined and chemically converted to moxidectin technical material. The fermentation of nemadectin and production of the moxidectin technical material take place at the Wyeth-Lederle S.p.A. plant in Catania, Italy. The moxidectin technical material is subsequently shipped to Fort Dodge Animal Health's manufacturing facility in Fort Dodge, Iowa, USA for the formulation, packaging and labeling of the finished product.

5. Identification of Chemical Substances:

5.1 Active Drug - Moxidectin

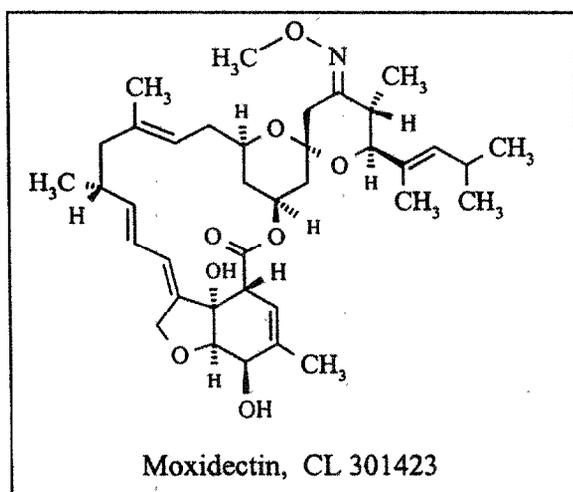
Nomenclature : moxidectin (USAN designation); 23-(O-Methyloxime)-F28249- α or 3-(O-Methyloxime)-F28249-alpha; CL 301,423

CAS Registration No.: 113507-06-5

Molecular Weight: 639.8

Molecular Formula: $C_{37}H_{53}NO_8$

Structural Formula:





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Physical Description:

Appearance - white to pale yellow powder

Purity - >90%

Melting point (liquefaction) - 145 to 154°C

Vapor Pressure - $< 3.2 \times 10^{-8}$ Torr

Volatility (% by volume) - negligible

n-Octanol/water partition coefficient - 58,300

UV visible absorption spectrum - 245 nm peak

Evaporation rate - negligible

Solubility in water - 0.51 mg/L

Glass transition - 110°C

Bulk density - 0.42 g/mL (untapped) and 0.56 g/mL (tapped)

Hygroscopicity - 0.6 to 1.1%

Solvation - Non-hydrating

Solution pH - pH 6.6 in 70% dioxane:30% water

Solubility in organic solvents (mL solvent/g moxidectin)

- dichloromethane - 1.64
- diethyl ether - 1.19
- ethanol (95%) - 0.81
- acetonitrile - 0.62
- ethyl acetate - 0.47
- formic acid - decomposed

5.2 Finished Product - CYDECTIN (moxidectin) Injectable Solution for Cattle

Physical Description - CYDECTIN (moxidectin) Injectable Solution is a colorless to pale yellow solution. It is a sterile solution and packaged in 50-mL, 200-mL or 500-mL bottles.

Composition - The finished CYDECTIN (moxidectin) Injectable Solution contains 1% (w/v) moxidectin as the active ingredient. The inactive ingredients make up the balance of the formulation.

6. Introduction of Substances into the Environment as the Result of Use

6.1 Administration

Moxidectin, the active ingredient of CYDECTIN Injectable Solution for Cattle, is a macrocyclic lactone based product. It is anticipated that the use pattern for this injectable



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product will closely follow that of established products within the general macrocyclic lactone group.

CYDECTIN (moxidectin) Injectable Solution for Cattle should be administered by subcutaneous injection under the loose skin in front of or behind the shoulder. Needles ½ or ¾ inch in length and 16 to 18 gauge are recommended for subcutaneous injection. Use sterile, dry equipment and aseptic procedures when withdrawing and administering CYDECTIN injectable solution. For multiple treatments, either automatic injection equipment or an aspirating needle should be used.

Due to their unique spectrum of activity, macrocyclic lactone products are used either for the control of ectoparasites, as an anthelmintic for the control of endoparasites or for the simultaneous control of both internal and external parasites. Macrocyclic lactone products are used with animals both in the feedlot and on pasture and are a critical part of any comprehensive parasite control program. The number of treatments and the timing of these treatments is based on the class of animals being treated, the epidemiology of the parasites being targeted and management practices in place at the facility. In cow-calf and stocker programs, the majority of treatments are directed towards parasite control in first season grazing animals. Treatment of second season grazing animals is less frequent and adult animals rarely receive treatment. In the feedlot, incoming animals will receive a single treatment for therapeutic control of internal and external parasites. As such, the target population for this CYDECTIN injectable solution is replacement females and steers grazing on pasture and cattle on-feed in feedlots. Treatment of adult females is relatively low.

The cattle population in the United States has remained relatively constant. A 1997-1998 USDA survey indicates that the U.S. cattle population consisted of approximately 34.8 million beef cows, 30.9 million calves and 25.7 million feedlot animals. These numbers are similar to those for the preceding 4 years of the survey. At the same time, the anthelmintic market has also remained relatively constant as indicated by data in the most recent survey by Wood McKenzie (2001) indicating a relatively mature market. The major change that has been observed in the cattle antiparasitic market has been the introduction of generic ivermectin products at a reduced cost. It is anticipated that the introduction of CYDECTIN injectable solution will not increase the use of macrocyclic lactone based products but will shift usage patterns away from existing products to the use of CYDECTIN.

6.2 Metabolism and Excretion of Moxidectin

The major route of excretion for cattle treated with moxidectin is the feces (see study PD-M 28-34). At 28 days post-treatment for cattle dosed subcutaneously, moxidectin in the feces

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accounts for 58% of the administered dose. Three percent of the treated dose was recovered in the urine during this same 28-day period. Six metabolites were extracted from steer fecal samples. Only 2 metabolites, CL189,056 and CL189,021, were identified at levels routinely above 2% of the administered dose. The major components of these metabolites were identified as the hydroxymethyl derivatives at C-29/C-30 (CL189,056) and C-14 (CL189,021). The remaining metabolites were mostly mono- or di-hydroxylated derivatives on the moxidectin parent molecule. Biological activity of the metabolites has been shown to be equal to or less than the parent compound.

The actual level of moxidectin in the feces of animals that had been treated with the 1% subcutaneous product was documented in report GASD 06-26.00. The experimental animals were treated with either moxidectin nonaqueous injectable or control vehicle. Fecal samples were taken on Day 0 (prior to treatment) and on Days 1, 2, 3, 5, 7, 10, 14, 17 and 21 post-treatment. The moxidectin content in all samples from treated and untreated animals was below the LOQ of 100 ppb. Despite the fact that all samples were below the LOQ of the method, a worst-case approach was chosen when calculating environmental impact of the use of moxidectin nonaqueous injectable. All calculations assumed that the entire recommended dose of 0.2 mg moxidectin/kg body weight is excreted as unaltered parent compound.

6.3 Metabolism Study of Moxidectin in Cattle

Study PD-M 28-34: Tissue residue depletion and metabolism of moxidectin were studied in cattle. Steers averaging 224 kg were dosed subcutaneously with the radioactive carbon-14 and deuterium labeled moxidectin at 0.2 mg/kg body weight. Total urine and feces were collected daily after treatment and the animals were sacrificed 7, 14 and 28 days later for the collection of tissues.

The total radioactivity recovered in the samples collected accounted for 72.7%, 70.7% and 76.9% of the administered dose at 7, 14 and 28 days after treatment respectively. These were distributed as follows: 29.8%, 17.6% and 11.6% in the carcass; 32.2%, 41.3% and 58.1% in the feces; 0.8%, 1.8% and 3% in the urine; and 9.9%, 10% and 4.2% in all other components sampled at the three sacrifice points, respectively. These data have demonstrated that the major route of excretion of moxidectin is in the feces.

Concentrations of total moxidectin related residues in the feces peaked at a level of 0.349 ppm on day 2 after treatment and were 0.133, 0.079, 0.038 and 0.041 ppm on days 7, 14, 21 and 28, respectively. The data are summarized in Table 1 below.

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Table 1. Kinetics of Moxidectin in Cattle Feces after Treatment

Time after treatment (Days)	Concentration of total radioactivity (ppm)*	Time after treatment (Days)	Concentration of total radioactivity (ppm)*
2	0.349	16	0.065
3	0.267	17	0.069
4	0.197	18	0.063
5	0.168	19	0.049
6	0.149	20	0.037
7	0.133	21	0.038
8	0.133	22	0.043
9	0.140	23	0.044
10	0.121	24	0.034
11	0.101	25	0.037
12	0.119	26	0.039
13	0.085	27	0.027
14	0.079	28	0.041
15	0.081		

* Calculated from study PD-M 28-34.

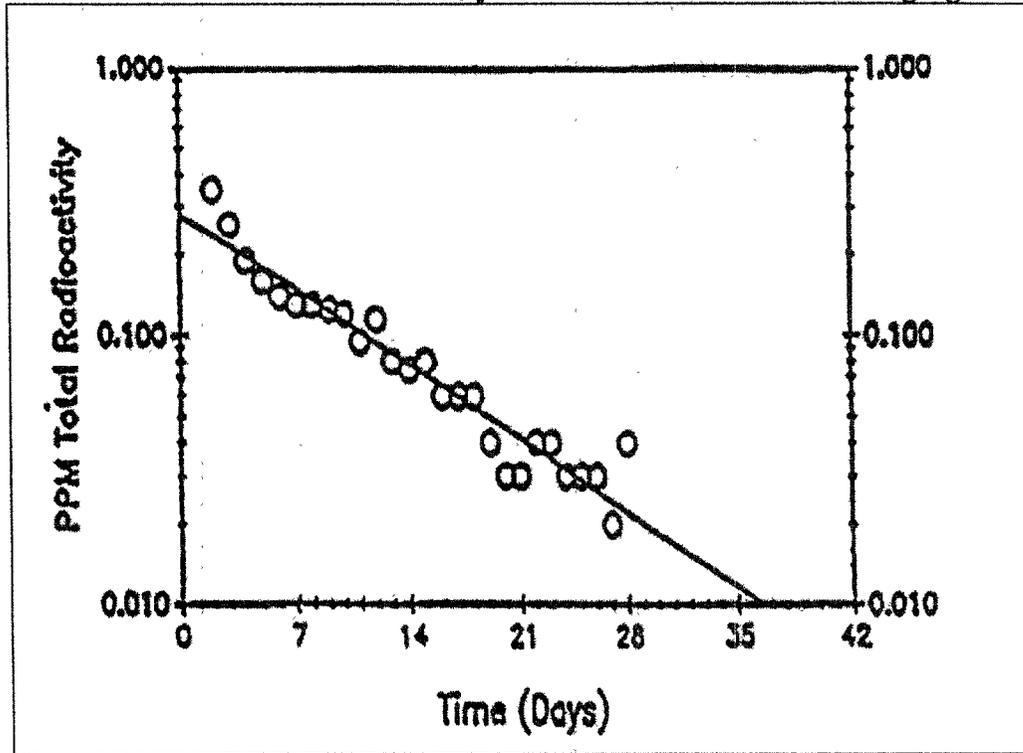
These values fit a first-order exponential decay equation to give an excretion half life of 7.6 days (Figure 1). Therefore, all moxidectin would be excreted from treated animals in the first 37 days after treatment. Manure produced after 37 days, containing no detectable levels of moxidectin (LOQ = 0.01 ppm), would reduce the overall concentrations of moxidectin in the manure produced by cattle still being maintained in the feedlot.



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Figure 1. Concentration of Total Radioactivity in the Manure from Steer Treated Subcutaneously with ¹⁴C-Moxidectin at 0.2 mg/kg



Further analysis of the feces at 2 and 7 days after treatment indicated that 91 and 93% of the radioactivity was extractable from the feces with methanol/water. HPLC profiles of this extract showed that moxidectin accounted for 26% and 22% of the total residue and that there was only one major metabolite (C-29/30 hydroxymethyl metabolite) which accounted for 25-34% of the total residue. The remaining minor (dihydroxylated) metabolites individually accounted for less than 10% of the total. *In vitro* studies using steer liver microsomes produced the same metabolites as found in the fecal extracts and confirmed that the principal fate of moxidectin in cattle is hydroxylation in the liver and excretion in the feces.

In summary, the excretion of moxidectin and its metabolites is primarily through the manure of treated cattle. The total moxidectin and its related residue levels in feces peak at 349 ppb at 2 days after treatment and decrease to less than 10 ppb by 37 days after treatment. Levels of moxidectin itself in feces are 22-26% of total residues and are below LOQ at all time points after treatment. Feces from cattle 37 days after treatment contains no detectable levels of moxidectin and, therefore, dilutes the overall concentrations of moxidectin in manure produced in a given cattle field.



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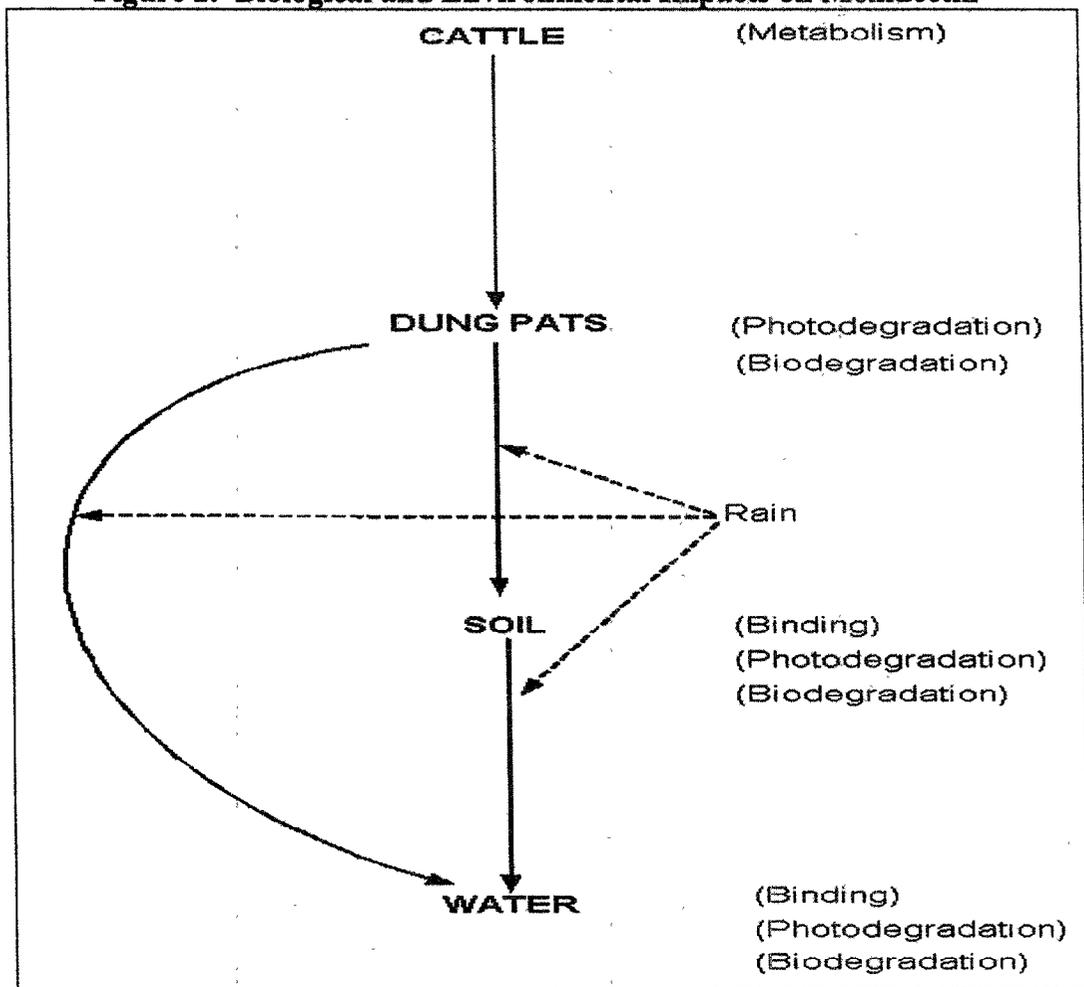
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7. Fate of Emitted Substances in the Environment:

Once excreted from treated animals, moxidectin primarily remains in the dung pats on the pastures or feedlot for a period of time (Figure 2). It may be subsequently deposited into soil and possibly washed off into water. Moxidectin can be inactivated by binding to soil and sediments, photodegradation and biodegradation. The impact of the environmental exposure on the chemistry and fate of moxidectin are discussed in this section.

Figure 2. Biological and Environmental Impacts on Moxidectin



7.1 Environmental Chemistry of Moxidectin

7.1.1 Study PD-M 28-21: The solubility of moxidectin in water and various organic solvents was determined using the shake-flask method. The water solubility was determined to be 0.51 mg/L. The solubility in various organic solvents is summarized in Table 2.

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Table 2. Solubility of Moxidectin

Solvent	(mL of solvent/g moxidectin)
Dichloromethane	1.64
Diethyl Ether	1.19
Ethanol (95%)	0.81
Acetonitrile	0.62
Ethyl Acetate	0.47
Formic Acid	decomposed

- 7.1.2 Study PD-M 28-10: The vapor pressure of moxidectin at 25°C was determined to be less than 3.2×10^{-8} torr, the limit of detection. Therefore, it is considered non-volatile.
- 7.1.3 Study PD-M 27-51: The ultraviolet-visible absorption spectra of moxidectin was determined in a series of water/acetonitrile solutions using several concentrations of moxidectin. An absorption peak was observed at 245 nm with slight absorption above 300 nm. Therefore, moxidectin might be degraded by sunlight because of its absorption of light in wavelengths found on the surface of earth.
- 7.1.4 Study PD-M 28-20: The *n*-octanol/water partition coefficient of moxidectin was determined to be 58,300 using the shake flask method. This finding suggests that moxidectin is lipophilic and also confirms its low aqueous solubility.
- 7.1.5 Study PD-M 28-17: The melting point of moxidectin was determined by the capillary tube method and found to be 145°C to 154°C.
- 7.1.6 Summary of the environmental chemistry of moxidectin: The chemical and physical properties of moxidectin directly influence the fate of moxidectin in the environment. For instance, the high melting point and very low vapor pressure indicate that moxidectin is non-volatile and will not spread away from areas of use through the atmosphere. The large *n*-octanol/water partition coefficient of moxidectin indicates that the compound is lipophilic, thus confirming its poor water solubility.

7.2 Environmental Fate of Moxidectin

After being excreted from cattle, the fate of moxidectin is influenced by various environmental factors including the aerobic soil metabolism, adsorption to soils and sediments, and photodegradation. These factors are discussed in this section.

- 7.2.1 Study PD-M 28-23: A soil degradation study was conducted by adding ¹⁴C-moxidectin to each of three soils and aging them under aerobic conditions for 63 days. During the aging

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period the ¹⁴C-moxidectin was extensively degraded, with 5.24%, 1.59% and 1.16% of the applied dose being mineralized (converted) to ¹⁴CO₂ in soils from Indiana, New Jersey and Wisconsin, respectively. After 63 days of incubation, moxidectin accounted for 47, 44, and 57% of the applied dose in the Indiana, New Jersey and Wisconsin soils. These findings indicate that moxidectin is biodegradable in soils. There were at least 10 degradation products formed, most of which were at trace levels. Half-lives of approximately two months under these conditions indicate that moxidectin is not expected to persist in the environment. The properties of the soils are given in Table 3.

Table 3. Composition of Solids Tested

Type & Texture	Composition					pH	C.E.C
	% Sand	% Silt	% Clay	% O.M.	% O.C.		
Sassafras sandy loam ¹	62.8	25.6	11.6	1.0	0.58	6.9	5.9
Piano loam ²	32.8	47.6	19.6	2.4	1.39	7.1	7.46
Tippecanoe silt loam ³	32.8	49.6	17.6	3.1	1.80	6.9	20.06

Soil origin: ¹New Jersey, ²Wisconsin, ³Indiana

O.M. = organic matter; O.C. = organic carbon; C.E.C. = cation exchange capacity

7.2.2 Study PD-M 28-7: The adsorption of moxidectin onto four different soils was investigated using the batch equilibrium technique. Initial concentrations of ¹⁴C-moxidectin of 0.044, 0.084, 0.455 and 0.983 ppm in 0.01 M calcium chloride were used. The soil and the moxidectin solutions were mixed, shaken continuously for two days at room temperature, centrifuged and the concentration of moxidectin in the adsorption solution was measured. Fresh 0.01 M calcium chloride was added and the desorption of moxidectin from soil was studied using the same procedures used in the adsorption phase. After the desorption phase, the amount of ¹⁴C-moxidectin remaining in the soil was determined. The adsorption coefficients, normalized for the % organic carbon in the soil (Koc), are shown in Table 4. The Koc values of these soil samples ranged from 18,000 to 41,000, indicating a strong binding of moxidectin to soils.



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Table 4. Adsorption of Moxidectin to Soils

Type & Texture	Composition					pH	C.E.C	Koc
	% Sand	% Silt	% Clay	% O.M.	% O.C.			
Buelah loamy sand ¹	80.0	15.6	3.6	0.5	0.29	6.5	3.4	41379
Sassafras sandy loam ²	62.8	25.6	11.6	1.0	0.58	6.9	5.9	28448
Piano loam ³	32.8	47.6	19.6	2.4	1.39	7.1	7.46	20215
Tippecanoe silt loam ⁴	32.8	49.6	17.6	3.1	1.80	6.9	20.06	18666

Soil origin: ¹Arkansas, ²New Jersey, ³Wisconsin, ⁴Indiana

O.M. = organic matter; O.C. = organic carbon; C.E.C. = cation exchange capacity

7.2.3 Study PD-M 27-24: The mobility of moxidectin (¹⁴C-labeled) was assessed in four different soils using soil thin layer chromatography. Soil coated (1 mm) plates were used with water as the mobile phase. The very small Retardation Factor (RF) values indicated that moxidectin-soil complex could not be separated by the thin layer chromatography (Table 5). All four soil types were given a 1 classification with moxidectin under the Helling method, indicating that the binding of moxidectin to soils is so tight that the complex is characterized as an immobile compound.

Table 5. Mobility of Moxidectin in Soils

Type & Texture	Composition					pH	C.E.C	RF
	% Sand	% Silt	% Clay	% O.M.	% O.C.			
Buelah loamy sand ¹	80.8	15.6	3.6	0.5	0.29	6.5	3.4	0.07
Sassafras sandy loam ²	62.8	25.6	11.6	1.0	0.58	6.9	5.9	0.08
Piano loam ³	32.8	47.6	19.6	2.4	1.39	7.1	7.46	0.07
Tippecanoe silt loam ⁴	32.8	49.6	17.6	3.1	1.80	6.9	20.06	0.07

Soil origin: ¹Arkansas, ²New Jersey, ³Wisconsin, ⁴Indiana

O.M. = organic matter; O.C. = organic carbon; C.E.C. = cation exchange capacity

Retardation Factor (RF) = distance traveled by compound/distance traveled by water front

7.2.4 Study PD-M 28-22: The photodegradation of moxidectin in aqueous solutions was studied using both sunlight and a high-pressure xenon-arc lamp which was filtered to remove light <290 nm to simulate sunlight. The sunlight study was conducted in NJ in late autumn (November). Due to the low solubility of moxidectin in water (i.e., < 1 ppm), acetonitrile (1%) was used as a cosolvent to help keep moxidectin in solution. Foil wrapped samples were used as dark controls. The initial concentration of moxidectin was

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measured and additional determinations made every two hours until termination of the study after 14 hours exposure. There was a reduction in the measured moxidectin concentration from 97% to 22% of the applied dose after 12 hour exposure to natural sunlight, and a reduction from 94% to 19% of the applied dose after 14 hour exposure to the xenon-arc lamp. The calculated half-lives were 6.8 hours and 5.6 hours, respectively. The half-life from the spring to early fall would be even more rapid due to the longer and more intense exposure to sunlight. This rapid photodegradation in water will rapidly degrade moxidectin entering the aquatic environment. Several photodegradation products were observed, but were not identified since each accounted for less than 10% of the applied dose.

- 7.2.5 Summary of environmental fate of moxidectin: Because of the very low water solubility, high n-octanol/water partition coefficient, high melting point, high degree of adsorption to soil, and biodegradation by microorganisms in soils, moxidectin is not expected to move from fields into surface water. Even being washed off from soil or feces, moxidectin will subsequently undergo photodegradation and bind to other suspended soil particles, plants and any materials in water. This secondary binding process will result in a continuous depletion of free moxidectin from the environment. In addition, because of the very strong binding to soil particles, the water bed would prevent the moxidectin from entering and contaminating groundwater.



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7.3 Calculation of the Predicated Moxidectin Concentration in Environment

This section is conducted by following the “Guidance for Industry for Environmental Risk Assessment Covering New Animal Drug Applications for Veterinary Use”, draft #10, dated August 21, 1996.

For evaluating the environmental impact of moxidectin, the “worst-case” approach was used to calculate the Predicted Environmental Concentration (PEC) in manure, soil and water. It was assumed that 100% recommended dose of 0.2 mg moxidectin/kg body weight is excreted from treated cattle as an unaltered parent compound. These PEC’s are the worst-case values because they are calculated based on the assumptions that moxidectin is not metabolized in cattle, not bound to soil particles and any other sediments, and not degraded by sunlight and microorganisms. Although the product will be used in cattle maintained on pasture or feedlots, calculations are based on a feedlot management system because this results in the highest concentration of fecal moxidectin.

Calculation of PEC in manure in feedlot systems:

$$PEC_{\text{manure}}(\text{max}) = \frac{\text{Total Dose Administered (mg/animal/day)} \times \# \text{ Days Treated}}{\text{Total Amount of Manure Produced during Manure Production Period}}$$

$$PEC_{\text{manure}}(\text{max}) = \frac{\text{Total Dose Administered (mg/animal/day)} \times \# \text{ Days Treated}}{\text{Kg Excreta/Day} \times \text{Manure Production Period (in Days)}}$$

$$PEC_{\text{manure}}(\text{max}) = \frac{0.2 \text{ mg/kg} \times 300 \text{ kg animal} \times 1 \text{ day of treatment}}{27.3 \text{ kg (wet)} \times 130 \text{ Days}}$$

$$PEC_{\text{manure}}(\text{max}) = \frac{60 \text{ mg}}{3549 \text{ kg (wet)}} = 0.0169 \text{ mg/kg} = 0.0169 \text{ ppm} = 16.9 \text{ ppb}$$

The manure is then spread to pastures or field as fertilizer and the PEC of moxidectin residue in soil is estimated as follows. The maximum PEC_{soil} is calculated assuming that an approximately 13,600 kg manure is applied to each acre of field with plowed soil weights of approximately 910,500 kg. The water content of manure is assumed to be 48%.

$$PEC_{\text{soil}}(\text{max}) = \frac{\text{Concentration in Manure (ppm)} \times \text{kg Manure Applied/Acre}}{\text{Weight of Soil in Plow Layer} \times \text{Water Content of Manure}}$$

$$PEC_{\text{soil}}(\text{max}) = \frac{0.0169 \text{ ppm} \times 13,600 \text{ kg Manure Applied/Acre}}{910,500 \text{ Kg Soil in Plow Layer} \times 0.48} = 0.526 \text{ ppb}$$



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This calculation is worst-case because it assumes that all moxidectin applied to cattle is excreted into manure as an unaltered moxidectin molecule during the 130-day period. The actual concentrations of moxidectin in soil would be reduced from this maximum PEC due to the metabolism in cattle prior to excretion and the degradation of manure during the storage prior to application to the fields.

Since it is possible that moxidectin can be washed off from fields into water, the PEC of moxidectin in water is estimated as follows. The maximum PEC_{water} is calculated assuming that 1% of the total drug per acre applied to 10 acres of soil moves into a 1 acre pond which is 2 m (6 feet) deep.

The mass of compound which enters the pond is calculated from:

$$\text{Mass of compound} = \text{PEC}_{\text{soil}(\text{max})} \times 9.1 \times 10^5 \text{ kg/acre} \times 0.01 \times 10 \text{ acres} = \text{PEC}_{\text{soil}(\text{max})} \times 9.1 \times 10^4 \text{ kg}$$

A one-acre pond which has a depth of 2 m has a volume of 8,094,000 liters.

$$1 \text{ acre} \times 4047 \text{ m}^2/\text{acre} \times 2 \text{ m} = 8094 \text{ m}^3 \times 1000 \text{ liters/m}^3 = 8,094,000 \text{ liters} = 8.1 \times 10^6 \text{ liters}$$

$$\text{PEC}_{\text{water}(\text{max})} = \frac{\text{Mass of product moved into water}}{\text{Mass of water in pond}} = \frac{\text{PEC}_{\text{soil}(\text{max})} \times 9.1 \times 10^4 \text{ kg}}{8.1 \times 10^6 \text{ liters} \times 1 \text{ kg/liter}}$$

$$\text{PEC}_{\text{water}(\text{max})} = \frac{0.526 \text{ } \mu\text{g/kg} \times 9.1 \times 10^4 \text{ kg}}{8.1 \times 10^6 \text{ kg}} = 0.0059 \text{ } \mu\text{g/kg} = 5.9 \text{ ppt}$$

The concentration of moxidectin in water would be reduced from this maximum PEC value due to the metabolism in cattle prior to excretion, degradation in manure during the storage prior to application to fields, very strong adsorption to soil and sediments.

The concentration in water, corrected for adsorption, is calculated from the relationship:

$$\text{Soil/Water Partition Coefficient} = K_d = \frac{\text{Concentration in Sediment}}{\text{Concentration in Water}}$$

$$K_d = \frac{\text{Mass of Compound in Sediment/Mass of Sediment}}{\text{Mass of Compound in Water/Mass of Water}}$$

$$K_d = \frac{(\text{MA}-\text{MCW})/\text{Mass of Sediment}}{\text{MCW}/\text{Mass of Water}}$$

Where MA = Mass of Compound Added to Pond; and MCW = Mass of Compound in the Water

Rearranging the equation and solving for the mass of the compound in water:

$$\text{MCW} = \frac{\text{MA} \times \text{Mass of Water}}{\text{Mass of Water} + (\text{Mass of Sediment} \times K_d)}$$



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$$PEC_{water} = \frac{MCW}{\text{Mass of Water}} = \frac{MA}{\text{Mass of Water} + (\text{Mass of Sediment} \times Kd)}$$

Assuming that the compound is adsorbed in the top 5 cm of the sediment:

The volume of sediment is: $0.05 \text{ m} \times 1 \text{ acre} \times 4047 \text{ m}^2/\text{acre} = 202 \text{ m}^3$

The mass of sediment is: $202 \text{ m}^3 \times 1500 \text{ kg/m}^3 = 3 \times 10^5 \text{ kg}$

The mass of water is: $8.1 \times 10^6 \text{ liters} \times 1 \text{ kg/liter} = 8.1 \times 10^6 \text{ kg}$

As was previously shown in the calculation of the $PEC_{water}(\text{max})$:

$$\text{Mass of compound} = PEC_{soil}(\text{max}) \times 9.1 \times 10^5 \text{ kg/acre} \times 0.01 \times 10 \text{ acres} = PEC_{soil}(\text{max}) \times 9.1 \times 10^4 \text{ kg}$$

$$PEC_{water} = \frac{MA}{\text{Mass of Water} + (\text{Mass of Sediment} \times Kd)}$$

$$PEC_{water} = \frac{PEC_{soil}(\text{max}) \times 9.1 \times 10^4 \text{ kg}}{\text{Mass of Water} + (\text{Mass of Sediment} \times Kd)}$$

$$PEC_{water} = \frac{0.526 \text{ } \mu\text{g/kg} \times 9.1 \times 10^4 \text{ kg}}{8.1 \times 10^6 \text{ kg} + (3 \times 10^5 \text{ kg} \times Kd)}$$

$$PEC_{water} = \frac{47.9 \text{ mg}}{8.1 \times 10^6 \text{ kg} + (3 \times 10^5 \text{ kg} \times Kd)}$$

Assuming the sediment contains 5% organic matter, which is equivalent to 2.9% organic carbon, based on the % organic carbon = % organic matter/1.724, then:

$Kd = 0.029 \times Koc$. The Koc values were 18,000 - 41,000. Using a conservative value of 20,000 for the Koc, the $Kd = 580$.

$$PEC_{water} = \frac{47.9 \text{ mg}}{8.1 \times 10^6 \text{ kg} + (3 \times 10^5 \text{ kg} \times 580)} = \frac{47.9 \text{ mg}}{(8.1 + 174) \times 10^6 \text{ kg}} = \frac{47.9 \text{ mg}}{1.82 \times 10^8 \text{ kg}}$$

$$= 26.3 \times 10^{-8} \text{ mg/kg} = 2.63 \times 10^{-7} \text{ mg/kg} = 2.63 \times 10^{-7} \text{ ppm} = 0.263 \text{ ppt}$$

This value would be reduced due to the degradation processes of moxidectin in both the soil and the pond.

In summary, the maximum PEC's of moxidectin in manure and soil are estimated as 16.9 ppb and 0.526 ppb, respectively. The maximum PEC of moxidectin in water is 5.9 ppt (without adsorption adjustment) and 0.263 ppt (with adsorption adjustment), respectively. These PEC's are the worst-case values because they are calculated based on 100% of the moxidectin dose being excreted from treated cattle without the adjustments of the metabolism in animals, degradation by sunlight and microorganisms, and adsorption to soil particles and other sediments.



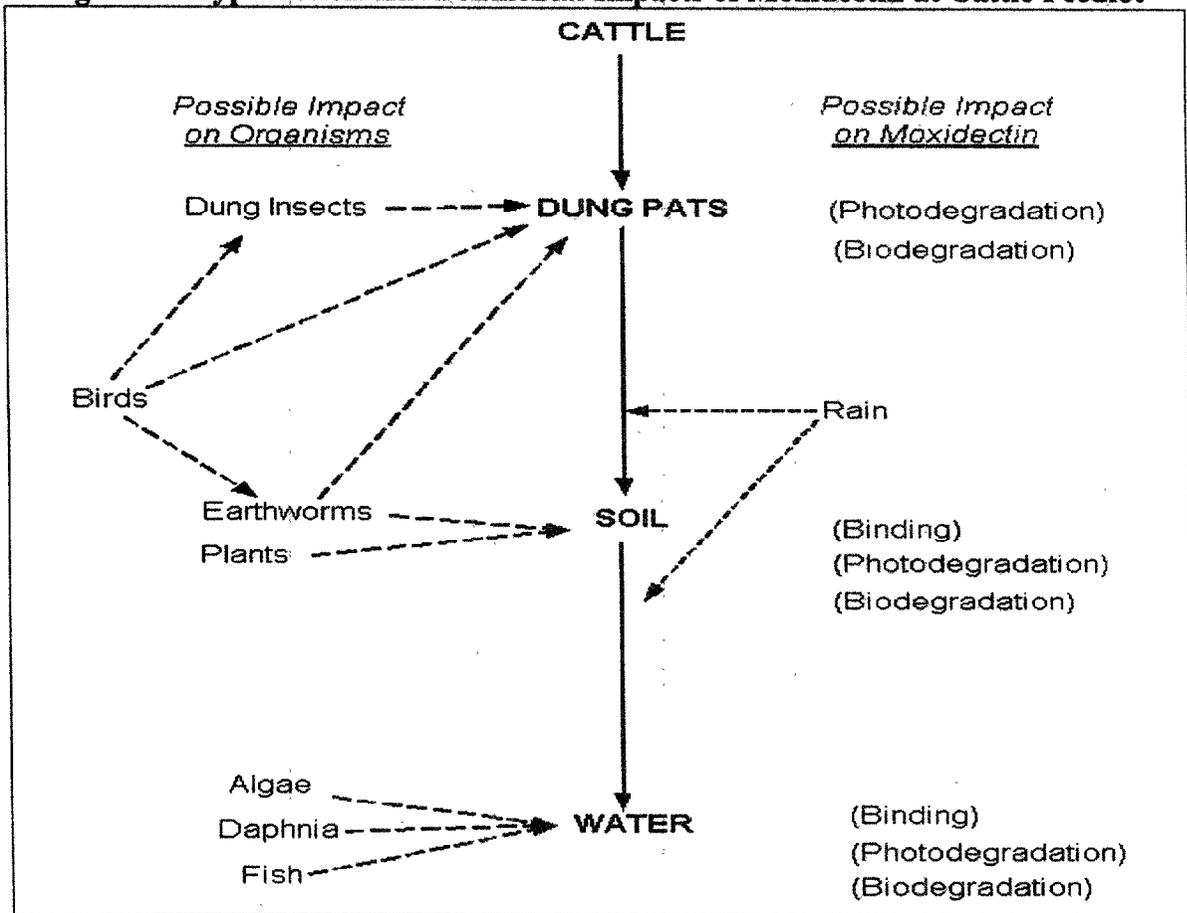
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8. Environmental Effects of Released Substances:

CYDECTIN (moxidectin) Injectable Solution for Cattle will be used for cattle on pastures/feedlots and is excreted from treated animals through manure. Therefore, the potential environmental effects of the excreted moxidectin would take place in cattle manure (dung pats), soil and water. As illustrated in Figure 3 below, the potential targets of moxidectin after being excreted from cattle include insects associated with cattle dung, avian, terrestrial and aquatic organisms. The possible impacts of moxidectin on these living organisms are discussed in this section.

Figure 3. Hypothetical Environmental Impacts of Moxidectin at Cattle Feedlot



8.1 Toxicity of Moxidectin to Dung Insects

8.1.1 Dung Ecosystem

The dung ecosystem is comprised of a diverse population of invertebrates and micro

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organisms. It consists of a patchy and ephemeral habitat, characterized by severe competition, and complex behavior in many similar species living together. Dung beetles comprise one of the animal populations of importance in the dispersal and breakdown of dung. Dung beetles are often exceedingly abundant. Thousands of individuals and dozens of species may be attracted to single droppings in both temperate and tropical localities. A number of species of dung beetles (especially Scarabaeidae) have been introduced throughout the world, including the US, to aid in the environmental recycling of the dung of domestic animals.

There are many ways of classifying dung beetles. A functional distinction is made between the dung dwellers (Aphodiinae) and those beetles which exhibit nesting behavior by tunneling or rolling dung to find a safe place for rearing their young (Scarabaeinae and Geotrupidae). The dung dwellers are generally considered more primitive in evolutionary terms, and largely leave their young to fend for themselves, while the nesters may invest considerable parental time in preparing and defending their brood masses. Generally the fecundity of nesters is less than that of the dwellers.

The majority of dung beetles belong to the coleopteran family Scarabaeidae. Hanski (1991) have identified some 1850 species of Aphodiidae, 5000 species of Scarabaeidae, and 150 species of Geotrupidae.

The vast majority of the coprophagous species in the sub-family Aphodiinae belong to the single genus *Aphodius*. They are the characteristic dung beetles of north temperate regions though they are present in tropical and subtropical regions as well. About 50 species of *Aphodius* have been listed as living in North Eastern America, and 25 of these have been retrieved from cattle dung (Hanski 1991; Gordon, 1983,).

Nesting is universal in Geotrupidae and reaches the highest levels of sophistication in Scarabaeinae.

In addition to dung beetles, the coprophagous flies play an important part in the breakdown of dung and must be considered in any assessment of effects on the dung ecosystem.

The environmental assessment of any effects of moxidectin residues in dung has included both dung dwellers and nesting species as well as a range of fly genera. Because of the complexity of the dung ecosystem, and in keeping with published recommendations for assessing disturbance to dung ecosystems (Moore and DeRuiter, 1993; Moore et al, 1993; Strong, 1993; Herd, 1995) the environmental assessment of effects of moxidectin is based on assessing effects on indicator species which are significant in the dung dispersal process.



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8.1.2 In Vitro Bioassay

8.1.2.1 Study 0693-B-US-29-98 (Report GASD 06-24.00): In an *in vitro* study on two indicator species of beetles, *Onthophagus gazella* and *Euoniticellus intermedius*, the toxic level (EC₅₀) of moxidectin in cattle feces to adult and developing stages was determined under controlled laboratory conditions. Pooled feces from two normal cattle were spiked with moxidectin at approximately 0, 150, 200, 300, 400 and 500 ppb. Fecal samples were placed in plastic containers containing soil and exposed to pairs of adult beetles on Day 0. The feces were removed from the containers on day 7, the adult beetles were collected from days 7 to 10, and their progeny beetles were collected from days 23 to 35. The numbers of living and dead parent and progeny beetles and their sex were recorded at each counting day. All intact brood balls remaining in the containers were counted and recorded on day 35.

The results from this *in vitro* bioassay demonstrated that moxidectin concentrations of up to approximately 500 ppb in cattle feces did not adversely affect the numbers of brood balls produced, the numbers of live, dead or total progeny, the percent emergence of the progeny, or the sex ratio of progeny beetles produced by adult *O. gazella*. Similarly, moxidectin at the same concentrations did not affect survival of adult *E. intermedius* beetles. Concentrations of 390 ppb moxidectin in feces significantly reduced reproduction and the emergence of *E. intermedius* progeny. Probit analysis of the most sensitive measure of these effects demonstrated that a concentration of 469.3 ppb of moxidectin in cattle feces produced a 50% reduction in the number of live *E. intermedius* progeny. The key findings in this study are summarized in Table 6:

Table 6. Toxicity Assessment of Moxidectin on Two Dung Beetle Species

Species	NOEC	EC ₅₀
<i>Onthophagus gazella</i> adult	> 500 ppb	Not determined
<i>Onthophagus gazella</i> progeny	> 500 ppb	2567.7 ppb
<i>Euoniticellus intermedius</i> adult	> 500 ppb	Not determined
<i>Euoniticellus intermedius</i> progeny	> 269 ppb	469.3 ppb

8.1.2.2 In collaboration with Doherty et al (1994), additional data were generated to assess the comparative larvicidal effect of moxidectin and abamectin against the scarabaeine beetle *Onthophagus gazella* and the Buffalo fly *Haematobia irritans exigua* De Meijere. In this study, the 1% injectable formulations of moxidectin and abamectin were incorporated into dung, because preliminary trials were unable to find a solvent for moxidectin technical material which was not toxic to the larvae of *H. i. exigua*. Dung for both trials was obtained from a steer held in a slatted pen in an insect proof enclosure. Pats containing 4, 8, 16, 32, 64, 128, 256 and 512 ppb were prepared by serial dilution of each formulation in dung. However, the four highest concentrations of abamectin were excluded since

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preliminary trials had shown that no larvae survived at concentrations of 16 ppb or greater. Each treatment was replicated five times, except for the control group, where there were 10 replicates. Five equal measures of dung, each approximately 150 g, were removed to provide the experimental pats. Each pat was placed on the surface of 3 kg of moist soil in a ventilated 4 L container.

Adult *Onthophagus gazella* were collected from the field and a laboratory colony was established from eggs which had been collected from the brood balls produced by these adults. The colony was maintained by the methods of Macqueen and Feehan (personal communication with Doherty). Dung and soil used for the trial and the colony were treated to eliminate unidentified rhabditiform nematodes, infestations of which appeared to reduce survival of adults and larvae and oviposition in previous colonization attempts. One pair of unmated beetles 5-7 days old was added to each container. Eight days later another pair of unmated beetles aged 9-12 days was added to each container. The containers were stored at 21-30°C for the duration of the trial. Fresh pats were added to each container 3, 5, 8, 11 and 13 days after commencement to allow beetles the maximum opportunity for dung burial and oviposition. The numbers of brood balls (each of which contained a single egg), adults, pupae and larvae in each container were counted 53 days after commencement. Data were analyzed by one-way analysis of variance. Treatments comprised entirely of zero values were excluded from the analysis. Least significant differences (5% level) were calculated where a treatment effect was indicated.

Neither moxidectin nor abamectin reduced oviposition by *O. gazella*. There was however a consistent trend towards increased oviposition with increased concentrations of moxidectin, although this was not significant ($P = 0.10$). All concentrations of abamectin, and 512 ppb of moxidectin reduced larval survival of *O. gazella*. Abamectin at concentrations of 16 and 32 ppb produced complete mortality.

In the assay with *H. irritans exigua*, the flies were obtained from a laboratory colony and maintained according to the methods of Thomas and Davis (1984) with minor modifications. Flies were allowed to oviposit on fresh dung, which was collected daily and left undisturbed for 24 hr. The dung was then moistened if necessary to a moisture content of approximately 80%, formed into pats and placed on a 2 cm layer of sand. The dung was held a further 6 days at 26-30°C, after which pupae were retrieved from the sand by flotation. Eggs were obtained by the method of Thomas and Davis (1984), washed into petri dishes and transferred onto damp filter paper. Each pat was placed on dry sand in a ventilated container. A batch of 100 eggs 2-6 hours old was placed on each pat and the containers held at 20-30°C for 7 days. Pupae were then harvested from the sand and dung by flotation and held for 7 days at 27°C after which adult eclosion was assessed. Larval survival of *H. i. exigua* was reduced by all concentrations of abamectin, and by concentrations of moxidectin of 128 ppb or greater. Moxidectin at 256 and 512 ppb



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produced survival at levels comparable to those at 4 and 8 ppb abamectin, respectively. Moxidectin did not affect the eclosion of adult *H. i. exigua*. This could not be measured for abamectin as there were no survivors at levels above 4 ppb. Concentrations of moxidectin 64-fold greater than abamectin concentrations were required to produce similar toxic effects. The key findings of this study are summarized in Table 7.

Table 7 Survival of larval and pupal *H. irritans exigua* and oviposition and larval survival of *Onthophagus gazella* in dung containing various concentrations of moxidectin and abamectin.

Treatment	<i>H. i. exigua</i> Mean % Pupation (SE)	<i>H. i. exigua</i> Mean % Eclosion (SE)	<i>O. gazella</i> Mean No Brood Balls (SE)	<i>O. gazella</i> Mean % Survival to Adult (SE)
Moxidectin ppb				
4	38 (9.7)	99 (0.8)	48 (2.8)	89 (4.1)
8	55 (4.3)	98 (0.8)	49 (10.4)	81 (5.1)
16	50 (6.5)	99 (0.7)	44 (2.7)	89 (4.4)
32	50 (9.0)	98 (1.0)	56 (5.1)	87 (3.6)
64	47 (5.3)	97 (0.9)	56 (6.4)	87 (4.0)
128	24 (3.2)	92 (3.4)	61 (6.1)	81 (3.2)
256	13 (1.8)	96 (2.5)	64 (1.8)	61 (5.3)
512	0 (0)	na	70 (11.1)	7 (2.5)
Abamectin ppb				
4	1 (0.8)	83 (16.7)	42 (5.9)	57 (6.8)
8	0*	na	53 (3.2)	5 (1.9)
16	0*	na	44 (6.8)	0*
32	0*	na	49 (6.0)	0*
64	0*	na	nt	nt
128	0*	na	nt	nt
256	0*	na	nt	nt
512	0*	na	nt	nt
Control				
	62 (8.2)	96 (1.4)	46 (6.2)	72 (4.2)

* excluded from analysis

na = not applicable

nt = not tested

8.1.3 Bioassays Using Dung from Treated Animals

Bioassays have been conducted to evaluate the effects of dung excreted from animals treated with moxidectin on various dung insect families. Animals in these studies were treated with commercial formulations at the recommended label dose rates.



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8.1.3.1 Family Aphodiidae

8.1.3.1.1 Study 0963-B-US-28-98 (Report GASD 04-28.00): This study investigated the effects of moxidectin and ivermectin residues in dung of treated cattle on non-target organisms of pasture at Montpellier, France, using 26 Aubruc breed heifers. These animals were divided into three groups and grazed on three separate pastures throughout the trial. Animals in these three groups were either controls, injected with moxidectin (0.2 mg/kg body weight), or injected with ivermectin (0.2 mg/kg body weight). Fresh feces were collected on days 0, 1, 2, 3, 5, 7, 10, 16, 20, 24, 34, and 38 after treatment. Bioassays were conducted on the dung beetle *Aphodius haemorrhoidalis* and on the fly *Neomyia cornicina*. For the dung beetle assay, young larvae were collected from the field and placed in small cavities made in the dung designated for use in the bioassay. For one month after seeding the dung samples, emerging adults were collected, identified and counted. As summarized in Table 8 below, the larvae of the dung beetle species *Aphodius haemorrhoidalis*, although they spent their entire life in contact with dung from treated cattle, were not significantly affected when reared in dung from cattle treated with either moxidectin or ivermectin, even in the first few days after treatment, when dung concentrations were the highest.

Table 8. Toxicity of Moxidectin and Ivermectin on *Aphodius haemorrhoidalis*

Days	Moxidectin			Ivermectin		
	Total Larvae	Adults emerged	Emergence (%)	Total Larvae	Adults emerged	Emergence (%)
2	54	48	88.9	54	47	87.0
3	54	41	75.9	54	36	66.7
5	54	43	79.6	54	47	87.0
7	54	41	75.9	54	42	77.8
10	54	40	74.1	54	44	81.5
16	54	38	70.4	54	50	92.6
20	54	43	79.6	54	44	81.5
24	56	42	75.0	54	51	94.4
28	56	52	92.9	56	52	92.9
34	56	51	91.1	56	49	87.5
38	56	46	82.1	56	49	87.5
Control	83	71	85.5			

For the dung fly assay, dung samples of 50 or 100 grams were used by placing 10, 20 or 50 fly eggs on the top of each sample in replicates of 3, 6 or 15, depending on the numbers of eggs deposited. Eclosed adult flies were counted for 15 days after seeding of the samples. As summarized in Table 9 below, larvae of *Neomyia cornicina* suffered high rates of mortality, and no adults emerged for at least 3 weeks following ivermectin treatment. In contrast, adult emergence was reduced by moxidectin only for the first three days after

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treatment and the rate of emergence of adult fly was indistinguishable from controls by 2 weeks post treatment.

Table 9. Toxicity of Moxidectin and Ivermectin on *Neomyia cornicina*

Days	Moxidectin			Ivermectin		
	Total Larvae	Adults emerged	Emergence (%)	Total Larvae	Adults emerged	Emergence (%)
1	150	29	19.3	150	0	0
2	150	38	25.3	150	0	0
3	150	62	41.3	150	0	0
5	150	106	70.7	150	0	0
7	150	78	52.0	150	0	0
10	150	106	70.7	150	0	0
16	150	121	80.7	150	0	0
20	150	132	88.0	150	0	0
24	150	120	80.0	150	82	54.7
28	150	129	86.0	150	87	58.0
34	150	134	89.3	150	138	92.0
38	150	121	80.7	150	109	72.7
Control	200	175	87.5			

8.1.3.1.2 Study 0866-O-FR-10-94 (Report GASD 03-32.00): This study further investigated the differential effects of moxidectin and ivermectin on the Coleopteran species *Aphodius constans* and the Dipteran species *Neomyia cornicina*. Animals were treated with either moxidectin oral drench at 0.2 mg/kg body weight, ivermectin oral drench at 0.2 mg/kg body weight, or not treated as controls. This bioassay was conducted at Montpellier, France, and utilized dung from sheep treated with the commercial oral formulations of each product. Dung was collected on days 0, 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 15, 16, 20, 24, 28, 34, and 38 days after treatment. Numerous replicates of 10 and 20 *Aphodius constans* larvae were placed into the dung samples. The emergence of adult beetles was counted and the rate of emergence was calculated to evaluate the effects of treatment residues for 38 days after treatment.

As summarized in Table 10 below, the rates of dung beetle emergence from the droppings of control group ranged from 56.7% to 76.1%. A significant difference in *Aphodius constans* emergence rates was observed between control and moxidectin groups for the first two days after treatment. However, from days 3 post-treatment to the end of the experimental period, the development and survival rates were not significantly different from controls. In the ivermectin groups, *Aphodius constans* emergence was almost zero for 5 days post-treatment. The difference remained significantly different from controls for six and seven days respectively for the 10 and 20 larvae series. After this period, there was no significant difference from controls. A significant lower beetle emergence rate was



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observed with ivermectin group than moxidectin group during the first 6 days post-treatment.

Table 10. Dung Beetle Bioassay: Emergence Rate of Adult *Aphodius constans*

Day	Control series 10+20 larvae			Moxidectin series 10+20 larvae			Ivermectin series 10 larvae			Ivermectin series 20 larvae		
	T	EA	% E	T	EA	% E	T	EA	% E	T	EA	% E
0	60	34	56.7									
1	-	-	-	60	12	20.0	60	0	0.0	-	-	-
2	120	77	64.2	120	42	35.0	-	-	-	120	0	0.0
3	-	-	-	80	48	60.0	60	0	0.0	20	1	5.0
4	80	53	66.3	80	53	66.3	60	0	0.0	20	0	0.0
5	120	89	74.2	180	107	59.4	60	1	1.7	120	0	0.0
6	180	127	70.6	180	120	66.7	60	20	33.3	120	36	30.0
7	-	-	-	180	114	63.3	60	30	50.0	120	60	50.0
9	-	-	-	180	126	70.0	60	37	61.7	120	70	58.3
10	-	-	-	180	134	74.4	-	-	-	120	72	60.0
11	180	133	73.9	160	114	71.3	60	48	80.0	120	75	62.5
13	180	117	65.0	180	113	62.8	-	-	-	120	81	67.5
15	180	117	65.0	180	126	70.0	60	26	43.3	120	83	69.2
16	-	-	-	180	117	65.0	60	28	46.7	120	80	66.7
20	180	137	76.1	180	110	61.1	60	41	68.3	120	77	64.2
24	180	108	60.0	180	113	62.8	60	36	60.0	120	76	63.3
28	180	123	68.3	180	122	67.8	60	53	88.3	120	70	58.3
34	120	78	65.0	180	108	60.0	60	32	53.3	120	75	62.5
38	180	128	71.1	180	129	71.7	60	36	60.0	120	90	75.0
Total	1940			2840			900			1840		
Total emerged		1321			1808			388			946	
Average % emergence			68.1			63.7			43.1			51.4

Note: T = Total; EA = Emerged Adults; %E = % Emergence

In the fly assay, replicates of 10 and 20 fly eggs were placed on the dung samples. Adult flies emerging from the dung were counted, and a rate of adult emergence was calculated. The rates of fly emergence from the control group ranged from 30.5% to 73% (Table 11). In the ivermectin group, almost no flies emerged for the first five days after treatment, and a significant difference from controls was observed on day 6. After this time, fly emergence ranged from 31% to 68.5%, and these values were not statistically significant from controls. In the moxidectin assay, however, a significant difference in emergence of *Neomyia cornicina* was observed only for the first two days after treatment. However, from day 3 until the end of the experimental period, the moxidectin dung was safe for *Neomyia cornicina* development, with survival rates (34% to 70%) not significantly different from controls (30.5% to 73%).



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Table 11. Fly Bioassay: Emergence Rate of Adult *Neomyia cornicina*

Day	Control series 10+20 larvae*			Moxidectin series 10+20 larvae			Ivermectin series 10+20 larvae		
	T	EA	% E	T	EA	% E	T	EA	% E
1**	200	1	0.5	200	0	0.0	200	0	0.0
2	200	88	44.0	200	11	5.5	200	0	0.0
3	-	-	-	180	106	58.9	200	1	0.5
4	225	144	64.0	200	140	70.0	200	0	0.0
5	200	109	54.5	200	107	53.5	200	0	0.0
6	200	130	65.0	200	122	61.0	200	23	11.5
7	200	132	66.0	200	128	64.0	200	100	50.0
9***	100	65	65.0	200	119	59.5	200	137	68.5
10	200	130	65.0	200	120	60.0	100	63	63.0
11	200	146	73.0	200	133	65.5	200	136	68.0
13	70	45	64.3	200	122	61.0	180	122	62.2
15	200	79	39.5	200	92	46.0	200	84	42.0
16	200	61	30.5	200	68	34.0	200	62	31.0
20	200	90	45.0	200	89	44.5	200	91	45.0
24	200	86	43.0	200	74	37.0	200	86	43.0
28	200	80	40.0	200	87	43.5	200	96	48.0
34	225	121	53.8	200	93	46.5	200	94	47.0
38	200	133	66.5	200	124	62.0	200	116	58.0
Total		3220			3580			3470	
Total emerged		1640			1735			1201	
Average % emergence		50.9			48.5			34.6	

Note: T = Total; EA = Emerged Adults; %E = % Emergence

* 25 eggs in days 1 and 34.

** in day 1, fly emergency in all control boxes (10 and 20 eggs) failed (too dry).

*** in day 9, fly emergence in control boxes with 10 eggs failed (too dry). The value retained for day 9 was the fly emergence from the day 9 control boxes with 20 eggs.

8.1.3.2 Family Scarabaeidae

8.1.3.2.1 Study 0693-B-US-28-98 (Report GASD 06-26.00): In this study, eight steers were randomly allocated to two groups of four animals each, and received a single treatment on Day 0 of either moxidectin 1% injectable at 0.2 mg/kg body weight or vehicle placebo. Fecal samples were collected from cattle on Day 0 (prior to treatment) and Days 1, 2, 3, 5, 7, 10, 14, 17 and 21 post treatment. A subsample from each animal at each collection was analyzed for moxidectin content. Three or four fecal subsamples from each animal collected prior to treatment, and 1, 2, 3, 5, and 7 days posttreatment were bioassayed with *O. gazella* and *E. intermedius* beetles. Fecal subsamples were placed on top of approximately 18 cm of sandy loam soil in a plastic container. Two pairs of adult beetles of the same species selected at random from the same generation of 10 day or older beetles

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were released into the container (Day 0 of Bioassay). Progeny beetles were collected daily from Day 23 to Day 35 for *O. gazella* and from Day 23 to Day 40 for *E. intermedius*. All intact brood balls remaining in the containers were counted on Day 35 for *O. gazella*, and Day 40 for *E. intermedius*. The numbers of living and dead parent and progeny beetles and their sex were recorded for each count day, and numbers of intact brood balls were recorded on the final count day.

Moxidectin levels of all fecal samples from treated and vehicle treated animals were below 100 ppb, the limit of quantification of the assay method. This was consistent with other studies which measured levels of moxidectin excreted in dung following treatment.

As summarized in the following Tables 12-15, there were no significant differences ($P > 0.05$) in the numbers of live or dead adult *O. gazella* or *E. intermedius* recovered from either group at each fecal collection day. For both beetles, there was little difference in the numbers of beetles collected from pretreatment and post treatment feces from any collection date. None of the collected beetles had any observable abnormalities. All progeny beetles recovered for both species had no observable abnormalities. For both species there was no significant difference ($P > 0.05$) between treatment groups for any of the parameters examined except for percent emergence of *E. intermedius* for feces collected prior to treatment. As this was the pretreatment sample, the difference was not due to treatment.

Since all fecal samples collected through 21 days posttreatment contained less than 100 ppb moxidectin, and the NOEC levels for *O. gazella* and *E. intermedius* have previously been determined to be > 500 and > 269 ppb respectively, this result was consistent with previous findings.



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Table 12. Arithmetic means of numbers of living and dead parent *Onthophagus gazella* recovered 7 to 10 days after 2 males and 2 females were added to containers with feces from cattle treated with moxidectin 1% w/v nonaqueous injectable (0.2 mg moxidectin /kg body weight) or moxidectin injectable vehicle ^a.

Fecal collection day (posttreatment)	Treatment Group	N ^b	Live	Mean number recovered		Total ^c
				Dead		
0	Vehicle	12	4.0	0		4.0
	Moxidectin	12	3.7	0		3.7
1	Vehicle	12	3.6	0		3.6
	Moxidectin	12	3.8	0		3.8
2	Vehicle	12	3.4	0		3.4
	Moxidectin	12	3.7	0.1		3.8
3	Vehicle	12	3.3	0		3.3
	Moxidectin	12	3.9	0		3.9
5	Vehicle	9 ^d	4.0	0		4.0
	Moxidectin	12	3.8	0		3.8
7	Vehicle	12	3.8	0.1		3.8
	Moxidectin	12	3.9	0		3.9

^a Means in the same column for each collection day are not significantly different (P> 0.05).

^b Number of replicates.

^c Mean was based on totals calculated for each replicate.

^d Three replicates from animal # 3134 were not used as this animal had been treated with mineral oil on Day 3.



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Table 13. Arithmetic means of numbers of living and dead parent *Euoniticellus intermedius* recovered 7 to 10 days after 2 males and 2 females were added to containers with feces from cattle treated with moxidectin 1% w/v nonaqueous injectable (0.2 mg moxidectin /kg body weight) or moxidectin injectable vehicle ^a.

Fecal collection day (posttreatment)	Treatment Group	N ^b	Mean number recovered		Total ^c
			Live	Dead	
0	Vehicle	16 ^d	2.4	0.4	2.8
	Moxidectin	16 ^d	2.9	0.2	3.1
1	Vehicle	12	3.3	0.3	3.6
	Moxidectin	9 ^e	3.4	0.1	3.6
2	Vehicle	12	3.0	0.2	3.2
	Moxidectin	12	3.2	0.1	3.3
3	Vehicle	12	3.3	0	3.3
	Moxidectin	12	2.3	0.2	2.5
5	Vehicle	10 ^f	2.4	0.1	2.5
	Moxidectin	14 ^g	2.9	0	2.9
7	Vehicle	16 ^d	2.1	0	2.1
	Moxidectin	16 ^d	3.1	0.1	3.2

^aMeans in the same column for each collection day are not significantly different (P> 0.05).

^bNumber of replicates.

^cMean was based on totals calculated for each replicate.

^dA total of four replicates for each animal, two at each assay date.

^eAnimal #3131 did not produce enough feces for any replicates at this time point.

^fThree replicates completed for three animals on one assay date, and a total of four replicates for one animal, two at each assay date. Three replicates from animal # 3134 were not used as this animal had been treated with mineral oil on Day 3.

^gThree replicates completed for two animals on one assay date, and a total of four replicates for each of two animals, two at each assay date.



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Table 14. Arithmetic means of numbers of living, dead and total progeny, numbers brood balls, percent emergence data and sex ratios of progeny produced by two pairs of *Onthophagus gazella* from feces from cattle treated with moxidectin 1% w/v nonaqueous injectable (0.2 mg moxidectin /kg body weight) or moxidectin injectable vehicle ^a.

Fecal collection day (posttreatment)	TREATMENT group	N ^b	LIVE progeny	Dead progeny	Total progeny ^c	TOTAL Brood balls ^{c,d}	Percent Emergence ^e	Sex ratio (M/M+F)
0	Vehicle	12	47.8	0.1	47.8	48.4	98.7	0.47
	Moxidectin	12	44.3	0.2	44.5	45.1	98.5	0.50
1	Vehicle	12	44.6	0	44.6	45.1	98.9	0.47
	Moxidectin	12	50.0	0.1	50.1	50.9	98.4	0.52 ^h
2	Vehicle	12	43.8	0	43.8	44.4	98.5	0.47
	Moxidectin	12	42.5	0.1	42.6	43.8	97.1	0.50
3	Vehicle	10 ^f	49.1	0	49.1	50.0	97.9	0.46
	Moxidectin	12	42.5	0	42.5	43.4	97.5	0.46
5	Vehicle	9 ^g	46.7	0	46.7	47.2	98.7	0.46
	Moxidectin	12	48.1	0.1	48.2	48.6	99.1	0.45
7	Vehicle	12	46.3	0	46.3	46.5	99.4	0.50
	Moxidectin	12	46.8	0	46.8	47.5	98.5	0.49

^a Means in the same column for each collection day are not significantly different (P > 0.05) except the footnote ^h.

^b Number of replicates.

^c Mean was based on totals calculated for each replicate.

^d Total progeny plus intact brood balls.

^e Total progeny/total brood balls * 100 was calculated for each replicate.

^f Two replicates were not used as <5 brood balls were produced in each container.

^g Three replicates from animal # 3134 were not used as this animal had been treated with mineral oil on Day 3.

^h Significantly different from respective controls.



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Table 15. Arithmetic means of numbers of living, dead and total progeny, numbers brood balls, percent emergence data and sex ratios of progeny produced by two pairs of *Euoniticellus intermedius* from feces from cattle treated with moxidectin 1% w/v nonaqueous injectable (0.2 mg moxidectin/kg body weight) or moxidectin injectable vehicle ^a.

Fecal collection day (posttreatment)	TREATMENT group	N ^b	LIVE progeny	Dead progeny	Total progeny ^c	TOTAL Brood balls ^{c,d}	Percent Emergence ^e	Sex ratio (M/M+F)
0	Vehicle	16 ^f	19.5	1.9	21.4	21.6	99.2	0.56
	Moxidectin	16 ^f	18.9	3.3	22.2	27.0	97.2 ⁱ	0.56
1	Vehicle	12	19.8	5.6	25.4	26.2	97.6	0.46
	Moxidectin	9 ^g	20.2	5.6	25.8	27.0	95.4	0.49
2	Vehicle	12	20.3	4.7	25.0	25.7	97.3	0.54
	Moxidectin	12	18.1	4.7	22.8	23.5	97.1	0.49
3	Vehicle	12	16.5	7.8	24.3	25.5	96.0	0.56
	Moxidectin	12	17.1	5.3	22.4	22.6	99.2	0.52
5	Vehicle	10 ^h	14.8	5.6	20.4	20.8	97.1	0.51
	Moxidectin	14 ⁱ	16.6	6.8	23.4	23.7	98.7	0.55
7	Vehicle	16 ^f	10.1	3.1	13.2	13.6	95.0	0.52
	Moxidectin	16 ^f	14.4	3.8	18.2	18.9	95.8	0.50

^a Means in the same column for each collection day are not significantly different (P > 0.05) except the footnote *j*.

^b Number of replicates.

^c Mean was based on totals calculated for each replicate.

^d Total progeny plus intact brood balls.

^e Total progeny/total brood balls * 100 was calculated for each replicate.

^f A total of four replicates for each animal, two at each assay date.

^g Animal # 3131 did not produce enough feces for any replicates at this time point.

^h Three replicates completed for three animals on one assay date, and a total of four replicates for one animal, two at each assay date, three replicates from animal # 3134 were not used as this animal had been treated with mineral oil on Day 3.

ⁱ Three replicates completed for two animals on one assay date, and a total of four replicates for each of two animals, two at each assay date.

^j Significantly different from respective controls.



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8.1.3.2.2 In collaboration with Wardhaugh et al (1999), a detailed study to assess the lethal and sub-lethal effects of moxidectin and eprinomectin residues on a dung breeding fly, *Musca vetustissima*, and on the scarabaeine beetle *Onthophagus taurus* was conducted by the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia. In this study eighteen heifers of mixed European breeds were divided into three groups of six animals. One group was used as untreated controls, the remaining groups were treated with the pour-on formulation of either moxidectin or eprinomectin at 0.5 mg/kg body weight. Each group was allocated to separate but adjoining paddocks. Approximately 3000 specimens of *Onthophagus taurus* were collected from the field. *Onthophagus taurus* was regarded as an indicator species for the effects of macrocyclic lactones, due to susceptibility to toxic effects of avermectins, and the relatively rapid rate of development when compared to species such as *Onitis alexis*. Disruption to the subsequent generation, even on a temporary basis, is greater in the rapid developing species. A sample of 20 beetles was dissected to determine their state of development and physiological age. Additional specimens were examined for tibial wear. Female beetles were classified as: Stage 1 – immature reproductively, Stage 2 – developing females, Stage 3 – mature females, and Stage 4 – evidence of egg resorption. Body fat was scored on a scale ranging from 0 (none) to 3 (full) similar scales were used for gut contents, and tibial wear. Some 91% of beetles examined were physiologically immature, and most appeared to be newly emerged. Accordingly, beetles were divided into groups of about 100 and subjected to a period of 9 days maturation feeding. Dung beetle assays commenced when the beetles had matured, and some 60% were ready to lay. Dung was collected from cattle on days 3, 7, 14, 21, and 42. Survival rates and the duration of egg-to-adult development were the two criteria used to test for lethal and sub-lethal effects of drug residues on the juvenile stages of *O. taurus*. Differences in the rates of survival and sexual development were used to assess drug effects in adult beetles.

a) *Mature Dung Beetle Experiment*: Beetles were sexed and subdivided into batches comprising 6 males and 6 females. Each batch of insects was placed in a secure 5L container holding a mixture of sieved soil and vermiculite. The vermiculite-soil mixture was steam sterilized prior to use. Each combination of drug treatment x dung collection was replicated 8 times. Broods were held at 26°C and checked 3 times weekly to ensure that the surrounding vermiculite remained moist until emergence was complete. Proportions emerging, and time to emergence were measured. As illustrated in the Figure 4 below, in the moxidectin and control treatments, brood survival was roughly constant in all dung collections, varying from 57.6% to 78.4%. There was no evidence of any adverse effect due to moxidectin residues. In the eprinomectin treated group, survival of larval *O. taurus* was inhibited in dung collected three days after treatment, and greatly reduced in day 7 dung.

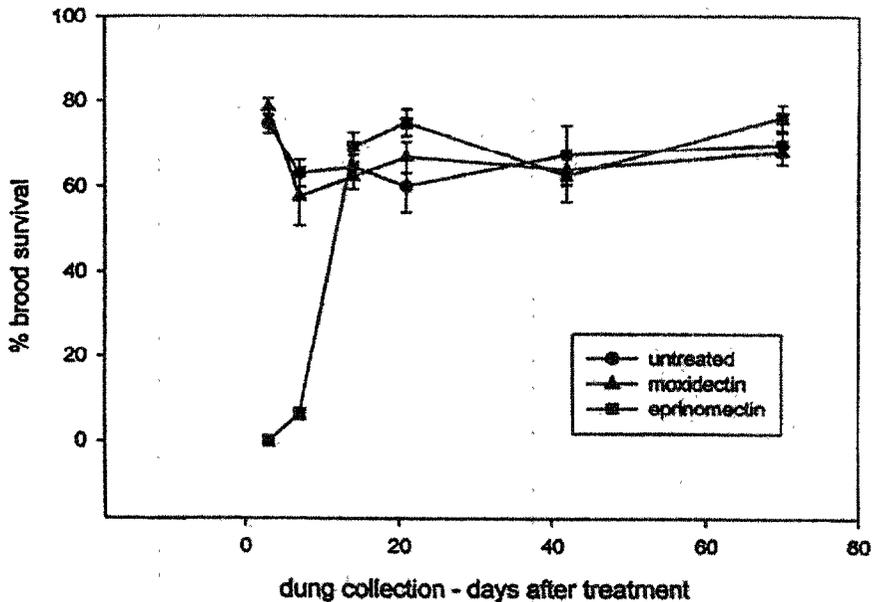


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Figure 4. Survival of Larval *Onthophagus taurus*



Development times from laying to adult emergence for eggs laid over a ten day period of feeding in each drug treatment were assessed. Development time in the moxidectin treatment was significantly shorter than that recorded in control dung, and marginally so in eprinomectin dung.

b) *Immature F1 Beetle experiment*: A number of parameters were assessed in the F1 generation, including adult survival, brood production and physiological status of females. An analysis of the proportion of beetles dying over the duration of the experiment showed a strong effect due to treatment. There was a statistically significantly higher mortality among the insects exposed to residues of eprinomectin ($z = 2.50$; $P = 0.013$) but no effects due to moxidectin ($z = 0.00$; $P = 1.00$). For period 1, when beetles were exposed to dung from either treatment group, brood production in the eprinomectin group was significantly lower than that recorded in both untreated dung ($P \leq 0.01$) and dung from moxidectin treated cattle ($P \leq 0.05$). Differences between control and moxidectin-treated dung were not significant ($P \geq 0.9$). Effects of eprinomectin treatment on brood production were still apparent at the end of the second period of feeding, i.e., after each group of insects had been allowed to feed for ten days on dung from untreated cattle ($P = 0.024$). This period of feeding was marked by a significant overall increase in the numbers of eggs laid ($P = 0.027$); beetles exposed previously to eprinomectin residues still produced fewer brood masses ($P \leq 0.02$). There was no evidence of any effect on the gut content, body fat or ovarian development in female beetles at the end of the trial period. It was concluded that



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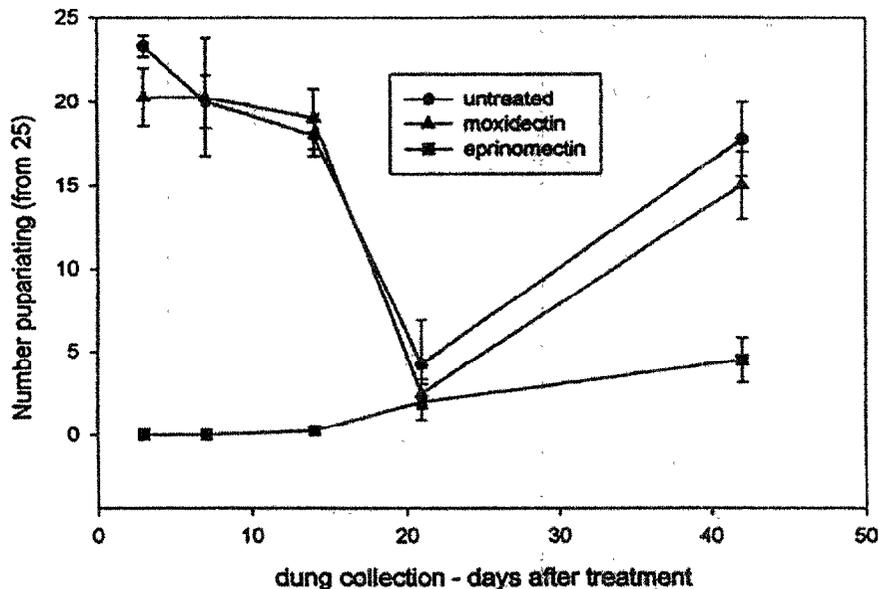
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10 days of feeding on untreated dung is sufficient to effectively mask or even reverse any adverse effects arising from initial exposure to residues of eprinomectin.

For the bushfly, *M. vetustissima* bioassay, drug effects were assessed using measurements of egg to pupal survival. Parent flies were kept in natural daylight and $26 \pm 1.0^\circ\text{C}$ throughout their development. Eggs were laid on the dung of untreated cattle, left at 26°C for about 8 hours, and then randomly divided into three separate groups (i.e., one group per treatment) and transferred to 18°C for hatching. For each dung sample, a batch of 25 newly emerged larvae was placed on a 150 mL ball of dung and left to develop in secure containers at $26 \pm 1.0^\circ\text{C}$. Newly hatched larvae were transferred with a fine paintbrush to a freshly excavated crevice on the dung surface. Brushes were changed between treatments to avoid cross-contamination between dung balls. Each combination of drug treatment x dung collection was replicated four times. Proportions surviving to the pupal stage were used to test for differences in toxicity between treatments.

As illustrated in Figure 5, in dung from eprinomectin treated cattle, survival was completely inhibited during the first week after treatment, but increased steadily with time thereafter. Over the same period, survival in dung from untreated cattle, and cattle treated with moxidectin, ranged from 60% to 93%, except in day 21 feces, where survival was less than 17%. The cause of this anomalous result is not known. Since evidence of a similar effect in the dung beetle assay was lacking, it seems logical to suppose that this anomaly was an experimental artifact e.g., an non-viable batch of larvae.

Figure 5. Survival of Larval *M. vetustissima*





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8.1.3.3 Family Geotrupidae

The family Geotrupidae is the least numerous in terms of species of all the dung beetle families.

8.1.3.3.1 Study 0876-E-FR-06-95 (Report GASD 04-03.00): This study was conducted in Montpellier France to investigate the dung beetle species *Anoplotrupes stercorosus* (Coleoptera: Geotrupidae) a tunneller dung beetle which is highly attracted to horse droppings. In addition, a fly bioassay was conducted using *Neomyia cornicina*.

This experiment involved 15 horses randomly allocated to one of three treatment groups. The first group was treated with moxidectin 2% equine gel orally at a dose rate of 0.4 mg/kg bodyweight, the second group with ivermectin paste at 0.2 mg/kg body weight, and the third group remained untreated controls. The horses were kept outside during the day, and housed in individual boxes overnight. Fresh dung was collected in the morning from each box on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 14, 16, 18, 21, 24, 28 and 35 post-treatment. In the beetle assay, ten *A. stercorosus* adults were placed on the dung and the mortality rate was measured for 35 days. As summarized in Table 16 below, results indicated that the beetle mortality rate was high throughout the study for all groups, especially after ten days of breeding. Survival was not significantly affected by either treatment, and no significant difference was observed between the moxidectin and ivermectin treatments.

Table 16. Mortality Rate (%) of *Anoplotrupes stercorosus*

Days After Seeding Beetles	Control	Moxidectin	Ivermectin
(0-10)	4.82	6.78 ns	4.17 ns
(10-21)	9.99	9.33 ns	8.35 ns

ns: the differences between treated and control groups and between treated groups were not significant.

In the bioassay using *Neomyia cornicina*, two replicates of ten eggs were placed on the dung samples, and the numbers of adult *Neomyia cornicina* emerging for 21 days were counted. The % reduction in emergence versus controls was calculated for each treatment group. As summarized in Table 17 below, results indicated that a significant negative impact of endectocide treatment was observed for the first 5 days after moxidectin treatment, and for 21 days after ivermectin treatment. There was a significantly lower emergence rates by ivermectin (0 – 5%) than by moxidectin (13 – 75%) from 4 to 21 days post-treatment. The total mortality was observed only for the first 2 days in the moxidectin group, whereas the ivermectin group mortality was total for 14 days after treatment.



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Table 17. Reduction in *Neomyia cornicina* emergence rate (%)

Day	Control % emergence	Moxidectin % emergence	Moxidectin x control	Ivermectin % emergence	Ivermectin x control	Ivermectin x moxidectin
0	--	60		66		
1	56	0	***	0	***	
2	61	0	***	0	***	
3	68	2	***	0	***	
4	74	13	***	0	***	***
5	65	35	*	0	***	***
6	64	45		0	***	***
7	56	46		0	***	***
8	44	52		0	***	***
10	59	75		0	***	***
12	52	59		0	***	***
14	56	54		0	***	***
16	55	47		5	***	***
18	59	65		1	***	***
21	51	54		5	***	***
24	49	47		31		
28	51	46		37		

Note: << -- >> = For Day 0, moxidectin and ivermectin samples were used as control, as the sampling occurred before treatment.

* Significant difference at p<0.05

*** Significant difference at p<0.001

8.1.3.4 Diptera

In addition to the studies described previously, a number of studies were conducted to assess the effects of moxidectin specifically on various fly species.

8.1.3.4.1 A study was conducted by Wardhaugh et al of the Division of Entomology at the CSIRO in Australia to assess any impact of moxidectin or ivermectin residues in dung of treated cattle on the bushfly, *Musca vetustissima*, and the housefly *Musca domestica* (Wardhaugh, Holter, Whitby and Shelley, 1996). The cattle used in this study were 12 month old crossbred (Murray Grey x Angus) heifers divided into three groups. One group was treated with moxidectin injectable at 0.2 mg/kg live weight, the second group with 0.2 mg/kg live weight of ivermectin injectable, and the remainder were untreated controls. After treatment, the heifers were held in adjoining 5 hectare paddocks of similar floristic composition and no prior history of macrocyclic lactone usage. Dung was collected from each group of cattle on days 3, 7, 14, 21, 28 and 35 days after treatment.

Adult insects were kept in natural daylight and 26±1°C throughout their development. Dung from control cattle was provided as a medium for oviposition. Newly laid eggs were

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maintained at 26°C for about 3 hours before being harvested and transferred to 18°C to complete their development.

For each treatment x dung collection, a batch of 25 newly emerged larvae was placed on a 150 mL ball of dung and left to develop in secure containers at 26±1°C. Each dung ball was placed on a 1 cm layer of vermiculite which provides a suitable substrate in which fully fed larvae can pupariate. Newly hatched larvae were transferred with a fine paintbrush to a freshly excavated crevice on the dung surface. The brush was changed between treatments to avoid cross contamination between dung balls. As a further precaution, dung with expected lowest residue levels (i.e., 35 day dung) was seeded first, and dung with expected highest residues (day 3 dung) was treated last. Each treatment x dung collection was replicated four times. After pupariation, each dung ball and the underlying vermiculite was examined for the presence of pupae. These were counted, and returned to their container for adult emergence.

The data was analyzed on survival to pupal and adult stages for both *M. vetustissima* and *M. domestica*. Since dung from ivermectin treated cattle inhibited survival of *M. vetustissima* larvae for the first 14 days post treatment, three-way comparisons of moxidectin, ivermectin and control dung were confined to collections made on days 21 to 35. Numbers of larvae pupating in each treatment group were compared by a two-way analysis of variance (ANOVA) with drug and days post treatment as the two factors. An additional two-way ANOVA compared pupal numbers in control and moxidectin dung over the entire collecting period. Similar procedures were used to compare estimates of survival to the adult stage. For each ANOVA, a Student-Newman Keuls test was used to do pairwise comparisons between dung types for each collection day.

A comparison of the numbers surviving to the pupal stage in the moxidectin and control treatments indicated no overall effect due either to treatment ($P = 0.668$) or to day of collection ($P = 0.246$). There was however a significant treatment x day interaction ($P = 0.007$), with survival in control dung showing a slight decrease over time. Pairwise comparisons of moxidectin and control dung on individual days confirmed the lack of significant effects due to drug treatment, except on day 14, when survival in moxidectin dung was significantly lower than that recorded in the corresponding control. However, this reduction was confined to a single replicate and is thus unlikely to be indicative of any meaningful trend. There were no effects on the number of larvae surviving to the adult stage. Chi-square tests were used to compare the proportions of pupae that emerged as adults in the control and moxidectin groups. This analysis showed that exposure to residues of moxidectin had no significant effect on survival during the pupal stage.

In dung from ivermectin treated animals, larval survival was inhibited during the first 14 days post treatment. A two-way ANOVA of data for the control, moxidectin and

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ivermectin treatments showed effects due to day ($P < 0.001$) and treatment ($P < 0.001$) to be highly significant for dung collected 21 to 35 days post treatment. Day-treatment interactions were also significant ($P < 0.001$). Pairwise comparisons using the Student-Newman Keuls test showed that the number of larvae pupating in ivermectin containing dung was significantly less than that recorded in the control and moxidectin treatments on days 21 ($P < 0.05$) and 28 ($P < 0.05$). By day 35, the mean number of larvae surviving to the pupal state did not differ significantly ($P > 0.05$). Over the same period (days 21 to 35) differences between control and moxidectin dung were not significant ($P > 0.05$). For the dung of days 28 and 35 no significant differences were found between control, moxidectin and ivermectin dung in the proportions of pupal that emerged as adults. The accumulated mortality of newly emerged females was either zero or negligible in all treatments.

The procedures used for assessing survival in *M. domestica* were similar to those used in the *M. vetustissima* assay. Eggs were obtained from a laboratory colony of *M. domestica* and transferred to 18°C for hatching. Newly emerged larvae were transferred to 150 mL balls of dung and left to develop at 26 ± 1 degree C. As illustrated in Figures 6 and 7 below, survival was assessed after pupariation (a) and again at adult emergence (b), respectively. Results indicated that dung from ivermectin treated cattle inhibited survival of juvenile *M. domestica* for at least one week after treatment. In comparison, dung from moxidectin treated animals was innocuous and residue effects were not significant.



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Figure 6. Effects of Moxidectin and Ivermectin on the Pupariation of *M. domestica*

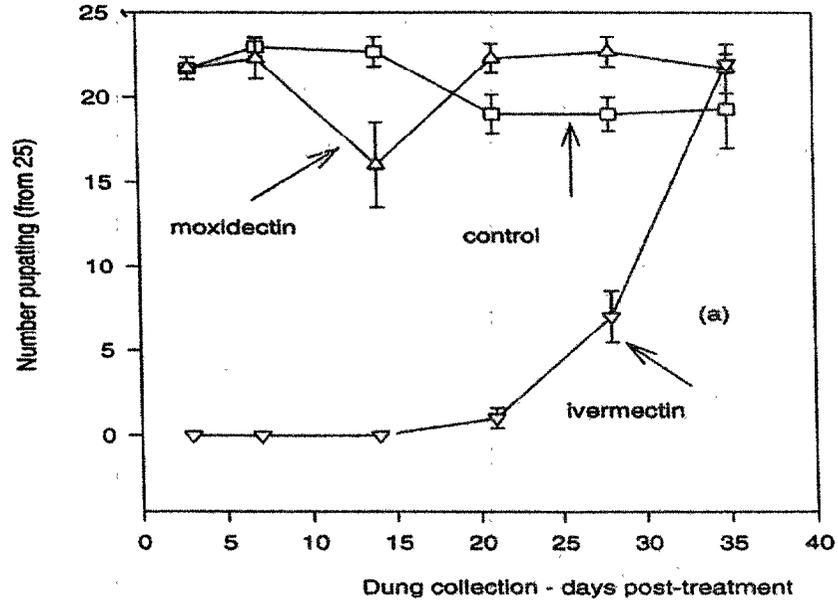
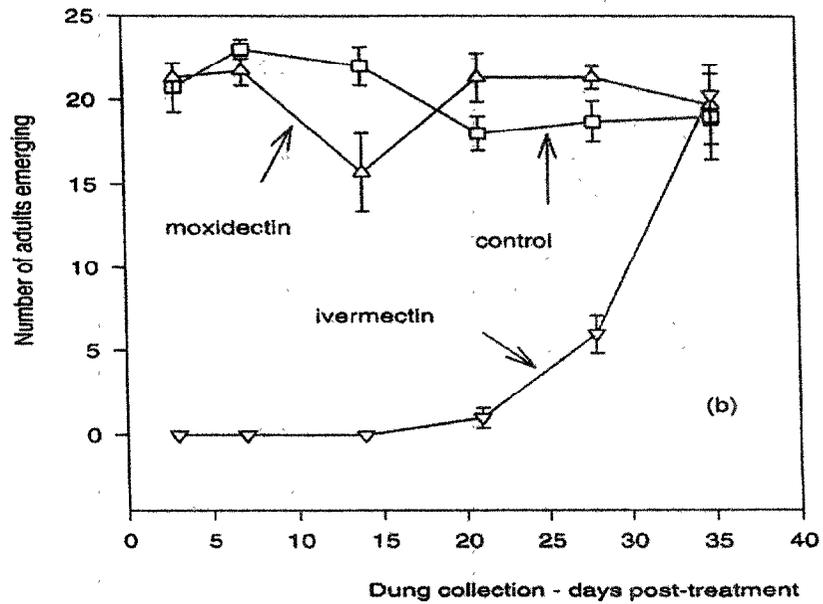


Figure 7. Effects of Moxidectin and Ivermectin on the Emergence of Adult *M. domestica*





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8.1.3.4.2 A study was conducted by Floate et al to compare the effects of various endectocide residues on three fly species, horn fly *Haematobia irritans*, house fly *Musca domestica* and stable fly *Stomoxys calcitrans* in Canada (Floate, Spooner, Colwell, 2001) Laboratory bioassays were performed in two experiments using colonies of house fly, horn fly and stable fly maintained in culture at the Lethbridge Research Centre.

In experiment one, 6 cattle were divided into three groups of two animals. Animals in the two treatment groups were treated with either moxidectin or ivermectin topically at a dose rate of 0.5 mg/kg live weight. The third group remained untreated controls. Fresh dung less than 3 hours old was collected from each pen immediately prior to treatments, and at weekly intervals for 9 weeks after treatment. For experiment two, 32 cattle were divided into four groups of eight. The treatments applied in this study were ivermectin, doramectin, moxidectin and eprinomectin at the recommended dosage of 0.5 mg/kg. Dung was collected from each pen immediately prior to treatment and at 1, 2, and 4 weeks after treatment.

Bioassays were performed by seeding 250 mL containers containing 100 mL of dung with either 50 newly hatched first stage larvae of house fly, or 50 eggs of either horn fly or stable fly per container. Lids were placed on the containers which were held at 25°C until emergence of adult flies. For each experiment, ANOVAs were performed using percentage survival as the dependent variable, and species, treatment and week as independent variables.

Results from the experiment 2 are summarized in Table 18 below indicating that there were significant interactions between species, treatment and week in both experiments ($P < 0.001$). Hornfly were the most susceptible of the three species to the larvicidal action of endectocides. This is consistent with the findings of Schmidt and Kunz (1980) who reported that the LC_{90} of ivermectin was 31-fold higher for horn fly than for stable fly.

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Table 18. Percent Survival of Flies Developing in Dung of Cattle Treated with Various Endectocides

	Week 0	Week 1	Week 2	Week 4
Doramectin				
Horn fly	42.5±3.1	0.0±0.0*	0.0±0.0*	0.0±0.0*
House fly	85.2±3.2	0.2±0.2*	26.4±1.9*	69.8±3.7*
Stable fly	63.5±3.4	0.0±0.0*	9.7±2.2*	43.2±2.5*
Eprinomectin				
Horn fly	70.0±5.6	0.0±0.0*	0.0±0.0*	23.1±3.7*
House fly	80.4±3.4	13.6±2.4*	77.2±2.2	82.6±1.8
Stable fly	72.4±2.9	2.3±0.8*	60.9±2.5*	57.0±4.3*
Ivermectin				
Horn fly	7.1±3.4	0.0±0.0*	0.0±0.0*	0.8±0.6*
House fly	99.0±1.7	4.0±0.9*	80.6±3.6*	99.0±0.7
Stable fly	58.3±4.4	24.0±2.6*	80.0±1.5	27.8±5.2*
Moxidectin				
Horn fly	49.2±4.5	36.4±3.9*	49.7±3.4	28.4±4.8*
House fly	88.0±3.1	76.4±6.8	86.4±2.2	80.2±4.1
Stable fly	75.2±3.5	74.0±1.8	74.4±1.9	54.1±6.6*

*, Significantly lower than Week 0 for that combination of species and treatment.

Based on the data, the compounds were readily separable into two groups on the basis of their larvicidal activities. The first group comprised doramectin, eprinomectin and ivermectin. These products suppressed development of horn fly for at least four weeks post application, with suppression by ivermectin observed for eight weeks post application in experiment one. Suppression of stable fly and house fly by these products ranged from one to five weeks. The second group contained moxidectin, which showed much less toxic effects on flies than the first group of compounds. Rank in the descending order of larvicidal activity is as follows: doramectin > ivermectin = eprinomectin >> moxidectin. The separation of these compounds by their larvicidal activities corresponds to their separation based on their chemical structures of the avermectins (doramectin, ivermectin, eprinomectin) and milbemycins (moxidectin)

8.1.4 Dung Colonization and Degradation

Despite the many studies which showed either no or minimal impact on indicator species of beetles and flies with importance in the dung degradation and colonization process, investigations were carried out to determine whether any impact could be found on colonization of dung pats, whether numerically or temporally in terms of the diversity of species, or in the rate of dung degradation. It was considered unlikely that any effects would be found, since in the case of the avermectins which have been shown to have significant effects on survival of dung insects, reported effects on dung colonization are variable, and in some cases reported as undetectable. Published, independent studies and a

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company report examining the impact of moxidectin on dung colonization and degradation are summarized in this section.

8.1.4.1 A study was conducted by Strong and Wall in a northern temperate climate (1994a, 1994b) in which the numbers of insects colonizing the dung of cattle injected with 0.2 mg/kg body weight of either moxidectin or ivermectin were compared with the colonization of the dung from control animals given no treatment, in a blind field trial.

Test animals were 12 yearling cattle, 11 were Limousin x Friesian cross, and 1 was a Belgian Blue x Friesian. They were divided randomly into three groups, and housed in pens at the University of Bristol. Dung was collected from each pen at 2, 7, 14 and 21 days after treatment, and set out in a randomized grid of standard 2 kg pats. A 12 x 12 m area of pasture was fenced off to exclude livestock throughout the trial. Inside this enclosure, 96 pat sites were marked out, 1 m apart in a 12 x 8 grid. At each designated pat site, a piece of plastic netting was placed on the grass. The pats were prepared by weighing out 2 kg of fresh dung, and placed into a cardboard former, 25 cm diameter by 5 cm deep, onto the plastic netting. The entire pat was covered with a piece of pen wire to prevent birds from damaging the pats. On each collection date, 8 pats were set out for each treatment group.

Pats were retrieved from each of the three groups after 7, 14, 21 and 42 days in the field, for analysis of insect fauna. When analyzed for invertebrates, the pats were gently broken up in a 12 liter bucket fitted with a spout of plastic tubing 5 cm in diameter. Water from a tap flowed into the bucket, and the effluent flowed from the spout through two stacking mesh sieves, size 2mm and 1 mm respectively. The contents of the finer sieve were transferred into a large white enamel tray approximately 1 cm depth of water. The tray was inspected for vertebrates, which were counted and identified into phyla.

Insect numbers were subjected to analysis of variance, with treatment, days after treatment and duration of exposure in the field as variables, and LSD multiple range tests.

Larvae from the dipterous families Anisopodidae, Anthomyiidae, Bibionidae, Fannidae, Muscidae, Psychodidae, Stratiomyidae, Sepsidae and Tipulidae were found in pats. Small numbers of adult Coleoptera from the families Scarabaeidae, Hydrophilidae, Carabidae and Staphylinidae, and large numbers of larval beetles, predominantly *Aphodius* spp were present. Ants and woodlice were common and were distributed irregularly beneath the pats of all three groups.

As illustrated in Figure 8 below, there were no differences in the number of adult *Aphodius* spp found in the dung from the three treatment groups with either time of exposure in the field or time after treatment. This demonstrated that adult beetles were attracted to all pats equally. However, the dung from animals treated with ivermectin prevented the

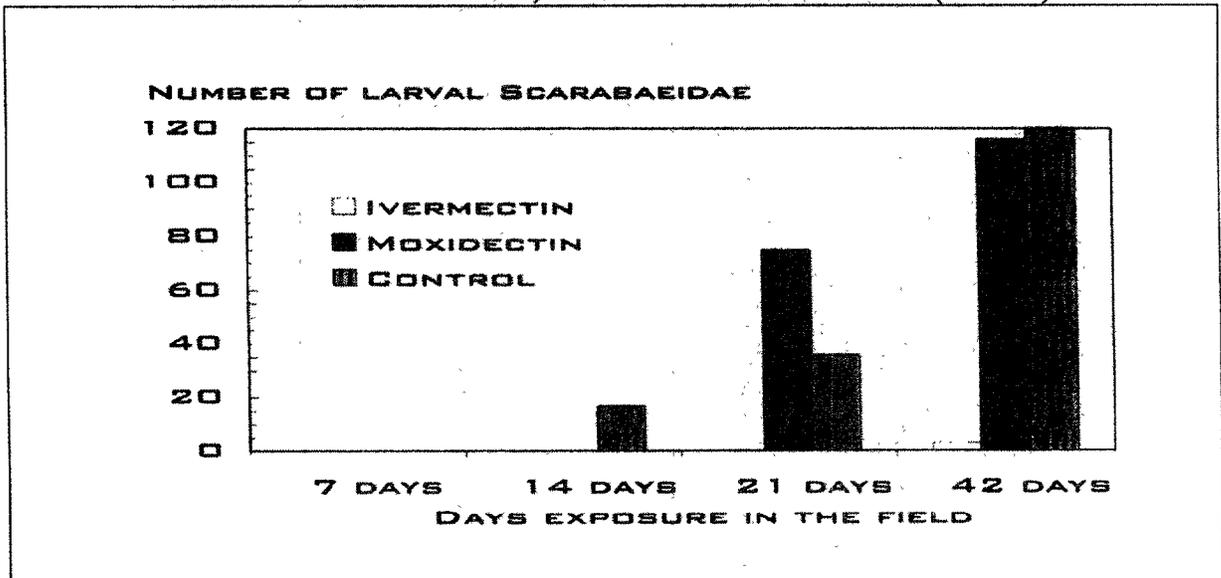
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development of larval *Aphodius* spp for at least 7 days after treatment. The dung from animals treated with moxidectin supported the development of *Aphodius* spp at all stages after dosing, as did dung from control animals. A multiple range test showed the ivermectin treated pats contained significantly fewer beetles larvae than the control ($P < 0.05$), while the moxidectin pats did not. After 42 days exposure in the field, the numbers of *Aphodius* spp larvae found in the dung from ivermectin treated cattle were still significantly below those found in the moxidectin and control dung ($P = 0.002$).

Figure 8. *Aphodius* (Coleoptera: Scarabaeidae) larvae, 7 days after subcutaneous treatment with ivermectin, moxidectin or no treatment (control)



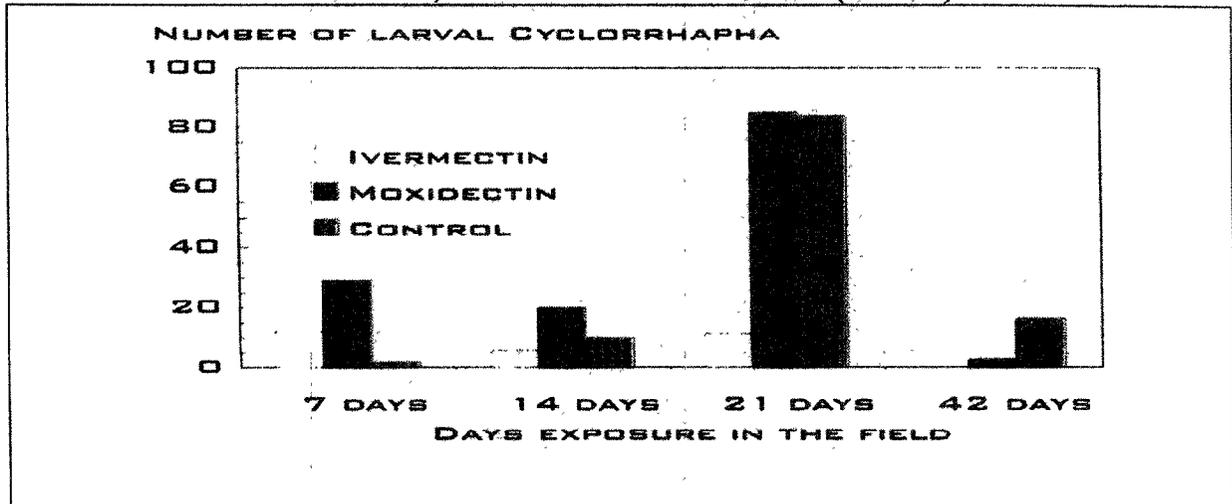
As illustrated in Figure 9 below, there were no significant differences in the number of cyclorrhaphous Diptera larvae in dung from control or moxidectin treated animals. In contrast, such larvae were largely absent from the dung collected from ivermectin treated cattle for up to 14 days after dosing, and the larvae appeared for the first time in dung collected 21 days after ivermectin treatment. However, there were still significantly fewer larvae than in dung from moxidectin treated or control cattle ($P = 0.01$). Nematoceros Diptera occurred in dung from all cattle, regardless of treatment, time of exposure in the field, or time after treatment at which the dung was collected.



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Figure 9. *Diptera Cyclorrhapha* larvae, 21 days after subcutaneous treatment with ivermectin, moxidectin or no treatment (control)



8.1.4.2 A study was conducted by Wardhaugh et al to investigate dung colonization under tropical conditions (Wardhaugh, Mahon and Bin Ahmad, 2001). One hundred female dairy cattle were used at the Institut Haiwan Screw-Worm Fly laboratory in Kluang, Malaysia. There were four treatment groups, moxidectin, doramectin, or eprinomectin applied topically at 0.5 mg/mg body weight, or ivermectin SR bolus. After treatment, each group of cattle was held in a separate paddock of a similar size (5 hectares) and had a similar grazing history. On three occasions after the cattle were treated, 30 or more dung pads from each of the treatment groups, and untreated controls, were examined for the presence of fly larvae (mainly *Musca inferior* and *Orthelia timorensis*). Larval size and abundance were scored on a scale of 1 to 3 (size: small, medium, large) (abundance: none, some, many). Pad age was classified as more or less than 3 days old. Freshly dropped pads and pads classified as more than a week old were not included in the survey. To analyze the data, young pads were assigned an average age of 2 days, and old pads were considered to be 5 days old, effectively resulting in six sampling occasions. Regression analysis was used to compare differences.

As summarized in Table 19 below, pads produced by ivermectin bolus treated cattle contained few or no fly larvae except on the first sampling occasion, when small numbers of larvae were observed in three pads. Residues of doramectin and eprinomectin appeared to reduce fly survival between days 3 to 9 after treatment, but by day 13 there was no evidence of any drug effects. In contrast, pads from moxidectin treated cattle followed a similar trend to those from untreated cattle, declining in the mid portion of the sampling period. This coincided with a sequence of heavy rainstorms. Analysis of the results showed that for the groups treated with the three avermectins, the proportion of pads infested with fly larvae was significantly less (in all cases $P < 0.001$) than that recorded for



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pats from untreated cattle. Pats voided by moxidectin treated cattle were not significantly different ($P > 0.2$) from those produced by untreated cattle.

Table 19. Proportion of pats infested with fly larvae at different times after treatment with various endectocides

Treatment	Proportion of pats with fly larvae at different days after treatment					
	0	3	6	9	13	16
Untreated	0.55	0.35	0.12	0.44	0.31	0.50
Moxidectin	0.60	0.21	0.25	0.14	0.69	0.44
Eprinomectin	1.00	0.07	0.00	0.00	0.36	0.58
Doramectin	0.47	0.10	0.00	0.00	0.32	0.18
SR bolus ^a	0.13	0.00	0.00	0.05	0.00	0.00
Rainfall (mm) ^b	4	30	17	58	42	29

^aSince bolus-treated animals were treated one week before those in other groups, 7 days should be added to the sampling observation times listed for this treatment.

^bTotal rainfall for preceding 3 days.

8.1.4.3 Study 0876-B-FR-29-94 (Report GASD 04-28.00): This study was conducted to assess the dung colonization and dung dispersal in Montpellier, France, under Mediterranean climatic conditions. Approximately 18 kg of cattle dung from animals previously treated with either moxidectin injectable or ivermectin injectable at 0.2 mg/kg body weight was used for this study. Samples weighing about 1 kg were deposited in a single pasture at 1 m intervals and identified as to group and sampling days. The dung pats were individually weighed, and placed on a rigid plastic screen anchored to the soil by two long nails. The samples were shaped into a flat circular pat approximately 18 cm in diameter. Samples were not protected from birds, this was deemed unnecessary as the bird population was sparse. The pats were placed in the field on the respective sampling days.

Two samples were collected at 7, 14, 21 and 28 days to evaluate dung colonization by insects. Ten other samples were kept in the field for two months, then collected and weighed to evaluate the rate of dispersal of dung. The samples were weighed, then disassembled to allow counting and identification of the insects present. The holes corresponding to the nesting activities of dung beetles were counted and recorded. The dung was oven dried and re-weighed to determine the rate of dispersal.

Only coprophagous insects living and feeding inside the dung pats, and present at the time of sampling were counted. Counts were limited to Coleoptera and Diptera. Dead insects were not included in the counts. Adult Coleoptera found were Scarabaeidae (mostly *Onthophagus* and *Bubas* spp) and Aphodiidae (mostly *Aphodius haemorrhoidalis*). Coleopteran larvae were *Aphodius haemorrhoidalis*.

Only Diptera larvae were counted, since adult flies do not actually live inside the dung.



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Predatory beetles, even when coprophilous and living inside pats (mostly Staphylinidae and Histeridae) were not counted, as they are generally predatory, and do not feed on dung.

In control dung, the total number of insects recovered per pat varied from 63.5 (Day 5) to 409.5 (Day 38), average number being 189.1 (Table 20 below). The moxidectin pats had high numbers of live insects, even in the first days after treatment, when residues would have been highest. The total number varied from 91.5 (Day 20) to 345 (Day 10), with the average number 214.8. This was not statistically significantly different from controls. In the ivermectin group, the number of insects was low inside the pats collected a few days after treatment, until day 5, averaging 36.3 per pat over days 2, 3 and 5. However, when counts from the whole timeframe are considered, the average number of live insects was 172.7, and not significantly different from controls.

Table 20. Total number of alive insects recovered from all dung pats from moxidectin-treated, ivermectin-treated and control cattle

Days after injection	Control	Moxidectin	Ivermectin
D 0	193.5	ND	ND
D+1	257.5	ND	ND
D+2	207.0	334.5	39.5
D+3	219.5	197.5	31.5
D+5	63.5	164.5	38.0
D+7	176.0	322.0	138.5
D+10	168.5	345.0	352.0
D+16	128.5	108.0	39.5
D+20	116.0	91.5	76.5
D+28	140.0	152.0	524.0
D+38	409.5	219.0	314.5
average of total from all days	189.1	214.8	172.7

ND = no data

As indicated in Table 21 below, there was a disruption of the normal proportions of species present in the ivermectin dung up to and including the day 16 samples. In control dung, the larvae of Diptera were the predominant species comprising 71.8% (range 57.3 to 94.9%), adult Coleoptera were 4.2% of the population, and larvae of *Aphodius* spp were 24%. The proportions were similar with moxidectin, being 78.8%, 4.0% and 17.8% respectively. In the ivermectin group, the toxic effects of residues depressed the numbers of Diptera for the first 16 days, so that they formed only 4.3% of the population. The adults and larvae of Coleoptera were dominant in this phase, forming 20.7% and 75.0% of the population. In dung collected 20 days or more after treatment, the proportions were closer to that of controls, being 63.4% for Diptera, 2.1% for adult Coleoptera, and 34.4% of larval Coleoptera. These field observations are consistent with laboratory bioassay results which



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showed that moxidectin residues have only a mild and transient effect on larvae of Diptera, while the effects of ivermectin residues are profound and of longer duration.

Table 21. Taxonomic groups of beetles and Diptera recovered in the whole trial from all moxidectin-treated, ivermectin-treated and untreated cattle dung pats.

Days after treatment	Control			Moxidectin			Ivermectin		
	Adult Coleoptera	Larvae Aphodius	Larvae Diptera	Adult Coleoptera	Larvae Aphodius	Larvae Diptera	Adult Coleoptera	Larvae Aphodius	Larvae Diptera
D 0	14.3	61.5	117.8	no data	no data	no data	no data	no data	no data
D+1	5.5	34.5	217.5	no data	no data	no data	no data	no data	no data
D+2	3.5	41.5	162.0	24.0	88.5	222.0	34.0	2.5	3.0
D+3	6.0	74.0	139.5	20.5	53.0	124.0	24.0	6.0	1.5
D+5	3.5	7.0	51.0	7.5	50.0	106.5	17.5	20.0	0.5
D+7	21.0	45.5	109.5	3.0	7.0	312.0	24.5	109.5	4.5
D+10	8.0	15.5	145.0	8.5	36.5	300.0	22.5	316.0	13.5
D+16	4.5	2.0	122.0	1.5	11.5	95.0	10.0	25.0	4.5
D+20	2.5	47.0	66.5	1.5	8.0	82.0	12.5	10.5	53.5
D+28	12.5	22.5	105.0	6.0	66.0	80.0	7.0	249.5	267.5
D+38	5.5	147.0	257.0	4.0	23.0	192.0	0.0	55.0	259.5
Total	86.8	498.0	1492.8	76.5	343.5	1513.5	152.0	794.0	608.0
% / pat	4.2	24.0	71.8	4.0	17.8	78.8	9.8	51.1	39.1
	Total Control	2077.6		Total Moxidectin	1933.5		Total Ivermectin	1544.0	

Deposits of the pats occurred on days D 0, D+1, ..., D+38 after injection.

Two pats were retrieved from each of these three groups after 7, 14, 21 and 28 days in the field (8 pats in total per series).

The numbers of insects were reduced to one pat in each series for each day after injection.

Despite the noted effects on the numbers and diversity of insects in dung in the colonization study, there were no significant differences ($P > 0.05$) seen in the rate of dung dispersal between the three treatment groups (Table 22 below). The average amount of dung dispersed in the control group averaged 19.99%, with a range of 10.9% for Day 5 to 32.1% for Day 28. In the moxidectin group, average dung dispersal was 23.6%, ranging from 15.3% on Day 5, to 32.8% on Day 10, a slightly higher rate than for controls. In the ivermectin group, average dispersal was 22.0%, ranging from 15.7% on Day 20, to 33.9% on Day 28.



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Table 22. Cattle dung dispersal: Loss of dry matter (%) after 2 months in the field (moxidectin-treated, ivermectin-treated and untreated cattle)

Days after injection	Control	Moxidectin	Ivermectin
D 0	21.98	no data	no data
D+1	29.85	no data	no data
D+2	17.23	22.59	21.78
D+3	11.24	21.86	19.60
D+5	10.92	15.28	24.45
D+7	25.62	22.99	16.60
D+10	18.45	32.77	24.36
D+16	20.94	22.42	25.72
D+20	20.44	25.17	15.70
D+28	32.09	25.07	33.88
D+38	19.08	24.20	15.98
average	19.99	23.59	22.01
standard deviation	7.27	4.53	5.89
variance	52.66	20.51	34.68

It is postulated that despite the effects on dung fauna for three days with moxidectin, and three weeks with ivermectin, the elimination of competition from some species, allows compensation for other species which face less competition, so that dung degradation is not affected.

8.1.5 Computer Modeling

Concerns have been expressed by scientists working in the field of evaluating environmental impact, that extrapolation from the results of simple bioassays, and field studies on colonization and degradation, is not an appropriate method for evaluating environmental effects of complex ecosystems such as the dung pat (Herd 1995; Moore et al 1993; Strong 1993). Recommendations have been made that computer simulation modeling is a more appropriate system, since it allows the assessment of a number of variables based on one bioassay data set (Moore and DeRuiter, 1993; Sherratt et al 1998). A model developed to assess effects of insecticides on the breeding success of dung beetles in the field has been developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO), a government research body based in Australia. This is the group which is responsible for the introduction of exotic dung beetle species into Australia over the past 30 years, in an attempt to deal with the problems of lack of dung degradation by native beetle species. A description of the model is provided in Wardhaugh, Longstaff and Lacey (1998).

Wardhaugh (1999) used this computer model to assess the potential impact of residues of moxidectin and eprinomectin on the reproductive success of two species of dung beetle, *Onthophagus taurus*, a rapid developing, multivoltine species of dung beetle, and *Onitis*



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alexis, a slow, developing univoltine species. The computer simulations were based on juvenile mortality effects for the two compounds, as determined by bioassay. The model uses age specific data on fecundity and survival in a Leslie matrix to compute the mortality of an age-structured population. For slow developers, it assumes that egg-to-adult development takes 80 ± 20 days, followed by a pre reproductive phase of 10 days. Females lay two eggs per day over a 10 week period, and move to a fresh pat every 2-4 days. Adult females have a half life of eight weeks, during which death rate remains constant. For fast developers, egg-to-adult development time is reduced to 40 ± 10 days. The half life of females is set at 4 weeks, with females laying two eggs per day over a 6 week period. For both fast and slow developers, the model assumes that immigration and emigration are equal and makes no allowance for density dependence.

To investigate the impact of treatment with moxidectin or eprinomectin when dosing occurs at different times of the year, simulations were run using treatment times of 7, 14, 28, 56 and 84 days after the median date of beetle emergence in spring. For each simulation, output from each drug treatment was standardized against that of the untreated control to provide a Relative Activity Index.

Because moxidectin has no detectable impact on beetle survival or development, its effect on beetle activity, as predicted by the model, cannot be distinguished from controls. The following 2 figures present the effects of on both slow-developing (Figure 10) and fast-developing (Figure 11) dung beetle, respectively. Simulations of eprinomectin data indicate substantial perturbations of beetle populations, depending on time and frequency of treatment. Fast developing species such as *O. taurus* and *E. fulvus* seem likely to be more severely affected than slow developers e.g., *Onitis alexis*, with maximum disturbance arising when treatment occurs some two weeks after the peak of spring emergence. Despite the short period over which dung is toxic, a single treatment around the time of peak egg laying is capable of reducing beetle activity across an entire season by as much as 35%. With slow developing species, the overall effect is less because they have an extended egg laying period and probably amounts to a reduction of about 25%.



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Figure 10. Response of slow-developing species of dung beetle to a single treatment of eprinomectin at different times after beetle emergence

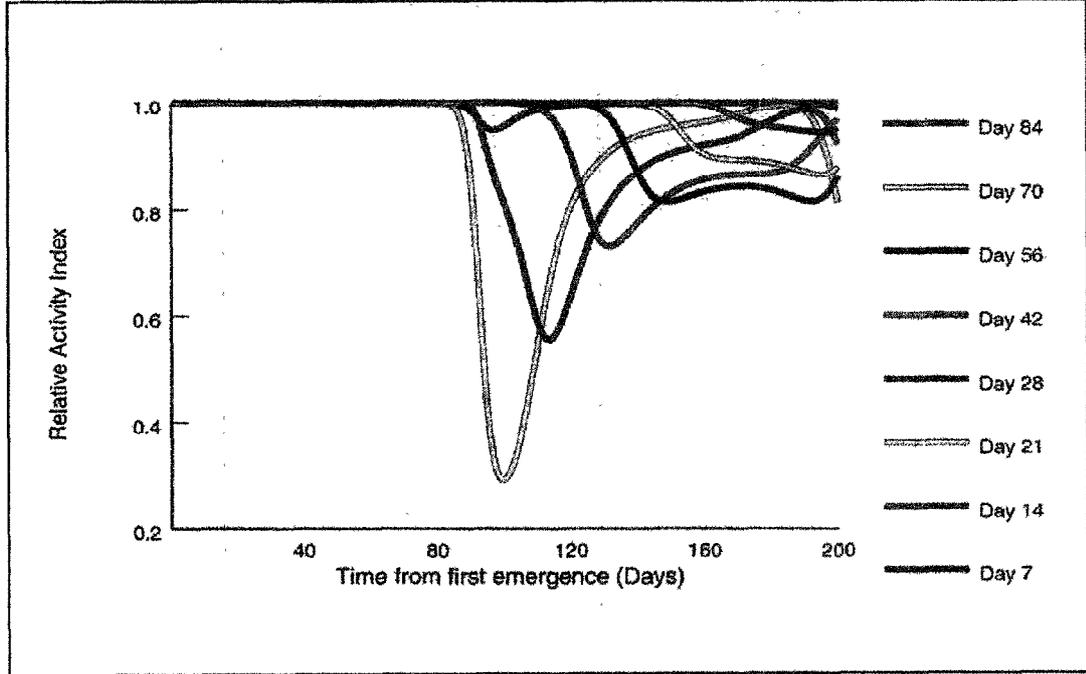
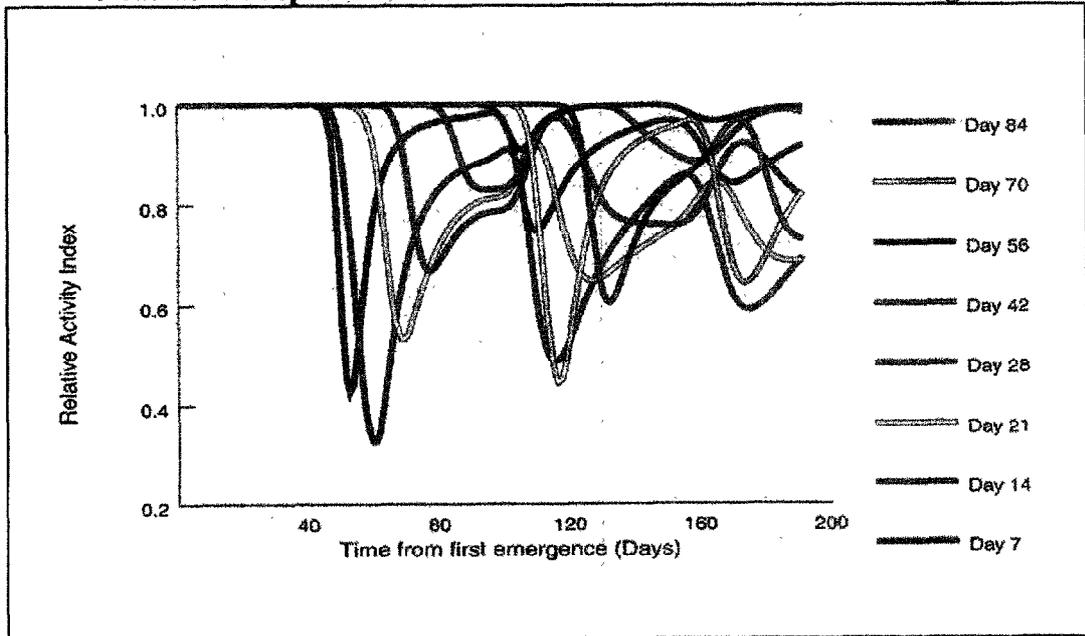


Figure 11. Response of fast-developing species of dung beetle to a single treatment of eprinomectin at different times after beetle emergence





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8.1.6 Differential Activity of Moxidectin Versus Avermectins Against Insects

Early in the development of moxidectin for commercial use in livestock, there was interest in exploring the mechanisms behind the observed differential activity against certain insects. In order to determine whether this is a function of the molecules themselves, or if it relates to formulation differences, or to effects of metabolism or pharmacokinetics in the animal, two *in vitro* binding studies were conducted to determine whether differences existed in binding at the γ -amino-butyric acid (GABA)-gated chloride channel receptor sites.

8.1.6.1 Cole and Casida (1992) described an assay using the tritiated 4'-ethynyl-4-*n*-[2,3-³H₂]-propylbicyclo-orthobenzoate [³H]EBOB in vertebrate brain and insect head to study several insecticides including macrocyclic lactones. A number of insecticides inhibit GABA mediated chloride conductance by binding to the non competitive blocker site(s) of the GABA-gated chloride channel. This site has been characterized primarily by the binding parameters of radiolabelled ligands. These radioligands measure a site relevant to the toxicity of many classes of insecticides and convulsants in mammals, i.e., potency in the radioligand binding assay with brain receptor is a good predictor of mammalian toxicity. However, several of the radioligands were of low insecticidal activity, and did not adequately measure the toxicologically relevant sites in insect membrane preparations. An understanding of the selective toxicity mechanisms for insecticides acting at the chloride channel requires comparative studies with a radioligand of high specific binding and affinity in mammals, other vertebrates and insects. Studies were conducted to evaluate candidate sites and revealed that the 4'-ethynyl and 4-*n*-propyl substituents confer exceptionally high potency as toxicants for mice and house flies and as an inhibitor for the mammalian brain receptor. The [³H]EBOB met expectations in binding with high affinity to the toxicologically relevant site in house fly head for seven different classes of insecticides. The findings in the subsequent binding studies help in understanding species specificity among target and non-target organisms for insecticide action.

For the insect studies, house flies (*Musca domestica* L.) and fruit flies (*Drosophila melanogaster* Meigen) were from cultures maintained in the Departments of Entomological Sciences and Molecular and Cell Biology of the University of California at Berkeley, respectively. The flies were anaesthetized with carbon dioxide, then frozen using dry ice to allow the heads to be harvested. Insect head membranes were prepared and re-suspended in 10nM phosphate buffer, pH 7.5, containing 300 nM NaCl (assay buffer). Incubation mixtures consisted of 750 pM [³H]EBOB final concentration, in assay buffer (0.5 mL) and candidate inhibitors. Specific binding was considered to be the difference between total ³H bound with 750 pM [³H]EBOB and nonspecific ³H bound on addition of 5 μ M unlabeled EBOB. Saturation experiments used increasing



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concentrations of [³H]EBOB alone or with 5 uM unlabeled EBOB for non specific binding. Each experiment was repeated at least three times to evaluate the variability of the tissues and technique.

As summarized in Table 23 below, the results with the macrocyclic lactones, avermectin B1a, and moxidectin are abstracted from the table 2 of the original publication. Considerable specificity was evident between vertebrate and insect preparations in their sensitivity to macrocyclic lactones. The insect preparations were 615 to > 714 fold more sensitive than the vertebrate preparations. Differences were found in the concentrations of active ingredient needed for binding, with avermectin B1a consistently binding at lower concentrations than those required for moxidectin. Avermectin and its analogues (channel openers) probably act in the GABA-gated chloride channel of house fly head at a site closely coupled to that of EBOB (a channel blocker). It appears that receptor site specificity may contribute to species selectivity.

Table 23. [³H]EBOB Binding Site in Vertebrate Brain and Insect Head
IC₅₀ in nM (mean±SE, n=3-5)

Inhibitor	Human	Dog	Mouse	Chicken	House Fly	Fruit Fly
Avermectin B1a	1741±1	230±37	1177±23	1770±322	2±0.3	2±0.3
Moxidectin	>10000	>10000	>10000	>10000	16±4	12±2

8.1.6.2 A second study was conducted by Deng and Casida (1992) to further assess the specific binding of moxidectin, avermectin B1a and a number of its analogues to house fly GABA-gated chloride channel.

[5-³H]avermectin B1a ([³H]AVM) binds in house fly membranes to a single saturable high affinity site. This radioligand is not displaced by GABA and several noncompetitive blockers of the GABA-gated chloride channel. The mode of action of avermectin B1a and its analogues was explored using radioligand binding studies with [³H]AVM and the closely related [³H]ivermectin ([³H]IVM).

Moxidectin, avermectin and 10 avermectin analogues were used in this study. House fly heads or thoraces/abdomens were collected by freezing the adults at dry ice temperature, shaking to separate the body parts, and sieving. The heads or thoraces/abdomens were homogenized in 250 mM sucrose and 10 mM Tris HCl buffer, pH 7.5, the homogenate was centrifuged at 500 g for 5 min and then the supernatant thereof at 130,000 g for 60 minutes to prepare the head or thorax/abdomen membranes. The [³H]AVM binding studies were conducted, and results obtained by liquid scintillation counting. Non specific binding was determined with 5 uM unlabeled AVM. IC₅₀ values for [³H]AVM displacement were determined by conducting the assays with a constant level of [³H]AVM (400 pM and



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varying concentrations of candidate inhibitors. The [³H]EBOB assays were carried out with head membranes. They differed from the [³H]AVM assays only in the radioligand concentration (780 pM for the [³H]EBOB versus 400 pM for the [³H]AVM) and the deletion of ethanol from the rinse solution for [³H]EBOB assays. All binding assays were repeated two or three times to verify the reproducibility of the results. Toxicity assays were conducted to determine if there was a correlation between the results of the binding studies, and actual insect toxicity. Adult female flies (SCR strain, ~20 mg each, 3-7 days after emergence were treated topically on the abdomen with piperonyl butoxide (PBO) (0 or 5 ug) in acetone followed 1 hour later by the AVM analogue, or moxidectin, applied in the same manner. Mortality determinations were done at 24 hours (with PBO) or 48 hours (no synergist). The IC₅₀ values were reproducible within 1.5 fold in repeated experiments. Results obtained are summarized in the table below.

As summarized in Table 24 below, the potencies of the lipophilic macrocyclic lactone compounds as inhibitors of [³H]AVM binding were good predictors of their insecticidal activities. This observation established the toxicological relevance of the binding assay. Both avermectin and moxidectin were toxic to houseflies potentiated with PBO, showing LD₅₀ of 0.01 and 0.02 ug/g respectively. The potentiation of moxidectin by PBO was > 100 fold, and it was proposed that this high level of synergy relative to the avermectins may be associated with its methoxime moiety. It is notable that the avermectin B_{1a} aglycone, and monosaccharide analogues showed much lesser activity than the parent compound. This indicates that the sugar chain is of importance in the insecticidal activity of the avermectins. The activity of moxidectin without PBO potentiation was considerably less than that of AVM B_{1a} and AVM B_{2a}.

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Table 24. Structure Activity Relationships of 12 Avermectin (AVM) Analogs for Topical Toxicity to Adult House Flies and Inhibition of [³H]AVM and [³H]EBOB Binding to House Fly Head Membranes

No.	AVM Analog ^a	24 hr with PB LD ₅₀ (µg/g)	48 hr no synergist LD ₅₀ (µg/g)	[³ H]AVM IC ₅₀ ^b (nM)	[³ H]EBOB EC ₅₀ ^{b,c} (nM)
AVM	AVM B _{1a}	0.013	0.1	27	1.7
1	Moxidectin	0.020	1.6	40	3.5
2	4''-Deoxy-4''-epi-(methylamino)-AVM	0.068	0.43	26	4.0
3	AVM B _{2a}	0.073	0.15	42	3.4
4	10,11,22,23-Tetrahydro-AVM B _{1a}	0.37	0.50	285	16
5	13-Deoxy-22,23-dihydro-AVM B _{1a} aglycone	0.58	3.4	315	84
6	22,23-Dihydro-AVM B _{1a} monosaccharide	0.62	10	900	133
7	AVM B _{1a} monosaccharide	1.0	>100	240	56
8	10-Hydroxy-10,11-dihydro-AVM B _{1a}	2.9	50	780	136
9	22,23-Dihydro-AVM B _{1a} aglycone	12	>100	1025	336
10	AVM B _{1a} aglycone	45	>100	395	195
11	22,23-Dihydro-AVM B _{1a} 4''-O-phosphate Na ⁺ salt	^d	^d	190	16

^aFor chemical structures see Asato and France (1988) for 1 and Fisher (1990) for the remaining compounds. Other designations are CL 301,423 for 1 and MK-243 for 2.

^bStandard error values (based on two or three experiments) averaged 24% of the mean for the IC₅₀s (n=12) and 19% of the mean for the EC₅₀s (n=12).

^cThe maximum inhibition attained was 72-80% for AVM, 1, 2, 4, and 7, 57-65% for 3, 5, and 8-10, and 45-50% for 6 and 11.

^dNot tested topically due to insolubility to acetone. Injected LD₅₀ 0.39 µg/g both with PB and with no synergist.

This study has established that AVM and moxidectin are potent non competitive inhibitors of EBOB binding and their actions at this site are associated with their insecticidal activity. This is the first validated assay of this type with insects.

8.1.7 Evaluation of Moxidectin and Non-target Dung Insects

The various studies undertaken demonstrate that moxidectin residues in dung of treated animals occur at levels below those which are toxic to indicator beetle and fly species which dwell or breed in dung. Two *in vitro* bioassays determined NOEL and toxic level for the dung beetles *Onthophagus gazella* and *Euoniticellus intermedius*, and for the dung breeding fly *Haematobia irritans exigua*. The NOEL was greatly in excess of the expected maximum excreted level of moxidectin in dung of cattle treated by subcutaneous injection with 0.2 mg/kg of moxidectin, which is the recommended label dosage. Levels of moxidectin 64 fold higher than abamectin were needed to elicit a comparable toxic effect.



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Further studies were conducted, using beetle and fly bioassays on dung from animals treated with commercial formulations of moxidectin, at the recommended commercial dose rate. These studies also included one or more of the avermectins, not only for outright comparisons, but also to demonstrate that the assays used were sensitive enough to detect adverse effects of macrocyclic lactones, if they were present. In all cases the assays were valid, in that toxic effects were evident with the avermectins, and these were consistent with previously published literature. The effects of moxidectin residues generally were not significantly different from controls, although in a number of studies, a slight improvement in viability of dung dwelling insects was observed. The studies covered indicator species of the three main families of dung beetles, the Aphodiidae, Scarabaeidae and Geotrupidae. The studies also assessed a range of dung dwelling flies, including three species of *Musca inferior*, *Neomyia cornicina*, *Stomoxys calcitrans*, *Haematobia irritans exigua* and *Orthelia timorensis*. *Haematobia irritans* was determined to be the most sensitive to effects of macrocycle lactones. Bioassays were not conducted on the parasitic beetles such as Staphylinidae or Hydrophilidae, since unlike with avermectins, there was no detectable effect on the prey of these species.

Assessment of potential effects of moxidectin was not limited to juvenile and adult lethal effects, but also included measurement of sub-lethal effects which may affect the reproductive performance of beetles into a subsequent generation. The beetles exposed to dung from moxidectin treated cattle were indistinguishable from controls.

Field evaluations of dung colonization and degradation were conducted in two different climatic regions, the northern temperate zone, and a warm Mediterranean zone, as was a colonization study in a wet tropical zone in order to confirm that, as expected from the above results, there was no effect on either colonization or dung degradation in any type of climate. As expected, the colonization and degradation rates for dung from moxidectin treated animals was indistinguishable from controls.

A further evaluation was done, using a computer simulation model, to predict any potential disruption to dung beetle populations. The model was run to assess not only differing effects based on beetle breeding cycle (univoltine versus multivoltine species) but also the potential impact of the timing of treatment of cattle in relation to median emergence time of beetle species. The computer prediction based on multiple simulations is that effects of moxidectin dung are indistinguishable from untreated controls.

The differential activity observed between moxidectin and the avermectins is consistent with expectations, based on *in vitro* molecular binding studies which show that avermectins bind to insect brain and thorax/abdoment receptors at lower concentrations than does moxidectin.



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The evaluation of moxidectin residue potential effects has been conducted in keeping with published recommendations for the assessment of disruptions to soil ecosystems, and indicates that no adverse effects are expected on insects which dwell in, breed in or feed on dung and therefore there is no disruption to colonization numerically or in diversity. The colonization and degradation of such dung is not distinguishable from control dung.

8.2 Toxicity of Moxidectin to Terrestrial Organisms

Low levels of moxidectin may migrate from dung pats of treated cattle to soil in the field. The toxicity of moxidectin to plants and earthworms is discussed in this section.

- 8.2.1 Study PD-M 28-24: Moxidectin was applied to 12 different plants, at a rate of 4 kg active ingredient/hectare, either through the soil (pre-emergence) or directly onto the plants (post-emergence). This application rate is many orders of magnitude greater than the levels of moxidectin which could be expected in fields from treating cattle at pasture. Tested plants in this study included *Abutilon theophrasti* (velvetleaf), *Ambrosia artemisiifolia* (common ragweed), *Avena fatua* (wild oats), *Brassica kaber* (wild mustard), *Calystegia arvensis* (hedge bindweed), *Cyperus rotundus* (purple nutsedge), *Digitaria sanguinalis* (large crabgrass), *Echinochloa crus-galli* (barnyardgrass), *Elytrigia repens* (quackgrass), *Ipomoea* sp. (morningglory), *Setaria viridis* (green foxtail), and *Sida spinosa* (prickly sida). The absence of any visible effect of moxidectin on the ability of the plants to germinate or damage leaves of growing plants indicated that moxidectin caused no impact on plants when manure from treated animals is applied to field or pastures.
- 8.2.2 Study ECO 91-118: A subacute toxicity test was conducted on earthworms using a mixture of ¹⁴C-labeled and non-labeled moxidectin in accordance with FDA Guideline No. 4.12 "Earthworm, Subacute Toxicity Test". The toxicity of moxidectin to earthworms (*Eisenia foetida*) was evaluated in a 28-day test in a mixture of manure and artificial soil. After a range finding test, eight concentrations of moxidectin were used ranging from 1 to 1280 mg/kg (nominal). Samples were prepared by mixing a solution of moxidectin in acetone (11.8 mL) with cow manure (50 g) and deionized water (27.3 mL). The manure slurry was held in a fume hood overnight to evaporate the solvent. The slurry was then mixed with artificial soil (1000 g, dry weight) using a mechanical mixer. Samples were held in 2 L covered glass beakers with 10 earthworms per test container. Samples were kept at 20±2°C, with four replicates per concentration. Observations of mortality were taken at 7, 14, 21 and 28 days after application. Results showed that the Lethal Concentration 50 (LC₅₀) was 37.2 ppm and the NOEC was 1 ppm. Behavioral and morphological changes were generally observed in earthworms at concentrations above 1 ppm, which also corresponded to the no effect level, as determined by observations of



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weight gain. These concentrations are much higher than the levels of moxidectin which could be expected in the soil.

- 8.2.3 Summary: When compared with the maximum moxidectin PEC of 0.526 ppb in soil under the “worst-case” situation, findings from the above toxicity studies (see the summarized Table 25 below) suggest that moxidectin residues in soil are highly unlikely to cause any adverse effects on plants and earthworms when the product is used according to the product label.

Table 25. Toxicity of Moxidectin on Plants and Earthworms

Test Species	Test Parameter	Results
Plant	4 kg/ha	No effects
Earthworm	28-day subacute LC ₅₀	37.2 ppm
	NOEC	1 ppm

8.3 Toxicity of Moxidectin to Avian Species

Birds may ingest the components of cattle dung pats, dung insects, plants and/or earthworms which may be contaminated with moxidectin. Therefore, the toxicity of moxidectin to various avian species was investigated.

- 8.3.1 Study 90-QD-156: The acute toxicity of moxidectin, when administered as a single oral dose, was determined for the bobwhite quail. The test was conducted in accordance with US EPA protocol FIFRA Guideline No. 71-1. Moxidectin was administered to 24-week old bobwhite quail (*Colinus virginianus*) at the dose range of 0 - 681 mg/kg body weight. Results concluded that the 21-day acute oral median lethal dose (LD₅₀) was 278 mg/kg body weight.
- 8.3.2 Study 90-DD-79: The acute toxicity of moxidectin, when administered as a single oral dose, was determined for the mallard duck. Moxidectin was administered to 33-week old mallard ducks (*Anas platyrhynchos*) at the dose range of 0 - 464 mg/kg body weight. The test was conducted in accordance with US EPA protocol FIFRA Guideline No. 71-1. The calculated 21-day acute oral median lethal dose (LD₅₀) was 365 mg/kg.
- 8.3.3 Study A90-42: The acute toxicity of moxidectin, when administered as a single oral dose, was determined for the chicken. Moxidectin was administered to 2-5 week old Peterson x Arbor Acres chicken at the dose range of 0 - 400 mg/kg body weight. Results concluded that the 14-day oral lethal dose (LD₅₀) was 283 mg/kg body weight
- 8.3.4 Summary: Based on the LD₅₀ levels of moxidectin for these 3 representative avian species as summarized in Table 26 below, birds would have to consume hundreds of kg of soil,



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feces and/or dung insects containing moxidectin in the feeding in order to reach the toxic level. Therefore, moxidectin is highly unlikely to cause any adverse effects on birds when used according to the product label.

Table 26. Toxicity of Moxidectin on Avian Species

Test Species	Test Parameter	Results
Bobwhite Quail	21 day acute oral LD ₅₀	278 mg/kg bw
Mallard Duck	21 day acute oral LD ₅₀	365 mg/kg bw
Chicken	14 day acute oral LD ₅₀	283 mg/kg bw

8.4 Toxicity of Moxidectin to Aquatic Species

While moxidectin binds tightly to soil, it is theoretically possible that moxidectin can be washed off into ponds or waterways from the feces of treated cattle or from soil contaminated with moxidectin. Therefore, the potential toxicity of moxidectin to living aquatic organisms is discussed in this section.

8.4.1 ECO 954-92-101: The effects of moxidectin on the growth of green algae (*Selenastrum capricornutum*) were studied over three days in accordance with OECD Guideline G 201. The effects were studied using a control, a solvent control and solution of moxidectin at nominal concentrations of 9.38, 18.8, 37.5, 75.0 and 150 µg ai/L in a synthetic algal assay nutrient medium. The mean measured concentration of moxidectin were 5.1, 10.0, 17.6, 39.5 and 86.9 µg ai/L, respectively. The highest concentration studied corresponding to the maximum solubility level in the test medium. The test was conducted under static, non-renewal conditions at 24±2°C with continuous illumination (4306 lux). Test vessels (500 mL Erlenmeyer flasks with 100 mL of test solution) were continually shaken. The effects on growth were evaluated by comparing the area under the growth curves in the treated solutions with the control groups. A statistical difference was noted between the blank and solvent controls. Therefore, the percentage growth inhibition was calculated against the growth in the solvent control. The 72-hour EC₅₀, based on measured concentrations was > 87 ppb which was the highest concentration tested. The NOEC of moxidectin to the green algae was not determined due to its limit of solubility in the test conditions. During the study there were significant decreases in the concentration of moxidectin from the treatment solutions. This decrease is consistent with the finding that moxidectin is rapidly photodegraded.

8.4.2 ECO 971-90-151 (Toxikon J9008029b): The toxicity of moxidectin to the water flea (*Daphnia magna*) was determined using a flow-through test over 48 hours of exposure. The test was conducted in accordance with US EPA guidelines, and used a control, a solvent control and solutions of moxidectin at nominal concentrations of 6.5, 11, 18, 30 and 50 ng ai/L. For each concentration studied, 20 daphnia were used with monitoring



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conducted at 24-hour intervals. The 48-hour LC₅₀ was 30.2 ppt (ng/L), while the NOEC was 11 ppt (ng/L).

- 8.4.3 ECO 971-90-149 (Toxikon J9008029c): The toxicity of moxidectin to the bluegill (*Lepomis macrochirus*) was determined using a flow-through test over 96 hours of exposure. The test was conducted in accordance with US EPA guidelines, and used a control, a solvent control and solutions of moxidectin at nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 µg ai/L. The mean measured concentrations of moxidectin were 0.52, 0.71, 1.1, 2.0 and 3.2 µg ai/L, respectively. For each concentration studied, 20 fish were used with monitoring conducted at 24-hour intervals. The 96-hour LC₅₀, based on measured concentrations was 0.62 ppb (µg/L), while the NOEC was < 0.52 ppb, the lowest concentration tested.
- 8.4.4 ECO 971-90-150 (Toxikon J9008029d): The toxicity of moxidectin to the rainbow trout (*Oncorhynchus mykiss*) was determined using a flow-through test over 96 hours of exposure. The test was conducted in accordance with US EPA guidelines, and used a control, a solvent control and solutions of moxidectin at nominal concentrations of 0.26, 0.43, 0.72, 1.2 and 2.0 µg ai/L. The mean measured concentrations of moxidectin were 0.15, 0.22, 0.43, 0.71 and 1.2 µg ai/L, respectively. For each concentration studied, 20 fish were used with monitoring conducted at 24-hour intervals. The 96-hour LC₅₀, based on measured concentrations was 0.16 ppb (µg/L), while the NOEC was < 0.15 ppb, the lowest concentration tested.
- 8.4.5 Summary: The toxicity of moxidectin to various aquatic species are summarized in the Table 27 below. Moxidectin is toxic to many aquatic species, with the water flea (*Daphnia magna*) being the most sensitive species with its 48-hour EC₅₀ of 30 ppt and NOEC of 11 ppt. The concentrations at which toxicity is observed in these tests would be regarded as the "worst-case" values because several factors, such as binding to sediment and suspended particulate matter, and photo-degradation, which greatly reduces moxidectin exposure in field conditions, were not factored into these studies. Even under this worst case scenario and based on the US EPA Feedlot and Run-off Model, the maximum moxidectin PEC in water is 5.9 ppt (without adsorption) and 0.263 ppt (with adsorption), which is lower than all LC₅₀ values for these species including the water flea, the most sensitive aquatic species.



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Table 27. Toxicity of Moxidectin on Aquatic Species

Test Species	Test Parameter	Results
Green Algae	72-hour EC ₅₀	> 87 ppb
Water Flea	48-hour EC ₅₀	30 ppt
	NOEC	11 ppt
Bluegill	96-hour LC ₅₀	0.62 ppb
	NOEC	< 0.52 ppb
Rainbow Trout	96-hour LC ₅₀	0.16 ppb
	NOEC	< 0.15 ppb

The moxidectin PEC of 5.9 ppt (without adsorption) and 0.263 ppt (with adsorption) in water was estimated under the worst case scenario in which the metabolism and degradation of moxidectin are not factored into the calculation. In reality, the concentration of moxidectin would be much less than these calculated estimates. Therefore, it is extremely unlikely that there would be any significant toxic impact on aquatic living organisms in aquatic ecosystems as a result of using the moxidectin injectable solution according to the product label.

8.5 Environmental Assessment Summary

The studies described in this Environmental Assessment (EA) indicate the use of CYDECTIN (moxidectin) Injectable for Cattle will not have harmful effects on the environment. The use of cattle endectocide products in the U.S. is not growing and is projected to remain stable in the future. As such, CYDECTIN Injectable will simply be administered instead of the other cattle endectocide products currently approved for use in the U.S., many of which have been shown to have potentially greater deleterious effects on the environment. CYDECTIN Injectable, like the other endectocide therapeutics, will generally be used only once per year. Cattle managers will routinely choose to use other classes of internal and external parasiticides if additional treatment is required over the course of the year.

Following the administration of CYDECTIN by injection, peak residues of moxidectin and metabolites in the animal's excreta are reached in 2 days and concentrations are below the level of detection in 37 days. Moxidectin is unlikely to move through the environment because it has a low water solubility and tightly binds to soil, sediment and organic matter. In addition, moxidectin is readily degraded in the environment by photodegradation and aerobic breakdown by soil organisms.

The potential toxicity of moxidectin was evaluated against 12 different plant species, earthworms, 3 avian species, and 4 aquatic species. Using the "worst case" approach to



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calculate the Predicted Environmental Concentration (PEC) in manure, soil and water, no detrimental effects would be expected towards plants, earthworms, avian species or the aquatic ecosystem. Moxidectin was tested, at levels expected to be found in the excreta, against a diverse array of dung insects (beetles and flies) and was determined to be essentially non-toxic. As such, the rate of dung degradation and manure accumulation on pastures will not be impacted by the use of CYDECTIN (moxidectin) Injectable for Cattle.

8.5.1 The summary of the environmental chemistry and environmental fate of moxidectin and environmental toxicity of moxidectin are tabulated in Table 28.

Table 28. Tabulated Summary of the Environmental Studies on Moxidectin
(A) Physical and Chemical Properties of Moxidectin

Melting Point	145-154°C
Vapor Pressure	< 3.2 x 10 ⁻⁸ torr.
Ultraviolet- Visible Absorption Spectra	Peak at 245 nm; slight absorption above 300 nm.
n-Octanol/Water Partition Coefficient	Kow = 58,300; (Log Kow = 4.766)
Solubility in Water	0.51 mg/L
Solubility in Organic Solvents	(mL of solvent/g moxidectin)
Dichloromethane	1.64
Diethyl Ether	1.19
Ethanol (95%)	0.81
Acetonitrile	0.62
Ethyl Acetate	0.47
Formic acid	decomposed

(B) Environmental Fate of Moxidectin

Photolysis Half-life in Water	6.8 hours (late autumn)
Laboratory Soil Biodegradation Half-Life	2 months
Soil Adsorption/Desorption Coefficient (Koc),	18,000 - 41,000
Mobility Class by Soil Thin-Layer Chromatography	1 (immobile)

(C) Environmental Toxicology of Moxidectin

Aquatic Species:		
Bluegill	96-hour LC ₅₀	0.62 ppb
	NOEC	<0.52 ppb
Rainbow Trout	96-hour LC ₅₀	0.16 ppb
	NOEC	<0.15 ppb
Water Flea	48-hour EC ₅₀	30 ppt
	NOEC	11 ppt
Green Algae	72-hour EC ₅₀	>87 ppb
Avian Species:		
Bobwhite Quail	21 day acute oral LD ₅₀	278 mg/kg bw
Mallard Duck	21 day acute oral LD ₅₀	365 mg/kg bw



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Chicken	14 day acute oral LD ₅₀	283 mg/kg bw
<i>Terrestrial Species:</i>		
Plant Phytotoxicity	Inactive at:	4 kg/ha
Earthworm	28-day subacute LC ₅₀	37.2 mg/kg medium
Dung Beetles:		
<i>O. gazella</i> - adult	NOEC	> 500 ppb
<i>O. gazella</i> - progeny	EC ₅₀	2567.7 ppb
<i>E. intermedius</i> - adult	NOEC	> 500 ppb
<i>E. intermedius</i> - progeny	EC ₅₀	469.3 ppb
	NOEC	> 269 ppb

9. Use of Resources and Energy:

The manufacture and disposal of moxidectin and the formulated nonaqueous injectable solution will not require any unusual amounts of resources or energy.

10. Mitigation Measures:

No adverse impact on the environment is expected from the proposed action; therefore, no mitigation measures are required. CYDECTIN (moxidectin) Injectable Solution for Cattle will be packaged in 50 mL, 200 mL and 500 mL polyethylene bottles. Consistent with the container disposal pattern for similar animal drug products, individuals purchasing and administering this product will be instructed to dispose of empty bottles and any residual content in an approved landfill or by incineration. Instructions for proper handling and container disposal are clearly stated in the “Environmental Safety” and “Disposal” sections of label of the CYDECTIN (moxidectin) Injectable Solution for Cattle.

Environmental Safety

Studies indicate that when moxidectin comes in contact with the soil it readily and tightly binds to the soil and becomes inactive. Free moxidectin may adversely affect fish and certain aquatic organisms. Do not contaminate water by direct application or by improper disposal of drug containers.

Disposal

Dispose of containers in an approved landfill or by incineration.

11. Alternatives to Proposed Action:

No potential adverse environmental impacts have been identified for this proposed action. As a result, no alternative actions are necessary.

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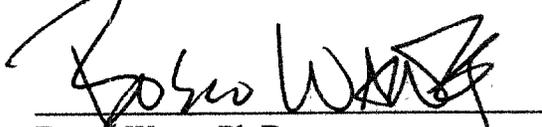
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Twenty years experience in basic research and five years experience in regulatory affairs.

13. Certification:

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



Bosco Wang, Ph.D.

Senior Product Registrations Manager

Animal Health Research Regulatory Affairs

14 June 2001

Date

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15. Appendices:

15.1 Material Safety Data Sheet

The Material Safety Data Sheet (MSDS) for Cydectin 1% Nonaqueous Injectable dated May 10, 1999 is provided in the following four pages.

MATERIAL SAFETY DATA SHEET


FORT DODGE

Cydectin 1% Nonaqueous Injectable

Fort Dodge Animal Health
800 5th Street NW
PO Box 518
Fort Dodge, IA 50501

Emergency Telephone No.: (515) 955-6033
General Information No.: (515) 955-4600
Preparation Date: 12 December, 1996
Revision Date: 10 May, 1999

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 **PRODUCT NAME:** Cydectin 1% Nonaqueous Injectable

1.2 **USE:** Used as an anthelmintic for cattle only

1.3 **SIZE:** 50, 200, and 500 mL vials

1.4 **SYNONYMS/TRADE NAMES:** Moxidectin

2. COMPOSITION / INFORMATION ON INGREDIENTS (chemical, active ingredient)

INGREDIENT NAME	SYNONYMS	CAS NO.	CONC	OSHA PEL/STEL	ACGIH PEL/STEL	OTHER PEL/STEL
*Moxidectin	NA	113507-0605	1.00%	Not Established	Not established	0.05 mg/m ³ (ACY)
Ethanol	Ethyl Alcohol	64-17-5	20.0%	1,000 ppm	1,000 ppm	NA
Inert ingredients, including propylene glycol and other non-hazardous compounds.	NA	NA	Balance	Not Established	Not Established	Not Established

EMERGENCY OVERVIEW

3.1 **POTENTIAL HEALTH EFFECTS:** May be harmful if ingested, producing gastrointestinal distress and possible central nervous system disorders. Treatment of overexposure should be directed at the control of symptoms and the clinical condition. This product may be toxic to young children through ingestion, particularly those less than 1 year in age, producing possible central nervous system effects.

3.1.1 ACUTE EFFECTS:

INHALATION: Inhalation is considered an unlikely route of exposure unless bulk quantities of the product are being handled. If inhalation of vapors occurs and causes respiratory distress, remove victim to fresh air and seek medical attention immediately.

INGESTION: Product may be harmful through ingestion. Seek medical attention immediately.

SKIN: Product is considered to be relatively non-irritating to the skin. Wash thoroughly with soap and water. If irritation persists, seek medical attention.

EYE: May be irritating to the eyes. Rinse thoroughly with water for 15 minutes and seek medical attention immediately.

3.1.2 **TARGET ORGAN EFFECTS (SUBCHRONIC/CHRONIC):** Gastrointestinal system, Central nervous system. No chronic effects are associated with exposure to this product.

3.1.3 **CARCINOGENIC EFFECTS:** This product is not considered to be carcinogenic.

3.1.4 **REPRODUCTIVE/TERATOGENIC EFFECTS:** There are no reproductive or teratogenic effects associated with exposure to this product. However, ingestion of sufficient quantities may present a risk of teratogenic effects due to the ethanol content.

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3.2 CARCINOGENICITY STATUS: This product is not listed as a carcinogen or suspected carcinogen by OSHA, IARC, or other organizations.

3.3 MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE: None known.

4. FIRST AID MEASURES

INHALATION: Remove victim to fresh air. Seek medical attention immediately.

INGESTION: Seek medical attention immediately. See Section 3.1 for ingestion concerns.

SKIN: Wash affected area thoroughly with soap and water. If irritation persists seek medical attention.

EYE: Rinse eyes thoroughly with water for 15 minutes, holding eyelids open with fingers. Seek medical attention immediately.

5. FIRE FIGHTING MEASURES

5.1 FLASH POINT: 88° F **METHOD:** Closed Cup

5.2 AUTOIGNITION TEMPERATURE: Not established.

5.3 FLAMMABILITY LIMITS: LOWER LIMIT: Not established. UPPER LIMIT: Not established.

5.4 UNUSUAL FIRE AND EXPLOSION HAZARDS: Fires involving this product may burn vigorously, and may release toxic vapors and smoke.

5.5 COMMON EXTINGUISHING METHODS: Water, foam, dry chemical, or carbon dioxide extinguishers.

5.6 FIRE FIGHTING PROCEDURES: Wear full protective gear, including SCBA. Use as little water as possible, and dike area to prevent runoff. If water enters a drainage system, advise authorities downstream. Use spray or fog – solid stream may cause spreading. Conduct fire fighting and rescue operations from upwind of the fire area. Evacuate people downwind who may come in contact with smoke, fumes, or contaminated surfaces. Do not decontaminate personnel or equipment or handle broken packages or containers without protective equipment described in Section 8, Exposure Controls. Decontaminate emergency personnel with soap and water before leaving the fire area.

6. ACCIDENTAL RELEASE MEASURES

Wear appropriate protective gear as described in Section 8, Exposure Controls. For small spills, such as those that would be associated with normal use of this product, do not wash spilled liquid to a drain. Absorb with paper towels, granular clay, or other suitable absorbent and place into a container for future disposal. Wash affected areas with soap and water. For large spills, contain the spill immediately using dikes, spill booms, or other appropriate containment devices. Absorb the spill with a suitable absorbent, and place into containers for future disposal. Do not allow the spill to enter rivers, lakes, streams, or sewer systems.

7. HANDLING AND STORAGE

Store product in a secure, dry, cool, well-ventilated area. Do not contaminate water, food, or feed by storage or disposal. Keep out of the reach of children. Not for use or storage in or around the home. Keep away from sources of ignition and protect from exposure to fire and heat. Use in accordance with label directions.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 VENTILATION: For normal handling of this product as described on the label, special ventilation provisions are not considered necessary. In situations where bulk quantities of the product are being handled, local exhaust ventilation is recommended.

8.2 RESPIRATORY PROTECTION: None recommended for use as directed on the product label. If sufficient ventilation cannot be provided to maintain exposures within acceptable limits (such as for handling bulk quantities of the product), use a NIOSH/MSHA approved full face or half mask respirator fitted with organic vapor cartridges (ethanol vapors).

8.3 PROTECTIVE GLOVES: Nitrile or other gloves impervious to organic solvents are recommended.

8.4 EYE PROTECTION: A face shield worn in conjunction with safety glasses is recommended if splashing of the product is likely. If the product is being handled in such a way that significant splashing is not likely, safety glasses are recommended.

8.5 OTHER: Protective clothing, such as Tyvek coveralls, is recommended if the product could spill or splash onto the skin.

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9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 APPEARANCE AND ODOR: Clear, pale yellow solution with a characteristic alcohol odor.

9.2 MELTING POINT: -117° C

9.3 BOILING POINT: 97° C

9.4 SPECIFIC GRAVITY / DENSITY: 0.99 g/mL

9.5 VAPOR DENSITY: 1.59

9.6 VAPOR PRESSURE: 19.8 mm @20° C

9.7 SOLUBILITY

- WATER: Completely soluble
- OTHER SOLVENTS: Soluble in most organic solvents.

9.8 DECOMPOSITION TEMPERATURE: Not Available

9.9 VISCOSITY: Not Available.

9.10 pH: Not Applicable

10. STABILITY AND REACTIVITY

10.1 STABILITY: This product is considered to be stable under normal conditions.

10.2 HAZARDOUS DECOMPOSITION PRODUCTS: This product may release toxic vapors when subjected to fire conditions.

10.3 CONDITIONS TO AVOID: Avoid high heat, flame, and other sources of ignition.

10.4 MATERIALS AND SUBSTANCES TO AVOID (INCOMPATIBILITY): Strong acids or bases.

11. TOXICOLOGICAL INFORMATION

11.1 ACUTE DATA: This product may be harmful through ingestion (see Section 3.1). It is considered to be relatively non-irritating through skin contact, and may be irritating in contact with the eyes.

INHALATION: No specific inhalation toxicity information available. However, inhalation of high concentrations of vapors can be expected to produce symptoms consistent with those associated with inhalation of organic solvent vapors, such as stupor, dizziness, nausea, and light-headedness.

INGESTION: Oral LD₅₀ (Rat) >3,800 mg/kg

EYES: May be irritating to the eyes.

SKIN: Dermal LD₅₀ (Rabbit) >2,000 mg/kg Considered to be relatively non-irritating to the skin.

11.2 TARGET ORGAN EFFECTS DATA (SUBCHRONIC/CHRONIC): Gastrointestinal system, central nervous system. No adverse chronic health effects are associated with exposure to this product.

11.3 CARCINOGENIC EFFECTS DATA: This product is not considered to be carcinogenic.

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11.4 MUTAGENIC EFFECTS DATA: There are no mutagenic effects associated with exposure to this product.

11.5 REPRODUCTIVE / TERATOGENIC EFFECTS DATA: There are no adverse reproductive or teratogenic effects associated with exposure to this product. However, ingestion of large quantities of the product may present a risk of teratogenic effects due to the ethanol content.

12. ECOLOGICAL INFORMATION

12.1 ECOTOXICOLOGICAL INFORMATION: This product is considered to be highly toxic to fish and other aquatic life.

12.2 CHEMICAL FATE INFORMATION: Not available.

13. DISPOSAL CONSIDERATIONS

Persons seeking to dispose of this product should contact a commercial hazardous waste disposal firm for specific waste disposal procedures. Incineration in a licensed and approved hazardous waste incinerator is recommended.

14. TRANSPORT INFORMATION

14.1 U.S. DEPARTMENT OF TRANSPORTATION (DOT): Classified as a flammable liquid per U.S. DOT regulations. Consult U.S. Department of Transportation regulations (49 CFR) for exemptions that may apply when shipping product in consumer packaging (consumer commodity).

14.2 INTERNATIONAL TRANSPORTATION REGULATIONS: Classified as a flammable liquid per International Transport regulations. Consult IATA Dangerous Goods Regulations for exemptions that may apply when shipping product in consumer packaging.

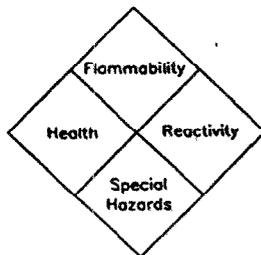
15. REGULATORY INFORMATION

15.1 FEDERAL REGULATIONS: None applicable.

15.2 STATE REGULATIONS: None applicable.

16. OTHER INFORMATION

16.1 HAZARD RATINGS



NFPA*:
 Health - 1
 Flammability - 3
 Reactivity - 0
 Special Hazards - 0

HMIS:	
Health	
Flammability	
Reactivity	
Personal Protection	

Health - 1
 Flammability - 3
 Reactivity - 0
 Personal Protection - See Section 8

* A hazard rating has not been developed by NFPA for this product. NFPA-derived rating is based on NFPA hazard evaluation criteria.

16.2 PREPARATION AND REVISION INFORMATION

Fort Dodge Animal Health, Department of Safety

The information and recommendations presented in this MSDS are based on sources believed to be accurate. However, Fort Dodge Laboratories, its Divisions and/or Subsidiaries assumes no liability for the accuracy, completeness, or suitability of this information. It is the product user's responsibility to determine the suitability of the information for their particular purposes.

This product should only be used by, or under the supervision of, a person trained and qualified to administer the product. Please refer to the package insert for indications or contraindications for use, and for dosage information.

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