UPDATED ENVIRONMENTAL ASSESSMENT FOR MERIAL'S AVERMECTIN-BASED PRODUCTS FOR CATTLE

MERIAL LIMITED

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1. INTRODUCTION

The approval of IVOMEC Injection for Cattle and subsequent avermectin-based products for cattle in the U.S. involved a full review of the environmental fate and effects data for these products by the U.S FDA's Center for Veterinary Medicine (CVM). Of the Environmental Assessments (EAs) prepared for Merial's avermectin-based products for cattle, the most appropriate Environmental Assessments to address effects to dung fauna and on dung degradation are IVOMEC Pour-On for Cattle, IVOMEC SR Bolus and IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle. The fate and effects discussions in these EAs are applicable to Merial's other avermectin products for cattle, including IVOMEC Injection and IVOMEC Cattle Paste. The EAs for IVOMEC Pour-On for Cattle, IVOMEC SR Bolus and IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle were prepared in the early to mid-1990s and they included reviews of the relevant literature on the effects of avermectins on dung-fauna and dung-degradation. However, there have been a number of relevant publications since these EAs were prepared. Therefore, this updated environmental assessment summarizes the environmental fate and effects data in the original EAs, reviews the subsequent literature and discusses what impact, if any, the the new literature has on the conclusions reached in the original EAs.

2. OVERVIEW OF PREVIOUS ENVIRONMENTAL ASSESSMENTS

A. IVOMEC® Pour-On for Cattle

The EA for IVOMEC Pour-On for Cattle (22 March 1990) included discussions on the introduction of ivermectin via manufacturing and use of IVOMEC Pour-On for Cattle into the environment. Also discussed were the studies that defined the fate and effects of ivermectin in the environment.

The fate studies included:

- photodegradation,
- fate in a feedlot runoff,
- fate in rain wash-off (correlation of wash-off with the soil Koc, pasture/nearby stream rain wash-off, direct introduction into a pond and direct introduction into a slowly moving stream), and
- the fate in soil and vegetation.

The effects tests included:

- aquatic toxicity (daphnia, fish, other aquatic species and bioconcentration in sunfish),
- avian toxicity,
- dung degradation, and
- dung-dependent insects.

The EA also included a discussion on handler safety. The EA addressed the toxicity of ivermectin to beetles, birds, aquatic invertebrates and man (occupational), the effects of ivermectin on the degradation of cow pats and the concentration of ivermectin in runoff water. The data in the EA indicated that use of IVOMEC Pour-On for Cattle would not lead to ivermectin levels in the environment that would affect aquatic organisms, avians, or vegetation and that ivermectin would not persist nor bioaccumulate. Laboratory studies were supplemented with a field trial where surface and subsurface water from a field containing cattle dosed subcutaneously with ivermectin were chemically and biologically assayed for ivermectin. The degree of retention of IVOMEC Pour-On for Cattle on the backs of cattle was demonstrated using simulated rain. These results allowed for the calculation of concentrations of ivermectin that might occur from cattle standing in a pond or slowly moving stream during rain. The calculated concentrations were then compared to measured effect levels for aquatic organisms. Similarly, the level of ivermectin in soil from the fertilization of fields with cattle manure was compared to the measured effect levels for terrestrial organisms.

Scenarios were also developed to model exposure of avians, including raptors. Ivermectin data were supplemented with data for the related compound, abamectin. In acute studies, the LD_{50} values for the bobwhite quail and mallard duck were greater than 2000 and 85 mg/kg body weight, respectively. The 8-day subacute LC_{50} values were 3102 and 383 mg abamectin/kg feed, respectively, for these same species. At sublethal concentrations, effects lasted only during the on-drug phase of the study. Birds appeared normal by 24 hours following return to basal diet. A definitive 18-week avian reproduction study was

also performed where male and female mallards were exposed to abamectin at levels of 3, 6 and 12 mg/kg diet for approximately 10 weeks prior to egg laying and continuing through the period. No treatment-related mortality or overt signs of toxicity (weight loss or decreased feed consumption, decreased numbers of eggs laid or numbers or hatchlings from live 3-week embryos) were observed.

Up to 1990, there were only a few published papers on any potential effects of ivermectin on dung degradation or on dung-dependent insects. The EA for IVOMEC Pour-On for Cattle summarized the results of the key papers. Wall and Strong (1987) concluded that degradation of dung pats from cattle treated with a sustained-release bolus was prolonged relative to control pats. Conversely, McKeand *et al.* (1988) found that the degradation rates of dung pats from untreated calves and calves treated with IVOMEC Pour-On for Cattle were similar. Schmidt (1983) found that dung from cattle subcutaneously treated with ivermectin degraded at the same rate as dung from untreated controls. Wall and Strong (1987) also noted decreased insect, but not earthworm, populations in artificially formed dung pats from bolus-treated cattle. The pats were prepared from feces collected on days 11 – 16 after administration of a bolus and were placed on pastures in the UK. Insects were counted in pats after 20 to 100 days on pasture. Wall and Strong also measured wet weights of the pats at the time of collection. They concluded that degradation in cattle-free pasture of 2000-g pats, prepared from feces containing ivermectin residues, was prolonged compared to that of pats prepared from control feces. These authors used differences in wet weight of control and experimental (i.e., ivermectin residue-containing) pats with time for an estimate of the difference in rates of pat decomposition, and speculated that ivermectin treatment could lead to an increase in the amount of pasture land fouled by dung. Results from field studies demonstrate that this speculation is not born out in reality. Since the control pats were "largely degraded within 100 days", the practical significance of a relative difference between small numbers is not clear. Additionally, any differences in moisture content between the control and experimental pats could have lead to the observations. When the data were presented using a more conventional plotting method (Strong and Wall, 1994a), it became clear that the originally reported data largely reflected the moisture content of the pats, not decomposition. Other researchers have discounted the importance of diminution of wet pat weights, with respect to pat degradation and environmental impact. Strong and Wall (1988) added ivermectin to manure and found that larval Scarabaeidae were found in dung containing up to 125 mcg/kg of ivermectin, but none in pats containing 250 or 500 mcg/kg. Some Diptera were unaffected by ivermectin in dung up to 500 mcg/kg. Strong and Wall estimated the ivermectin level in feces of bolus-treated cattle to be about 400 mcg/kg, a level affecting larval Scarabaeidae and some but not all Diptera. Schmidt (1983) noted emergence of several Diptera species was reduced from the manure of subcutaneously dosed cattle, but he did not examine Coleoptera species. Strong and Brown (1987) suggested that dung-degrading beetles would not be killed under the conditions of Schmidt's study, because manure from subcutaneously dosed cattle would contain less residues than those found in feces from bolus-treated cattle. The peak levels of ivermectin-related residues observed in feces in a radiolabeled study with the IVOMEC Pour-On for Cattle formulation were 80 mcg/kg between 3 and 7 days post dose. This level is below levels causing effects on larval Scarabaeidae or Diptera in the Strong and

Wall (1988) study and below peak levels following subcutaneous or bolus dosing. The EA therefore concluded that the literature studies published to date indicated that feces from cattle dosed with IVOMEC Pour-On for Cattle would not have a detrimental impact on dung pat degradation or on dung-dependent insects.

The NADA for IVOMEC Pour-On for Cattle was approved on 4 December 1990. The agency determined under 21 CFR 25.24(d)(1)(i) that approval of the NADA 'did not individually or cumulatively have a significant effect on the human environment' and that an environmental impact statement was not required.

Since the NADA for IVOMEC Pour-On for Cattle was approved, additional reports on the levels of ivermectin in the feces of dosed cattle have appeared in the literature. The analytical method used in the IVOMEC Pour-On for Cattle Environmental Assessment was radiochemical detection of total residue after combustion of feces. This method combusts a weighed sample and captures the resulting tritiated water. No extraction methods are used and the recovery of radioactivity is essentially 100%. The recovered radioactivity is compared to that from a control sample, so the method specifically quantifies the total amount of radiolabeled parent compound and metabolites in the sample. The method is sensitive, reproducible and essentially free from interference from endogenous compounds. In contrast, the method used in Herd *et al.,* (1996) and other papers was the HPLC of feces extracts with fluorescent detection of the major, H_2B_{1a} component. This method is subject to variable extraction and recovery efficiencies, subject to the efficiency of the chromatographic separation and subject to potential interference from fluorescence by co-eluting endogenous compounds, the concentration of which can vary from animal to animal.

The table below lists the limits of detection and/or quantification and variability cited in the relevant studies. The variability was not indicated in the published papers except for Payne *et al.* (1995). Herd *et al.*(1996)'s analytical method improves on those of Nessel *et al.* (1989) and Sommer *et al.* (1992), but not on the method of Payne *et al.* (1995) or on the detection limit or robustness afforded using radiochemical methods. Therefore, the use of the radiochemical method in the IVOMEC Pour-On for Cattle Environmental Assessment is the most conservative approach and is equally if not more valid than any of the HPLC methods.

Summary of Analytical Methods Used to Determine Ivermectin in Cattle Feces

Study	Feces Assay Method	LOD, mcg/kg wet wt.	LOQ, mcg/kg wet wt.	Variability (relative std. dev.)
Environmental Assessment	Radio-chemical combustion	0.2	0.2	<5%
Nessel, *et al.* 1989	HPLC-fluorescence	10	-	Not reported
Sommer, *et al.* 1992	HPLC-fluorescence	50	-	Not reported
Payne, *et al.* 1995	HPLC-fluorescence	1	2	5%
Herd, *et al.* 1996	HPLC-fluorescence	-	5	Not reported

Herd *et al.* (1996)'s paper provides additional data on ivermectin residues in plasma and feces. Herd *et al.*'s plasma and feces levels differ from those in the IVOMEC Pour-On for Cattle Environmental Assessment and other papers. However, the magnitude is not inconsistent with those in the Environmental Assessment. Data in the IVOMEC Pour-On for Cattle Environmental Assessment indicated the total drug residue in plasma and feces following treatment of cattle with IVOMEC Pour-On reached a plateau and then slowly decreased (see table below). Data presented by Herd, *et al.* and Sommer *et al.* indicated the H_2B_{1a} levels in feces quickly peaked, then rapidly decreased. A similar behavior was seen in the plasma results in Herd's paper. Plasma data presented recently by Gayrard *et al.* (1999) is similar to the plasma data presented in the Environmental Assessment. The reasons why the feces and plasma profiles observed by Herd, *et al.* and Sommer *et al.* differ from those in the Environmental Assessment and in Gayrard *et al.* may be due to diet, husbandry and/or animal-to-animal variability. Diet is known to significantly alter the absorption and excretion profile of subcutaneously injected ivermectin (Cook *et al.* 1996). The degree of confinement used in each study can also be a factor. There are a number of other parameters that can also affect absorption and excretion of ivermectin following administration of the pour-on formulation and therefore there will always be some variability between studies. The variability in the table below is not of a magnitude to influence the conclusions reached in the IVOMEC Pour-On for Cattle Environmental Assessment.

Peak Concentrations of Ivermectin in Plasma and Feces after Treatment of Cattle with IVOMEC Pour-On for Cattle

	Plasma		Feces (wet)	
Study	Peak, ng/mL	days	Peak, mg/kg	days
Environmental Assessment [a]	6 - 7	2 - 7	0.08	5 - 7
Sommer *et al.* 1992	nd	nd	1.5 [b]	1
Herd, *et al.* 1996	33	2	2.8	2
Gayrard *et al.* 1999	12	3.4	nd	nd

[a] data from Study CA-218, IVOMEC Pour-On for Cattle radioresidue depletion study
[b] not reported, but estimated from residue in dry feces * 0.15 to account for moisture

Herd *et al.* (1996) did not measure percent excretion of ivermectin in feces following treatment with IVOMEC Pour-On for Cattle. Estimates based on his data are similar to those in the IVOMEC Pour-On for Cattle Environmental Assessment. The percentages of absorption/excretion calculated by Gayrard (15%) and from Herd's data (20%, by Gayrard *et al.*) also agree with the absorption/excretion data in the IVOMEC Pour-On for Cattle Environmental Assessment (see table below). Sommer *et al.* (1992) estimated 45% excretion for IVOMEC Pour-On for Cattle, but they also estimated 89% excretion of H_2B_{1a} following subcutaneous injection. Since H_2B_{1a} represents only about 40-45% of the total excreted residue following subcutaneous injection (Halley, *et al.* 1989), Sommer's analytical method is likely overestimating H_2B_{1a} levels by a factor of 2 for both subcutaneous and topical applications. Total excretion of ivermectin following treatment with IVOMEC Pour-On for Cattle is therefore less than that following subcutaneous injection, e.g., 20% (from Herd's data) x 0.5 mg/kg b.w. = 0.1 mg/kg b.w. for IVOMEC Pour-On for Cattle versus 100% x 0.2 mg/kg b.w. = 0.2 mg/kg b.w. for subcutaneous ivermectin treatment.

Percent Absorption/Excretion of Ivermectin after Treatment of Cattle with IVOMEC Pour-On for Cattle

Study	% Absorbed/Excreted, IVOMEC Pour-On for Cattle
Pour-On EA, 1990 [a]	14
Sommer *et al.* 1992	45 [b]
Herd, *et al.* 1996	20 [c]
Gayrard *et al.* 1999	15 [d]

[a] data from Study CA-218, IVOMEC Pour-On for Cattle radioresidue depletion study

[b] nd, but estimated by Sommer, *et al.* (1992) as 45% based on feces AUC

[c] nd, but estimated by Gayrard, *et al.* (1999) as 20% based on Herd's plasma AUC

[d] nd, but estimated by Gayrard, *et al.* (1999) as maximally 15% based on plasma AUC

Both Herd and Sommer show the residue levels in feces after pour-on application are at or below levels following subcutaneous injection by 5 to 7 days post-dose. Roncalli (1989) demonstrated that a sub-tropical species of dung beetle fails to develop in feces of cattle treated subcutaneously with ivermectin for as much as 14 days post-dose. Sommer *et al.* (1992) indicated that emergence of a temperate species of dung beetle is affected for 1 – 2 days after either topical or subcutaneous treatment with ivermectin, but not affected at 13 – 14 days after either treatment. Sommer, *et al.* (1992) further determined that larval development of other species of dung-dependent insects were either unaffected by either treatment route, or that effects after topical treatments were shorter in duration or of the same duration as those after subcutaneous treatment. Therefore, the higher peak level of ivermectin residues in the excretion profiles observed by Herd and Sommer following treatment with IVOMEC Pour-On for Cattle does not indicate that dung fauna would be affected to any greater magnitude or for a longer duration than after subcutaneous treatment.

B. IVOMEC SR Bolus

Subsequent to the approval of IVOMEC Pour-On for Cattle, NADA 140-988 E0022 for the IVOMEC SR Bolus was approved on 25 November 1996.

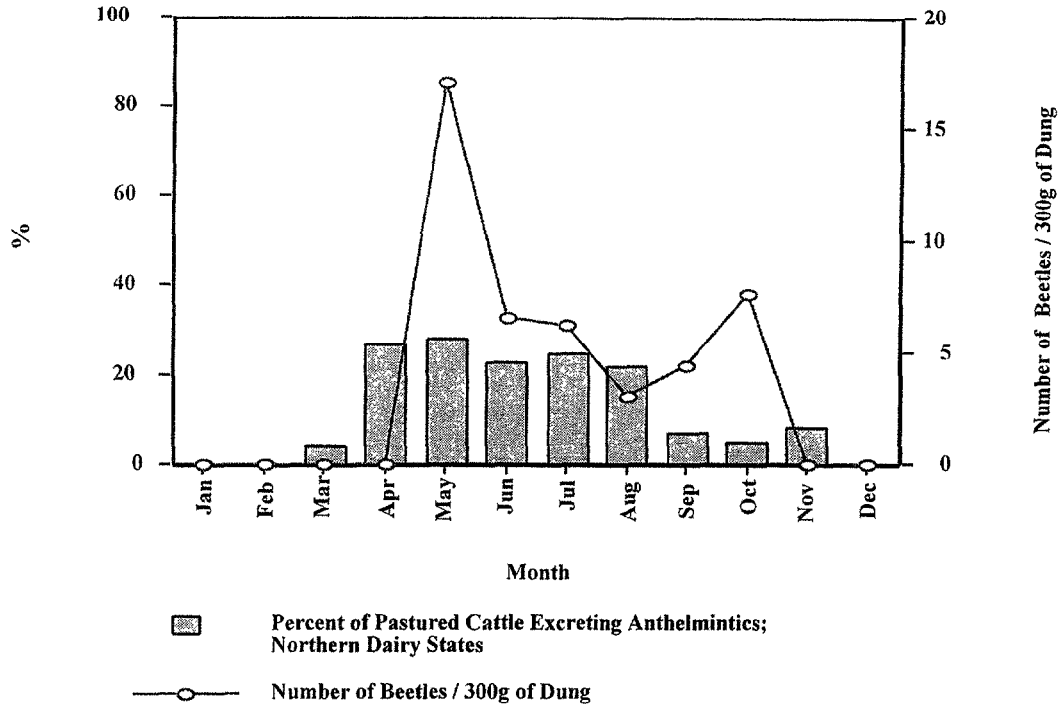
As part of the Environmental Assessment of the IVOMEC SR Bolus for Cattle, a paper entitled "Seasonal Patterns of Cattle Anthelmintic Use in the United States" was prepared by Dr. A. L. Eller, Jr., Virginia Polytechnic and State University. In it, Dr. Eller assessed the projected seasonal use of all anthelmintics in pastured cattle by class, region of the U.S. and month. Dr. Eller made his assessment by contacting cattle extension specialists and/or extension veterinarians in each of ten regions of the U.S. One regional cattle extension specialist from each region then coordinated information from specialists/veterinarians in each state within his region. The regional specialists confirmed the accuracy of the information. The numbers of cattle by class on pasture in each state were based on the official USDA cattle inventory. Both theoretical maximum and estimated actual treatments were reported. The estimated actual reflects the expert opinions of the regional specialists for use of all anthelmintics, not just avermectins. The theoretical maximum treatment, calculated as a worst-case, assumes all eligible cattle are treated at the maximum recommended frequency with anthelmintics. The regional experts agreed that the theoretical maximum treatment is not a realistic estimate of anthelmintic use in practice. In fact, the estimated actual use numbers agreed with available estimated sales data for anthelmintics. The estimated actual numbers indicate that even for seasons of peak anthelmintic use, in regions where parasite challenge is important, 75-90% of the cattle on pasture are <u>not</u> treated with an anthelmintic within any given month.

Another part of the Environmental Assessment of the IVOMEC SR Bolus for Cattle was the report "Hazard Assessment of the Effects of IVOMEC SR Bolus Use in Pastured Cattle on Dung Beetles (Coleoptera:Scarabaeidae)". This hazard assessment included a review of the key literature, including articles published through 1994 and discussed:

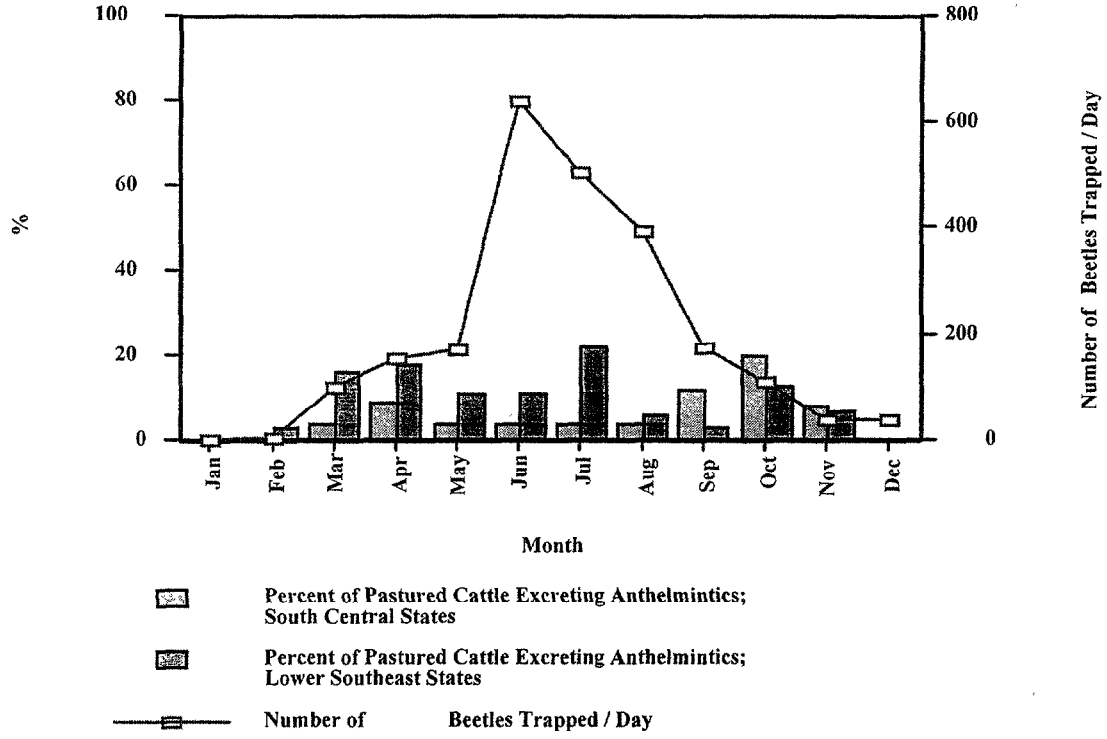
- Mobility of dung beetles
- Use of dung for feeding and reproduction
- Dung beetle activity and reproduction
- Role of dung beetles in degradation of cattle dung and in its removal from pastures
- Widespread distribution of beetles associated with cattle dung and open pastures
- Effect of ivermectin residues on dung beetles
- Use and exposure scenarios: estimated actual and theoretical maximum use of anthelmintics, and
- A hazard assessment for the use of the IVOMEC SR Bolus for Cattle.

This Hazard Assessment assumed that ivermectin residues would be excreted for 5 consecutive months from cattle treated with the IVOMEC SR Bolus and for 1 month after treatment with IVOMEC Injection or IVOMEC Pour-On for Cattle. Thus, the hazard assessment of IVOMEC Pour-On for Cattle was included in the hazard assessment of the bolus. The data for estimated actual percentages of cattle excreting anthelmintics on pasture in the regions of the U.S. where monthly dung beetle activity was known in 1994 are reproduced below.

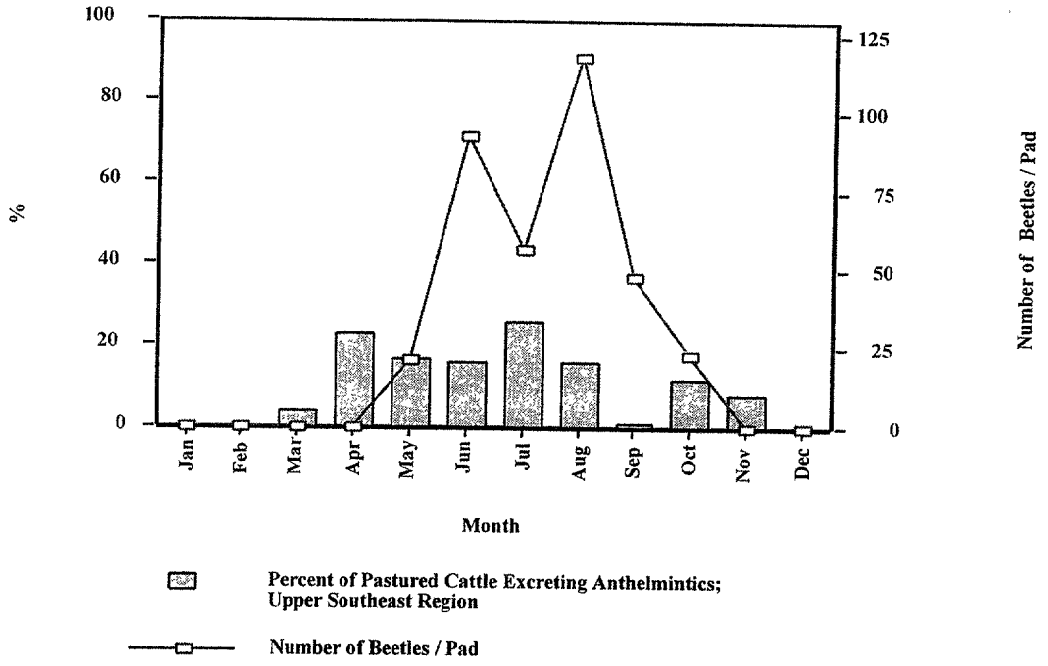
Comparison of the Estimated Actual Percentages of Pastured Cattle Excreting Anthelmintics in the Northern Dairy States versus Numbers of Dung Beetles by Month in Minnesota



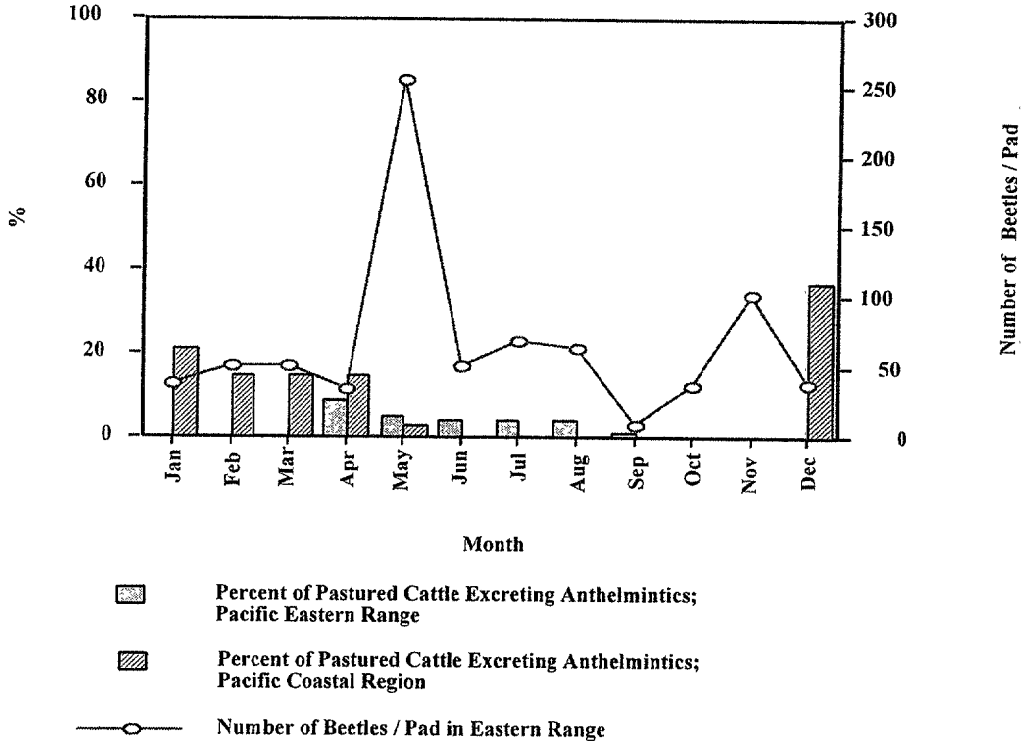
Comparison of the Estimated Actual Percentages of Pastured Cattle Excreting Anthelmintics in the South Central and Lower Southeast Regions versus Numbers of Dung Beetles by Month in Texas



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



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



Based on the estimated actual scenarios in the regions where dung beetle activity data are available, less than 40% of the larval dung beetle populations would be exposed in any given month to anthelmintic residues, and only a fraction of the anthelmintic use would be with the IVOMEC® SR Bolus for Cattle. In many cases there is asynchrony between the months of greatest beetle activity and the months with the greatest percentages of cattle excreting anthelmintic residues in the estimated actual scenario. But even if as much as approximately 66% (the greatest percentage of cattle excreting anthelmintic residues in the theoretical maximum scenario) of the dung beetle larvae were exposed during the peak month of reproduction/larval development to dung containing anthelmintics, this still would result in negligible long-term impact on dung beetle populations. This is because of the operation of various compensatory mechanisms.

The conclusions reached in the 'Hazard Assessment of the Effects of IVOMEC SR Bolus Use in Pastured Cattle on Dung Beetles (Coleoptera:Scarabaeidae)' were that use of the IVOMEC SR Bolus in conjunction with use of other avermectins, including IVOMEC Injection and IVOMEC Pour-On for Cattle, would not have a significant impact on dung beetle populations.

The hazard assessment concluded that:

- Anthelmintic use is highly variable within a region and throughout the year.
- High anthelmintic usage rates would be expected to be scattered throughout a region; used by some, but not all, farm managers.
- Not all eligible cattle will be treated.
- Most dung beetle species that are found on open pastures in temperate regions are dung generalists that are capable of using dung from a variety of species.
- Although ivermectin residues in dung may inhibit larval development, a high percentage of emergence can be expected from dung excreted approximately two to three weeks post-dose following subcutaneous or topical dosing.
- Ivermectin residues in dung of cattle do not affect numbers of colonizing adult dung beetles.
- Usage of anthelmintics in pastured cattle in most regions of the U.S.A. does not coincide with peak periods of dung beetle reproduction.
- In regions where treatment and reproduction of beetles may be coincident, the percentage of animals treated is low and sufficient dung would be available for reproduction.
- Repopulation of areas with reduced populations is expected to occur because of density-dependent reproduction within the area and migration of highly mobile dung beetles into the area
- The estimated actual scenario and the theoretical maximum scenario are conservative, in that they assume that avermectins are the only anthelmintics used. The toxicity timetable is also conservative, since projected effects are based on data for *O. gazella*, not for the less-sensitive *Aphodius* spp.
- Based on the estimated actual scenarios in the regions where dung beetle activity data are available, much less than about 40% of the larval dung beetle populations would be exposed in any given month to anthelmintic residues while cattle are on pasture, and only a fraction of the anthelmintic use would be with avermectins. In some

regions, up to about 40% of the cattle are treated with anthelmintics, but this occurs just prior to winter housing, thus ivermectin residues would not be excreted while cattle are on pasture and while dung beetles are active in those regions.

- The observed use patterns would not result in a long-term impact on dung beetle populations because of the low percentage of cattle treated and the operation of the discussed compensatory mechanisms.
- Even if there were a locale in which all of the cattle were treated during a month of major dung beetle reproductive activity, the compensatory factors would be expected to attenuate any effects upon populations of dung beetles. Thus, there will not be a long-term impact upon these populations.
- The occurrence of the theoretical maximum scenario over a large area is highly unlikely, as the recommended anthelmintic treatment of all target cattle is, in fact, not observed in practice in any of the regions examined. Few ranchers or farmers will disregard the economics involved in the amount of labor needed to treat animals with anthelmintics and the cost of these products. Scattered treatments of this nature could occur; however, their impact would be minimal, given the fact that cattle treatment and high dung beetle activity are not coincidental.
- The animal husbandry practices that have been identified ensure that there are ample supplies of dung that does not contain residues of avermectins at toxic levels, even if anthelmintics were used at the theoretical maximum levels. Hence, there will be no impact upon dung beetle populations even in those few locales, within a region, where anthelmintic usage is at the theoretical maximum.

The conclusions reached in the 'Hazard Assessment of the Effects of IVOMEC SR Bolus Use in Pastured Cattle on Dung Beetles (Coleoptera:Scarabaeidae)' were that use of the IVOMEC SR Bolus would not have a significant impact on dung beetle populations.

The IVOMEC SR Bolus hazard assessment also summarized key literature up to 1994 that indicated:

- Dung beetles are highly mobile. Their migration between pastures and immigration from refugia are certain, and this assures that a reservoir of dung beetles for colonization of pats will be maintained and available.
- Dung beetles that use cattle dung on open pastures in the U.S. are dung generalists. *Aphodius* species are the dominant dung beetles in northern temperate regions. In the U.S., *Aphodius* species of European origin are mostly generalists, preferring open pastures and bovine dung. In contrast, the native species tend to occupy non-pasture areas and utilize dung of native wildlife rather than that of recent arrivals, i.e., cattle. It is unlikely that native American *Aphodius* dung beetles will be exposed to dung containing residues of avermectins.
- Density-dependent reproduction (egg laying by females, and development of larvae in pats) among dung beetles is a compensatory mechanism that can mitigate against the possibility of population decreases caused by a lower than normal number of ovipositing females. These dung beetle characteristics can mitigate against an adverse impact upon dung beetle numbers caused by a variety of factors, by permitting the succeeding generation to rebound to former densities.

- Differing beetle populations, food-manipulation habits and seasonally dependent population densities affect the contribution of dung beetles in degrading dung or in removing it from pasture surfaces in the U.S., but, overall, dung beetles play, at most, a minor role in dung pat degradation in the U.S.
- Dung beetle species associated with cattle dung and open pastures are widespread across the U.S. Because of this widespread distribution, a localized elimination of beetles, for whatever reason, would not threaten the survival of a species.
- Larval development of most dung beetles found in the continental U.S. will occur normally with dung excreted by cattle treated subcutaneous or topically with ivermectin two to three weeks post-dose. *Aphodius* spp., the dominant species of dung beetles in the northern temperate region, is not as sensitive to ivermectin residues in dung as is *O. gazella,* a subtropical, brood-ball-forming species that is easier to cultivate in the laboratory.

The EA for the IVOMEC SR Bolus also included the results of studies with the bolus in the U.K, Missouri USA, and Germany. The study in the U.K. (Wratten *et al.* 1993) conducted by scientists from the University of Southampton at the Merck farm in Hoddesdon, Hertfordshire was a two-grazing season study that measured persistence of dung on pastures, earthworm populations and soil organic matter. The treatment groups included the bolus; subcutaneous ivermectin at 3, 8 and 13 weeks after turnout; an oxfenbendazole pulsed bolus; and untreated control. The study compared the persistence of dung pats from ivermectin-treated and control calves grazed separately on adjacent paddocks over two grazing seasons (April/May to October) in the U.K. and the quality of the pastures grazed by the two groups of calves. The pasture was divided into two blocks of four paddocks, each paddock being approximately 1.1 hectares. Twelve Hereford/Friesian cross calves each weighing approximately 100 kg were turned out in each paddock for the first grazing season (May 11 to October 12, 1988, 154 days) during the first year and 20 similar animals were turned out into each paddock in the second season (April 26 to October 3, 1989, 160 days). The stocking density was reduced to the same extent on each paddock in the latter part of each season as the availability of grass diminished. Each paddock within a block of four was grazed by similar calves given either no routine anthelmintic therapy; ivermectin from a sustained-release intraruminal bolus administered at turnout and delivering approximately 8 mg ivermectin/day for 90 days in the first year (approximately 0.08 to 0.05 mg/kg bodyweight/day) and 120 days in the second year (approximately 0.08 to 0.045 mg/kg bodyweight/day); ivermectin injected subcutaneously at 0.2 mg/kg, three, eight and 13 weeks after turnout according to the dosing schedule recommended in the U.K. and other parts of Europe for first-year animals; or oxfendazole delivered at the recommended dose level from an intraruminal pulse-release bolus (750 mg at five intervals of approximately 21 days each) administered at turnout. Two groups of calves, the one without routine anthelmintic therapy and the other treated with the anthelmintic oxfendazole, acted as controls. Within the blocks of paddocks, one treatment was assigned randomly to each paddock and was constant for both seasons. All the paddocks were managed in the same way before and during the study with respect to cultivation, applications of fertilizer, irrigation, grazing and parasitic nematode infestation. Vehicle movements across the paddocks were restricted to the period between the final disappearance of dung pats in January after the first grazing

season and the beginning of the next season. During the period after the two-year grazing phase of the study until the final soil and earthworm samples had been collected in June and November of the third year, the pastures were grazed only by cattle that had not been treated with ivermectin. The functionality of the ivermectin bolus was confirmed by fecal EPG count data. The results of the study indicated that there were no treatment-related differences between groups in the rate of dung deposition (weight of dung collected at monthly intervals) and accumulation of dung on the pastures, i.e., no significant difference ($P > 0.05$) in the dry weights of cumulative standing dung, during either grazing season.

As part of the same study, the rate of decomposition/degradation under natural conditions of dung pats from calves was investigated by locating 40 fresh pats in each paddock in July and September of each season. By July, the ivermectin bolus had been operational for two months, hence there was drug residue in the dung. Ten of these natural pats were collected in each paddock immediately following deposition, as were ten each at monthly intervals for three months. The dry weight of each collected pat was determined and a mean value calculated for each paddock at each time point. The collection procedure was repeated with pats deposited in September, more than 30 days after the IVOMEC SR Bolus was no longer delivering ivermectin. The results from this experiment (both July and September depositions, ivermectin-containing and ivermectin-free pats, respectively) show that weights of the pats decreased with time, and the rate of decrease was not affected by treatment ($P > 0.05$). Similar results, i.e., no significant ($P > 0.05$) differences among treatments for dung deposition rates, weight of dung collected at monthly intervals, or rate of decomposition/degradation of natural dung pats, were observed during the second grazing season.

Other key components of the U.K. trial involved determining pasture and soil qualities. Organic matter content of soil was determined periodically during the study. Pasture quality was determined by taking transects of fields, monitoring the development of grazing avoidance patches, and ascertaining whether the areas of the patches differed among treatment groups. No significant differences ($P > 0.05$) were found among treatments for either pasture or soil qualities for either year.

The study conducted in Missouri indicated there was no treatment-related effect upon percent weight loss by the pats. Two pats from each of three different days of pat deposition (dosing day, 14 and 28 days post dose) from both control and bolus-treated calves were photographed periodically for >300 days or more than 10 months for determination of pat areas. The data for pats deposited on the day of dosing (hence ivermectin residues not in dung) illustrated that ivermectin-free pats showed large differences and variations in percent of initial area with time. Areas of pats deposited on days 14 and 28 post dose demonstrated that there were no treatment-related effects upon reduction of dung pat areas over time.

Similar results were found in the study conducted in Lauterbach, West Germany. The surface areas of fecal pats deposited on days 21/22, 70 and 119 post treatment from control calves and those given an IVOMEC SR Bolus were followed for over eight months. Degradation of the pats from the IVOMEC SR Bolus-treated calves appeared to

be somewhat reduced compared to that for pats from control calves beginning one and one-half to two months post initiation of treatment. However, statistical analysis of these data revealed no difference ($p>0.10$) between treatments with respect to average surface area or change in area over time for dung pats deposited on Days 21/22 or 70. After adjusting for initial differences, control pats deposited on day 119 were slightly larger than ivermectin pats 7 to 49 days after deposition and slightly smaller 63 to 147 days after deposition; the difference was less than 1 cm^2 at 175 days. By 8-9 months, both sets of pats were essentially degraded. Further, the decrease in organic matter content, an indication of rate of dung pat disappearance of control and ivermectin residue-containing pats, was treatment-independent. Based upon these results, ivermectin treatment is not expected to increase pasture fouling and loss of new growth because of smothering.

This Hazard Assessment for the IVOMEC SR Bolus was reviewed by three independent dung beetle experts (Dr. Roger Moon, University of Minnesota, Dr. Richard Anderson, University of California Berekely and Mr. Richard R. Blume, USDA). All three agreed with the conclusions in the Hazard Assessment and that the use of the IVOMEC SR Bolus, in conjunction with use of other avermectins including IVOMEC Injection and IVOMEC Pour-On for Cattle, would not have a significant impact on dung beetle populations. The Hazard Assessments were presented to the FDA and their reviewer, Dr. R. D. Gordon, USDA.

The agency carefully considered the potential environmental effects of the approval of this product and concluded that the approval will not have a significant effect on the human environment and that an environmental impact statement was not required.

C. IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle

The EA for IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle (4 November 1996) also included discussions on the introduction of eprinomectin via manufacturing and use of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle into the environment. Also discussed were the studies that defined the fate and effects of eprinomectin in the environment.

The fate studies included:

- photodegradation,
- mobility in soil,
- aerobic degradation in soil
- hydrolytic stability

The effects tests included:

- aquatic toxicity (daphnia and fish),
- avian toxicity,
- phytotoxicity (terrestrial plants and algae),
- antimicrobial toxicity,
- earthworm toxicity, and

- dung beetle toxicity.

The EA addressed the toxicity of eprinomectin to beetles, birds, and aquatic invertebrates, the effects of eprinomectin on the degradation of cow pats and the concentration of eprinomectin in runoff water from fertilized fields. The degree of retention of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle on the backs of cattle was demonstrated via efficacy in clinical trials using simulated rain. The physiochemical properties of eprinomectin allowed for the calculation of concentrations of eprinomectin that might occur from cattle standing in a pond or slowly moving stream during rain. The calculated concentrations were then compared to measured effect levels for aquatic organisms. Similarly, the level of eprinomectin in soil from the fertilization of fields with cattle manure was compared to the measured effect levels for terrestrial organisms. The data in the EA indicated that use of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle would not lead to eprinomectin levels in the environment that would affect aquatic organisms, avians, or vegetation and that eprinomectin would not persist nor bioaccumulate.

The acute toxicity of eprinomectin was determined for the northern bobwhite and the mallard as 272 mg/kg and 24 mg/kg, respectively. The subacute LC_{50} values for eprinomectin, when administered via the feed in an eight-day dietary study, were 1813 ppm for the northern bobwhite and 447 ppm for the mallard duck, respectively. At the lowest concentration studied (100 ppm eprinomectin), sublethal effects were observed during the on-drug phase of the study. However, all birds appeared normal 24 hours following their return to the basal diet. Based on the acute and chronic data, scenarios were developed to model exposure of avians, including raptors. The scenarios showed that primary poisoning of birds, exposed through their diet, is highly unlikely as are chronic (reproductive) effects from use of this product. Secondary poisoning of raptors and poisoning of carrion-feeders are also unlikely even under worst-case assumptions.

Since the treatment of dairy cattle with anthelmintics and ectoparasiticides was not addressed in the assessment of the IVOMEC SR Bolus for Cattle, the assessment of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle also included the report "Anthelmintic and Ectoparasiticide Use in Lactating Dairy Cows: An Assessment of the United States Dairy Industry" by Patrick C. Hoffman, Associate Professor-UWEX, Department of Dairy Science, University of Wisconsin-Madison. Dr. Hoffman compiled the results of the responses from dairy specialists from universities in five major dairy regions of the US concerning:

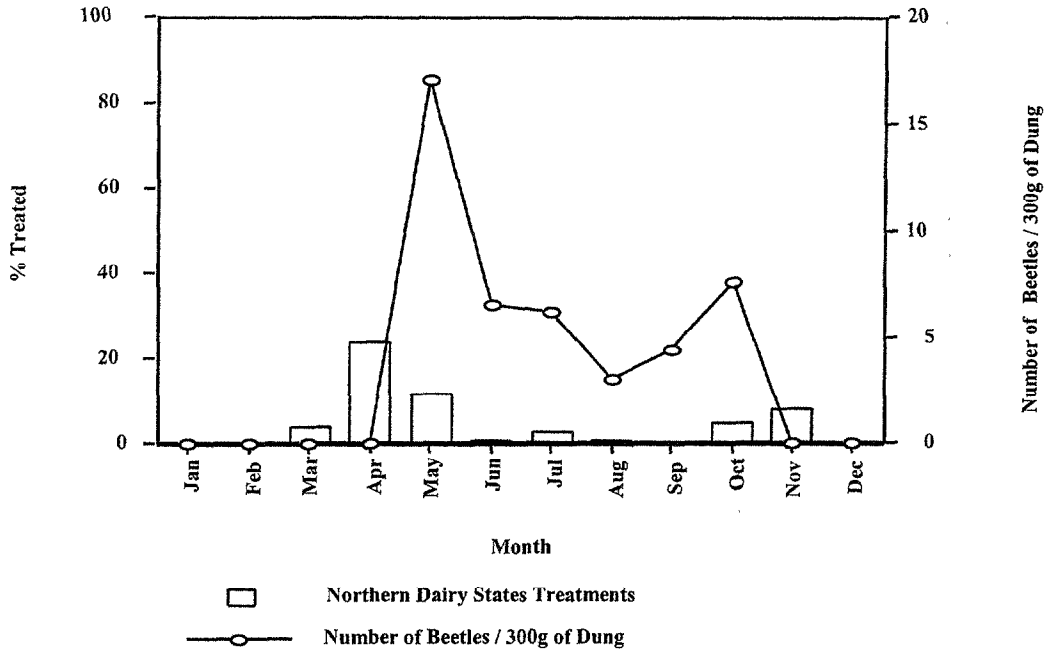
- Dairy management systems
- Anthelmintic use in lactating dairy cows
- Ectoparasiticide use for control of mange, lice, grubs, ticks and horn flies in lactating dairy cows
- Seasonal pattern uses of anthelmintics and ectoparasiticides in lactating dairy cows
- Potential use of a new product that controls both internal and external parasites in lactating dairy cows

Each of the dairy specialists contacted veterinarians, consultants, feed dealers and parasitologists within their region to prepare their response.

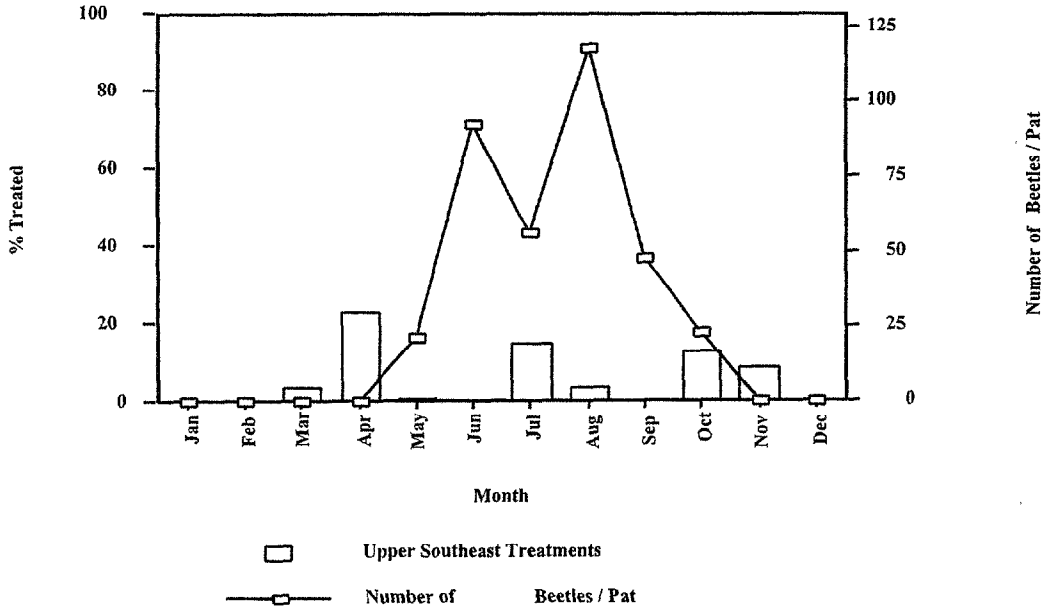
The overall assessment of any impact of eprinomectin residues in cattle dung upon dung beetle populations is based on the estimated actual usage of anthelmintics and ectoparasiticides. The use patterns are then compared with the activity patterns for dung beetles in those regions for which such activity data are available.

The data for estimated actual use of all anthelmintics in the regions of the U.S. where monthly dung beetle activity was known in 1994 are reproduced below. The plots were based on the assumption that ivermectin residues affecting emergence of dung beetle larvae would be excreted for 1 month after treatment with IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle. This assumption would also be applicable, and conservative, for subcutaneous, topical or oral formulations of ivermectin.

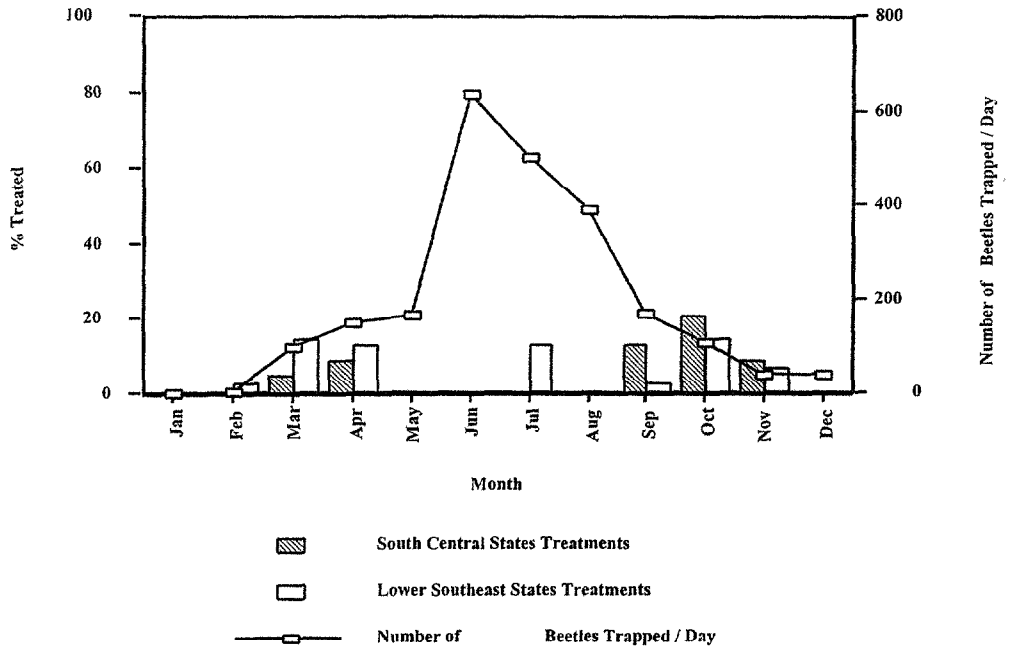
Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics versus Numbers of Dung Beetles by Month in Minnesota



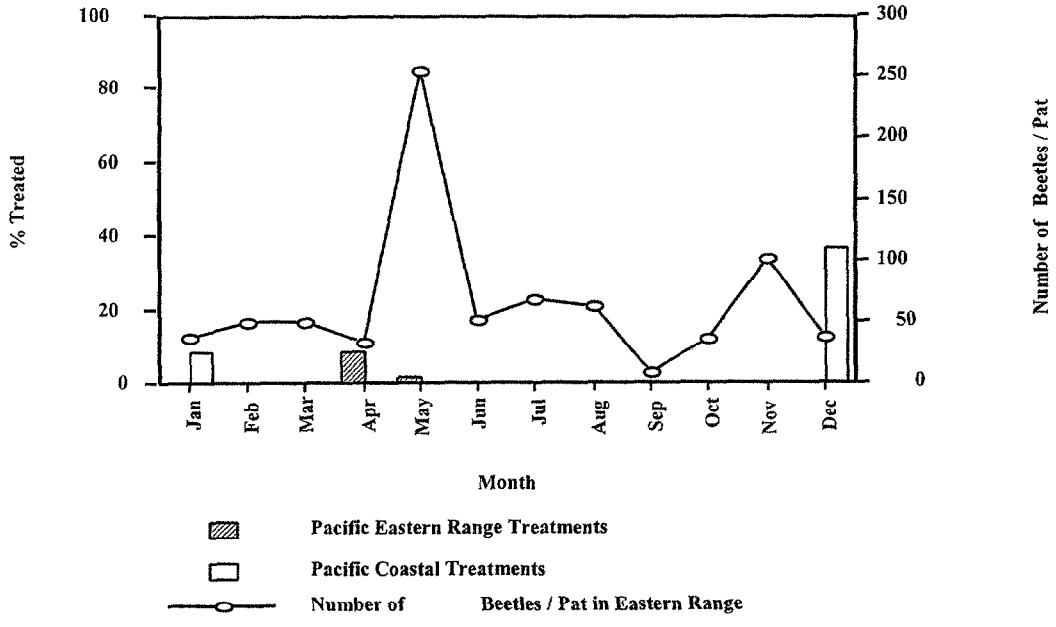
Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics versus Numbers of Dung Beetles by Month in Missouri



Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics in South Central and Lower Southeast Regions versus Numbers of Dung Beetles by Month in Texas



Comparison of the Estimated Actual Percent of Cattle on Pasture Treated with Anthelmintics versus Numbers of Dung Beetles by Month in California



Based on the estimated actual scenario in the EA for IVOMEC EPRINEX Pour-On, much less than 40% of the dung beetle larvae populations would be exposed to feces from cattle treated with anthelmintics or ectoparasiticides. Even if as much as 40% of the larval dung beetles were exposed to dung containing inhibitory levels of eprinomectin during the peak month of reproduction/larval development, this would not result in a long-term impact on dung beetle populations because of the operation of various compensatory mechanisms.

These compensatory mechanisms are based on two behavior characteristics of dung beetles that facilitate recolonization and compensate for any temporarily reduced populations of adult dung beetles that might result because of reduced emergence of a new generation. One of the characteristics is the mobility of adult dung beetles that allows them to move readily between locales and recolonize an area which may have for any reason a low population density. Studies demonstrated that dung beetles will be attracted to dung from at least one mile away, and migration of dung beetles over long distances has been well documented. In-flying dung beetles from other areas and refugia will reproduce using the readily available non-toxic dung pats being excreted by cattle treated weeks previously. The second characteristic that will aid in maintaining the dung beetle population is density-dependent reproduction. Lowered densities of dung beetles in pats can lead to increased egg laying and brood ball production per female, thus in part compensating for lower numbers of egg-laying females. In addition, even if there are fewer eggs per pat, an enhanced success rate for larval development occurs because of reduced competition among the larvae for food and habitat space. Both of these behavior patterns will serve to maintain the population of dung beetles in a locale where use of IVOMEC EPRINEX Pour-On might cause a decrease in the number of adults in a succeeding generation.

Even if there were a locale in which all of the cattle were treated during a month of major dung beetle reproductive activity, the compensatory factors would be expected to attenuate any effects upon populations of dung beetles. Thus, there will not be a long-term impact upon these populations.

The NADA for IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle (141-079) was approved 16 April 1997. The FDA concluded that the EA provided adequate information to determine that the manufacture and use of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle would not be expected to cause a significant impact on the environment.

D. Summary, including recent ecotoxicity studies with dung flies

Since the use of IVOMEC Pour-On for Cattle, IVOMEC Injection and IVOMEC Cattle Paste were included in the assessments for IVOMEC SR Bolus and IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle, the scenarios, assumptions and assessment conclusions outlined above for IVOMEC SR Bolus and IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle are applicable for Merial's other avermectin-based products for cattle. This includes the conclusion that use of the products would not have a significant impact on dung beetle populations. If dung beetle populations are not affected, then higher tropic

levels that feed or depend on dung beetles will not be affected. Therefore, studies looking for any effects of ivermectin on higher trophic levels under actual product-use conditions are not warranted.

Likewise, use of Merial's avermectin-based products for cattle would not have a significant impact on dung fly populations. Based on several clinical field trials, IVOMEC Pour-On for Cattle is efficacious against horn flies (*Haematobia irritans*) and the product carries an efficacy claim for 28 days. These trials were conducted under commercial practices in paddocks where control and treated cattle were separated from each other and from other cattle by approximately 500 meters to restrict flies from moving between paddocks. Horn flies breed and lay their eggs in fresh cattle dung. The time period from egg laying to adult emergence is about 4 days. Cattle are obligate hosts and adult horn flies frequent the neck and back of cattle, areas where the pour-on is applied. These factors make the horn fly a sensitive indicator species to any effects caused by endectocides administered topically to cattle. Horn fly populations on the cattle in the field trials were below 90% efficacy levels after twenty-eight days post-treatment, indicating that IVOMEC Pour-On for Cattle affects horn fly development for only a limited duration.

Although IVOMEC Pour-On is efficacious in reducing the populations of adult hornfly, populations of adult hornfly are unaffected by the presence of ivermectin in dung during the excretion period of the IVOMEC SR Bolus when some untreated animals are present. In one study (ASR 14003), there was no difference between the numbers of hornfly in the control group (25 animals) compared to the animals treated with an IVOMEC SR Bolus (100 animals). Similarly in another study (ASR 13847) there was no discernible difference between the populations of *Haematobia irritans* on bolus treated (8 animals) and control animals (8 animals). In both studies, the plasma levels of ivermectin (as measured by the H_2B_{1a} component) decreased after the day 120 assay, but were still detectable at day 134/135. Also in both studies, the numbers of flies increased with time on both bolus-treated and control cattle over the duration of the studies. Therefore, treatment of 80% of the cattle (in ASR 14003) with the IVOMEC SR Bolus did not prevent the population of *H. irritans* from increasing during the study. In a third study (ASR 11091), where forty bolus-treated cattle were grazed on pastures well separated from pastures grazed by 20 control cattle, hornfly burdens on bolus-treated cattle were less than those on control cattle for the duration of the 120-day study. Mean numbers of hornflies were never zero on the treated cattle, indicating that where all cattle receive a bolus and are isolated from any untreated cattle, numbers of adult hornflies may be reduced relative to controls, but not eliminated. Based on the US husbandry and bolus-usage data, situations where all cattle would be treated with a bolus and would be isolated from untreated cattle or other animals that could provide refugia for dung fauna would be extremely rare.

In a study where dung from eight calves treated with IVOMEC SR Bolus was compared with that from eight comparable untreated calves, Barth *et al.* (1993) observed an identical spectrum of Coleoptera species throughout 120 days in both groups. No differences in the numbers or frequency of adult Coleoptera species or soil nematodes were observed. The

numbers of some species of dung-specific and soil nematodes were elevated in the pats from the ivermectin-treated cattle, indicating some species take advantage of the reduction in the numbers of other nematodes.

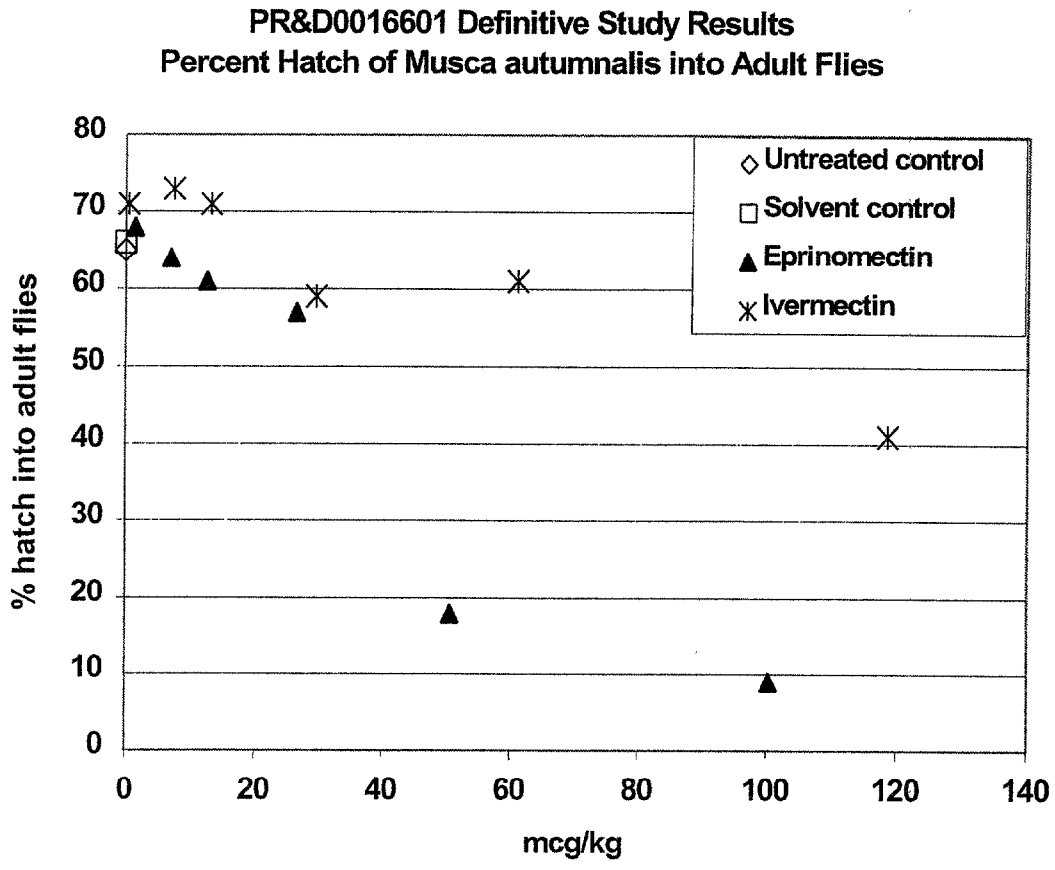
A laboratory study was also conducted to determine median effect concentrations (EC_{50}) and the no-observed effect concentrations (NOEC) of eprinomectin and ivermectin on the dung dwelling larvae of *Musca autumnalis,* a common dung fly. Negative control (solvent only), untreated control (no solvent added) and radioactivity control samples (highest levels of radioactivity but no unlabeled materials) were also included. The study was carried out under static conditions; the eprinomectin and ivermectin was added to bovine feces to which first instar larvae of *M. autumnalis* were added. The impact of the eprinomectin and ivermectin on maturation to adults was assessed.

In the range-finding study, radiolabeled eprinomectin and ivermectin were used to verify appropriate test concentrations and to determine homogeneity of mixing and stability of the test materials. Combustion analysis indicated that the test materials were reasonably evenly distributed throughout the dung. In addition, the levels of eprinomectin and ivermectin determined by combustion were similar to the nominal levels applied to dung. Study termination concentrations were slightly higher than initial levels, which may have resulted from loss of moisture over the duration of the study. HPLC data for extracts from the highest concentration samples confirmed that only parent compounds were detected, confirming the stability of the test material for the duration of the study. In the first range-finding study, 25 larvae of *M. autumnalis* (1-2 days old) were added at each concentration level to one test vessel containing 100 g of bovine dung. However, only 32% of flies emerged from the solvent-only control treatment. As the emergence rate of the control treatment was below acceptable limits, the test was not valid. A second range-finding study was therefore initiated with replication of the solvent control and each test concentration (n=3). Replicates of an untreated control (no solvent) were also included in the second range-finding study. Formulation procedures for the second range-finding study followed those used in the first range-finding study, using non-radiolabeled test materials because of a limited supply of radiolabeled materials. Analysis was not conducted on dung from the second range-finding study. One-day old larvae of *M. autumnalis* were used in the second range-finding study. Emergence from controls, both untreated and solvent (ethanol), were greater than 76% in all replicates in this study. Therefore, this second range-finding study was valid and the solvent appeared to have no effect on emergence.

Based on the results from the second range-finding study, the definitive study was conducted using the following nominal concentrations for eprinomectin and ivermectin: 100, 50, 25, 12.5, 6.25 and 0 (solvent control and untreated control) mcg/kg dung. Replication was further increased with five replicates at each test concentration and six replicates each of the solvent and untreated controls. Controls containing the highest level of radioactivity but no added non-radiolabeled material (radioactive controls) were also included for each compound to ensure that the radioactivity had no effect on larval development and adult emergence. The definitive study used one-day old larvae of *M. autumnalis* and used radiolabeled test materials to determine the exact concentrations of

the test materials. Mean emergence from solvent and untreated controls were 66 and 65%, respectively; therefore, the definitive study was valid.

The results from the definitive study are shown below. Measured concentrations of eprinomectin were 100, 51, 27, 13 and 7.0 mcg/kg wet-weight for eprinomectin. The concentration of eprinomectin in its radioactive control was 1.4 mcg/kg wet-weight. The EC_{50} for eprinomectin was calculated to be 41.6 mcg/kg (with 35.0 – 47.9 as the 95% C.I.) by applying the standard technique of maximum likelihood estimation to the probit model. The NOEC was 26.6 mcg/kg, calculated using Fisher's Exact Test. Measured concentrations of ivermectin were 119, 61, 30, 13 and 7.5 mcg ivermectin/kg wet-weight. The concentration of ivermectin in its radioactive control was 0.46 mcg/kg wet-weight. Mean emergence was 71, 73, 71, 59, 61 and 41% respectively. Although 50% reduction of emergence relative to control emergence (e.g., 50% of 65-66% 33%) was not achieved, the EC_{50} for ivermectin was calculated to be 78 mcg/kg (with 51.8 – 158.5 as the 95% C.I.) by the probit model. The NOEC was 61 mcg/kg, by Fisher's Exact Test.



The EC_{50} and NOEC values determined with *M. autumunalis* can be compared to concentrations of ivermectin determined in the feces of dosed cattle to estimate the duration of effects for this insect. Herd *et al.* (1996) measured a maximum fecal concentration of 2.8

± 1.2 mg ivermectin/kg wet feces, 2 days after dosing with IVOMEC Pour-On for Cattle. The level of ivermectin in feces fell to about 1.4 mg/kg by day 3 (value taken from plotted data). By day 28, ivermectin residues had fallen to 0.006 ± 0.004 mg/kg (Herd *et al.*, 1996). Sommer *et al.* (1992) also measured the levels of ivermectin in the feces of cattle after pour-on administration. Ivermectin levels were reported on a dry-weight basis. Wet-weight levels are estimated here by assuming an 85% water content (Barth, 1993) in fresh feces (i.e., wet-weight levels = 0.15 x dry-weight levels). A maximum of 1.4 mg ivermectin/kg was measured by Sommer *et al.* (1992) on day 1, falling to about 0.42 mg/kg by day 5 (value taken from plotted data) and below the limit of detection (0.0075 mg/kg) by days 13 - 14. Neither of these references report enough data to determine when the measured ivermectin concentrations fall below the EC_{50} (0.078 mg/kg) and NOEC (0.061 mg/kg) levels, but the levels were below those values at the next time points, day 13 – 14 in Sommer *et al.* (1992) and by day 28 in Herd *et al.* (1996). Thus, no emergence of adult *M. autumunalis* is expected from feces excreted between days 1 and about 13 –14, some emergence between days 14 and 28 and the same emergence rate as from control feces after 28 days.

As indicated in the EAs for IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle, the mean concentrations of eprinomectin B_{1a}, which comprises greater than 90% of eprinomectin, are below 50 mcg/kg in fresh cattle dung at day 7 post dose and below 10 mcg/kg by day 14 post dose. For the dung fly *M. autumnalis* where EC_{50} and NOEC values for eprinomectin are 41.6 and 26.6 mcg/kg, respectively, effects on numbers of *M. autumnalis* would be predicted through 7 days after dosing. That is because the mean concentration of eprinomectin B_{1a}, 42.1 mcg/kg wet weight, at 7 days after dosing is approximately equal to the EC_{50} in the fresh dung, 41.6 mcg/kg. By day 14, the next assay time, the concentration of eprinomectin B_{1a} in fresh dung decreased to levels below the NOEC of 26.6 mcg/kg in the feces of all of the 9 cattle in the study. Therefore, the numbers of *M. autumnalis* emerging from fresh cattle dung pats would be predicted to increase rapidly between 7 and 14 days after treatment of cattle with IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle. By day 14 and beyond, no effects on numbers of emerging *M. autumnalis* would be predicted.

Projected effects on numbers of *M. autumnalis* for 7 days after dosing with IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle and decreasing effects on *M. autumnalis* populations between days 7 and 14 is about the same as the 7-day duration of efficacy for IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle to control *Haematobia irritans*, the horn fly. Horn fly populations on cattle in field trials with IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle begin to increase shortly after seven days post-treatment, indicating that eprinomectin affects horn fly development for only a limited duration.

The projected duration of effects from IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle on numbers of dung flies is similar to the projected duration of effects of eprinomectin on dung beetles. The NOEC for eprinomectin, determined in the dung beetle study with *O. gazella* and *E. intermedius*, was 65 mcg/kg for both species. The EC_{50} values were not determined, but no beetles emerged at the next higher concentration tested, 166 mcg/kg. The mean concentration of eprinomectin B_{1a} in fresh cattle feces was below the NOEC value, 65

mcg/kg, to both species of dung beetles by day 7 post dose. Additionally, the concentration of eprinomectin B_{1a} was projected to be below the NOEC value in fresh cattle dung from all cattle in the trial by about day 10 post dose. *M. autumnalis* is slightly more sensitive to eprinomectin than are either of the two dung beetle species tested, but the duration of projected effects is about the same because of the rapid decrease in eprinomectin concentrations in fresh dung.

The animal husbandry practices that were outlined in the hazard assessments ensure that there are ample supplies of dung that does not contain residues of avermectins at toxic levels, even if anthelmintics and ectoparasiticides were used at the theoretical maximum levels. Hence, there will be no impact upon dung fauna populations even in those few locales, within a region, where anthelmintic and ectoparasiticide usage is at the theoretical maximum. Although there are reductions in the numbers of some dung-dependent insects species in dung pats for a duration after treatment of cattle with avermectin-based compounds, complete elimination of immature Coleoptera and Diptera is not observed, even in pats of cattle treated with the sustained-release bolus (Barth et al., 1993, 1994a). The total number of species and, in some cases, the total number of insects are also unaffected (Barth *et al.*, 1993, 1994a). This, in addition to the presence of sources of dung other than from treated cattle and the presence of constant or increased numbers of unaffected or opportunistic species in the dung practically eliminates the possibility that any higher trophic species that depends solely upon larvae in dung for its food would be affected even within a short temporal period within a small spatial region.

3. REVIEW OF KEY DUNG-FAUNA AND DUNG-DEGRADATION LITERATURE 1993-2000

The literature review focuses on the following areas of potential concern

(1) Effects and duration of effects on dung fauna.
(2) Excretion profile of ivermectin and utilization of dung by dung fauna.
(3) Soil organisms.
(4) Higher trophic levels.
(5) Fungi.
(6) Dung degradation.

No change to the scope or conclusions of the original EAs for IVOMEC Products for Cattle including IVOMEC EPRINEX Pour-On is indicated by new the literature. No significant risk to pasture organisms or processes is indicated.

A. Effects and duration of effects on dung fauna

This section of the review is divided into two subsections. The first section discusses articles concerning pat-level studies, investigating effects of laboratory or field exposure to ivermectin residues in dung on particular species or stages of dung fauna. The second section discusses articles concerning population and community level effects, where authors studied effects on populations and communities, or incorporated an exposure assessment into a risk assessment, to investigate effects on a population and community scale.

1. Pat-level

Mahon *et al.*, (1993) found that residues in sheep dung excreted 1 day post-treatment affected the pest blowfly *Lucilia cuprina*. Mortality, female ovarian output, and egg retention was significantly different in flies fed dung excreted by sheep 1 day after treatment, whereas egg hatch was unaffected. No effects were observed in flies fed dung collected at later times. Use of ivermectin was therefore considered to be ineffective at controlling this pest, as populations were not expected to be impacted by the effects.

McCracken and Foster (1993) used multivariate analysis to examine the effects of ivermectin on invertebrates in artificial 1 kg cattle dung pats in the U.K. An injectable formulation of ivermectin was diluted with water and mixed with control dung to produce levels of 0, 0.5, 1 and 2 mg of ivermectin per kilogram of dung. The pats were placed in stratified random block plots on pastures adjacent to fields containing cattle. Pats, and the soil beneath (4 cm depth), were taken at 15, 30, 45, 60 or 90 days after placement. Placement dates were in May, June, August and September. Initially, there were 60 pats per collection group, but 73 of the original 228 pats were not visible on the day of sampling and another 21 were excluded from further analysis because they contained less than 3 taxa. Data from soil samples from beneath 57 pats were used for analysis. The study concentrated on differences between pats with regard to the numbers and types of Diptera and Coleoptera present and the numbers of earthworms. Few differences were detected between the three levels of ivermectin used in the study, so experimental pats were regarded as either treated or controls. Eight distinct assemblages of taxa were found in the pats. Most (54%) of the between-pat

variation in the invertebrate communities was attributed to duration of exposure after placement, while 30% was attributed to time of year of placement and only 16% to the presence or absence of ivermectin. The greatest (42%) variation in the invertebrate communities in the soil beneath the pats was again attributed to the duration of exposure, with 35% attributed to the presence or absence of ivermectin in the pat and 23% attributed to the seasonality of placement of the pat. The most marked change to fauna associated with the presence of ivermectin was inhibition of larval development and/or pupation of cyclorrhaphan fly larvae. No solvent controls were included in this study.

Strong and James (1993) investigated larval mortality, pupation, and adult emergence of the yellow dung fly *Scatophaga stercoraria* from ivermectin in cattle dung. Formulated ivermectin (containing 40% glycerol formal and 60% propylene glycol as excipients) was diluted with ethanol so that addition of 200 mL of the ethanol and ivermectin formulation to 200 g cattle dung (from untreated cattle) achieved ivermectin levels ranging from 250 mcg/kg to 0.48 mcg/kg. Controls contained ethanol, but neither of the excipients. Of the effects measured, the lowest EC_{50} was 1 mcg/kg for adult emergence. The excipients were present at much higher concentration than ivermectin in the feces, but are not expected to be excreted in the feces of treated animals. Therefore this study did not have an adequate solvent control. Fly larvae have been found to be sensitive to organic solvents (Doherty *et al.*, 1994) and earthworms appear more sensitive to ivermectin in formulation than to ivermectin in cattle dung (see section 3.C below). Therefore the observed toxicities are more likely to have been due, in part, to the excipients or to a synergy (altered solubility or enhanced uptake) between the compounds present. This is supported by pat-level field studies in Canada (Floate, 1998a) where no significant difference was observed in the number of *S. stercoraria* emerging from dung collected from cattle before (control) and at weekly intervals after treatment with IVOMEC Pour-On.

Similar methodology was used by Strong and James (1993) to assess fluctuating asymmetry and abnormalities in the main veins of the wings of emerging adult flies, exposed to ivermectin at sub-lethal concentrations (0.5 mcg/kg). An important difference, however, was that effects from excipients in the formulation (40% glycerol formal, 60% propylene glycol), were accounted for in controls. About 2-fold greater variation was observed in the lengths of the main veins of flies emerging from dung containing ivermectin, relative to ethanol and excipient + ethanol controls. Also, 23% of flies emerging from dung containing ivermectin displayed abnormalities of main wing veins (ranging from small new veinlets to completely new cells), whereas no abnormalities were observed in dung from ethanol and excipients + ethanol controls. These non-lethal effects are consistent with the mode of action of ivermectin and are not expected to be detrimental to the organism. That these sub-lethal effects do not cause an adverse effect is demonstrated by results of Canadian field studies (Floate, 1998a), where no significant difference was observed in the number of *S. stercoraria* emerging from dung collected from cattle before (control) and at weekly intervals after treatment with IVOMEC Pour-On.

A field study conducted by Barth *et al.* (1994a) investigated effects on dung fauna and dung degradation arising from subcutaneous treatments of cattle with levamisole (5 mg/kg) or ivermectin (0.2 mg/kg) 3, 8, and 13 weeks after turnout. The range of Coleoptera,

nematodes, and earthworms was unaffected by treatment, and the numbers of Coleoptera, soil nematodes, and earthworms were unaffected by either treatment. There were no treatment effects on numbers of earthworm species, on numbers of adult or juvenile earthworms or on earthworm biomass associated with 63-day-old pats voided 0, 3, 7, 14 or 28 days after treatments. The lack of effects on the numbers of juvenile earthworms in fecal pats indicates that ivermectin, under typical use patterns, has no chronic effects on earthworms. However immature Diptera and some dung-specific nematodes were present in lower numbers in pats deposited up to 28 days and 14 days after treatment, respectively, in feces from cattle treated with ivermectin compared to the pats of cattle treated with levamisole. No difference in dung pat degradation (as determined by measurements of surface area, weight, organic matter content and monitored by photography) was observed between ivermectin and levamisole treatment groups.

Strong and Wall (1994b) investigated effects of ivermectin residues in cattle dung on colonization, survival and development of insects in June and July in the UK. Artificial, 2-kg pats were formed from dung collected 2, 7, 14 and 21 days after treatment of the cattle and from control dung. Eight pats from each group were randomly allocated to sites in a field and were protected from birds. On days 7, 14, 21 and 42 following placement, two entire pats from each group were removed, weighed and assayed for invertebrates. Dung beetles were predominately *Aphodius* spp. and numbers of adults were not different between pats from control and treated cattle. This indicates no difference in attraction to pats containing ivermectin residues relative to control pats and no toxicity to adults. Larval *Aphodius* spp. were unable to survive in 7-day post dose pats but there were no differences between numbers or dry weights of *Aphodius* spp. larvae in control pats and pats collected 14 days after ivermectin treatment. Very low numbers of dipteran larvae colonized pats, regardless of treatment group, in the early stages of the trial and this was attributed to the low spring temperatures experienced that year. However the data indicates that cyclorrhapous larvae colonized dung in significant numbers 21 days after treatment with ivermectin. No treatment related effects on nematocerous dipteran larvae were observed; 120 and 17 larvae were found in pats excreted on day 2 and 21 after ivermectin treatment, respectively, whereas 0 and 17 larvae were found in pats excreted on day 2 and 21 from untreated cattle.

Gover and Strong (1995) examined the effects of different concentrations of ivermectin in cattle dung on the fly, *Neomyia cornicina*. The LC_{50}, based on cumulative mortality over one week in adult flies fed feces containing ivermectin for 24 hours, was 0.139 mcg/g faeces (wet weight). Ingestion of faeces containing ivermectin at 0.15 mcg/g reduced the proportion of females that oviposited and reduced the percentage of eggs that hatched, but did not reduce the number of eggs per female.

In a review article, Herd (1995) considers potential ecological and agricultural consequences of endectocide use, focusing on ivermectin. The IVOMEC SR Bolus was considered the most potentially ecotoxic ivermectin formulation, due to the extended period of activity. The larger dose supplied by the pour-on formulation and the higher fecal residues (Sommer and Steffansen, 1993) suggest that the pour-on presents a greater ecotoxicological risk than the injectable formulation. However the analysis did not account for the retention of ivermectin on the hide of the treated animal, nor did it consider that fecal residues observed in cattle

treated with the pour-on formulation fall below those observed in cattle treated with the injectable formulation within 7 days of treatment. Consequently, the pour-on formulation may only exert greater effects than the injectable formulation within 7 days of dosing. The reduced bioavailability and rapid excretion of oral horse and sheep formulations were considered to present the least likelihood of ecotoxicological effects. Biological assays of ivermectin were described and their utility questioned, because lethal rather than sub-lethal effects are typically measured. Pat-level effects on dung fauna are described in general, but no exposure assessment is presented to allow assessment of how pat-level effects might potentially affect dung insect populations and communities. An earthworm study conducted by Gunn and Sadd (1994) (discussed in section 3.C) indicates toxic effects to earthworms not observed in four previous studies. However, the reviewer does not take into account that the study conducted by Gunn and Sadd concerned the toxicity of formulated ivermectin to earthworms. Effects from excipients were not accounted for in controls and would not be present in the dung from treated animals on pasture. Therefore the data from Gunn and Sadd do not represent effects from use on pasture and it is likely that the presence of excipients contributed to the differences in effects observed by Gunn and Sadd, compared to other studies (see section 3.C). Studies investigating impacts on dung degradation are discussed, but no conclusions are drawn.

Krüger and Scholtz (1995) found that dung from cattle treated with IVOMEC injection prevented development of *Musca nevilli* Kleynhans, a dung-breeding fly which is a vector of the filarial nematode *Parafilaria bovicola*, for up to 4 weeks and reduced development for up to 7 weeks in South Africa. The fertility of adults which fed on dung excreted for up to 8 weeks from treated cattle was also reduced relative to the fertility of flies fed control dung. The authors note that no potential ecotoxicity is expected from use of ivermectin to control parafilariasis, as treatment is typically given 70 days before slaughter when animals are usually on feedlots.

Fincher (1996) investigated effects from ivermectin residues in the dung of cattle treated with IVOMEC Pour-On, on the adult emergence of two species of dung beetles *Euoniticellus intermedius* and *Onthophagus gazella*, and on the horn fly *Haematobia irritans* (IVOMEC Pour-On has an efficacy claim of 28 days against *H. irritans*). Dung was collected from an untreated control and treated cattle prior to treatment, on the day of treatment, and weekly thereafter for the following 8 weeks. Adult emergence was reduced for 5-6 weeks for *H. irritans*, for 1-2 weeks for *E. intermedius* and for 2-3 weeks for *O. gazella*. There were no effects on the number of brood balls produced by either dung beetle species.

Gover and Strong (1996) investigated the toxicity and attractiveness of dung from cattle treated with the IVOMEC SR Bolus to the dung fly *Neomyia cornicia*. Day 7 mortality was 93% higher in flies fed dung from treated calves (collected 21-22 days after treatment with the IVOMEC SR Bolus) than flies fed dung from untreated calves. The day 7 mortality in flies fed dung from the bolus-treated calves corresponded to an ivermectin concentration of 0.57 mg/kg. Females showed no preference for the feces of dung from treated or untreated calves. These bioassay results indicate levels in dung from the IVOMEC SR Bolus were somewhat lower than measured by Alvinerie *et al.* (1998) (1.18 mg/kg). This difference may

be partially due to differences in weight of treated calves (278 kg and 210-230 kg in the studies of Gover and Strong (1996) and Alvinerie *et al.* (1998), respectively).

Strong *et al.* (1996) compared the effects of fenbendazole and ivermectin residues on dung-colonizing Coleoptera and Diptera after administration by sustained-release bolus to cattle. Cattle were housed and maintained on a diet of hay and feed concentrate, dung was collected 20 and 21 days after treatment then formed into artificial pats and randomly allocated on a grid location in cages on a field. Invertebrates were sorted and counted from pats retrieved 7, 14, 21 and 42 days after field placement. No difference in the numbers of adult Coleoptera (*Scarabaeidae*, *Hydrophilidae* and *Staphylindae*) was found between control and pats from either treatment group. Numbers of *Scarabaeidae* larvae varied with time on the field and were reduced in pats from ivermectin-treated cattle. A variety of Diptera larvae were found in dung from control and fenbendazole-treated cattle, but were absent in dung from IVOMEC SR Bolus-treated cattle. Dung from bolus-treated cattle had no significant repellent or attractive effect.

Wardhaugh *et al.* (1996) studied effects of drug residues in the faeces of cattle, maintained on a pasture diet and treated with ivermectin (subcutaneous administration) on larvae of the bush fly, *Musca vetustissima* and the house fly, *Musca domestica*. Larval development of *M. vetustissima* was inhibited for 14 days after treatment and reduced for 28 days by ivermectin residues in cattle dung. Dung collected on days 3, 7, 14 or 28 after dosing and fed for 8 days to newly emerged females was not toxic. Ivermectin residues also inhibited larval development of *M. domestica* for 7 days post dose. Similarly, Wardhaugh and Mahon (1998) observed larval development of *M. vetustissima* was completely inhibited for 8 days after treatment with ivermectin, reduced at 16 days but not affected at 32 days after treatment.

Krüger and Scholtz (1997) studied the brood ball production, adult emergence, developmental time, and reproduction of the dung beetle *Euoniticellus intermedius* and the adult emergence, development time, and adult weight of the dung beetle *Onitis alexis* in dung from cattle treated with injectable ivermectin. Control and treated cattle were housed, maintained on a diet of hay and lucerne, and dung collected 1, 2, 3, 4 and 7 days after treatment and weekly thereafter for a period of 7 weeks. Pairs of adult beetles were then supplied with manure from treated or control cattle. Adult emergence of *E. intermedius* was inhibited in dung collected 2 to 7 days after treatment, and reduced in dung collected 1 and 14 days after treatment, whereas adult emergence of *O. alexis* was reduced in dung collected 2 to 7 days after treatment. Dung collected 28 days and 21 days after treatment prolonged the development of *E. intermedius* and *O. alexis*, respectively. Adult *E. intermedius* that emerged from dung collected 1-14 days after treatment had reduced reproductive success for 1 week, compared to beetles that emerged from control dung. Brood ball production of *E. intermedius* was unaffected by residues in dung, with the exception of dung collected 3 days after treatment, and the live weight of *O. alexis* was unaffected by ivermectin residues. A field study conducted by these workers (Krüger and Scholtz, 1998b) found no reduction in numbers of both species in pastures stocked with cattle treated with ivermectin (subcutaneous administration) 7 days previously, highlighting that pat-level effects studies do not necessarily indicate population effects.

Floate (1998a) observed that the adult emergence of Diptera *Sepsis* sp. and *Coproica mitchelli*, one species of eucloid wasp, and Coleoptera *Cercyon quisquilius* and *Cercyon pygmaeus*, were effected by cattle dung excreted up to 12 weeks post-dosing with IVOMEC Pour-On, formed into large (0.5 L) artificial pats. All other dung fauna species were less affected or unaffected. The relative species sensitivities were consistent with previous studies; cyclorrhaphous flies were more sensitive than nematocerous flies (some of which were unaffected by treatment), and dung beetles were typically least sensitive. Floate (1998a) also noted the typical variability in species sensitivity, e.g. contrary to the generally greater sensitivity of other cyclorrhaphous species, no effect on *Scathophaga steroraria* and *Scathophaga furcata* were observed. The lack of effects on *S. steroraria* under field conditions contrasts with the high sensitivity of this species to ivermectin residues in laboratory tests (Strong and James, 1993), and emphasizes that laboratory testing does not necessarily indicate effects from field exposure.

It should be noted that feed effects were not accounted for in Floate's (1998a) study and may have affected the duration of effects in two ways. (1) Cattle were grain-fed and this is likely to substantially (about 5-fold) increase the levels of ivermectin in the feces relative to levels observed in feces from pasture-fed cattle (Cook *et al.*, 1996). (2) A non-pasture diet has been shown to reduce the utilization of cattle dung by beetles (Dadour and Cook, 1995). In Floate's experiment, cattle were penned, changed onto a diet of either alfalfa or barely silage, and then treated. It is therefore possible that the change to a non-pasture diet at the start of the study could cause a change in properties of the feces with time, in approximate coincidence with excretion of ivermectin, and that this feed effect might reduce the utilization (and therefore adult emergence) of dung by the insect community. Pre-treatment feces were used as controls, and therefore do not account for effects from change in diet. The authors were aware of the second potential feed effect, and carried out an additional experiment to investigate this. In the additional experiment, control cattle were maintained on the same diet, for the same length of time, and samples collected at the same intervals (and paired with) samples collected from treated animals. Data shows that adult emergence in control feces was greatly reduced from dung collected 6–16 weeks (in 3 of the 4 species) and 2–16 weeks (in the 4th species) "post-treatment", relative to adult emergence from dung collected 0 – 4, or 0 weeks "post-treatment", respectively. A very significant feed effect is indicated and shows that adult emergence from dung excreted by untreated cattle at 6–16 weeks was far less than adult emergence from dung collected earlier. While the data does not allow either feed effect to be quantitatively accounted for in the other experiments, both would act to increase duration and scope of effects in the study relative to effects from use on pasture. Therefore, this study likely reports the combined impact of ivermectin treatment and a non-pasture diet on dung fauna, and does not reflect effects from cattle treated on pasture.

Floate and Fox (1999) investigated effects of ivermectin in a study of a wasp parasitoid, *Muscidifurax zaraptor*, whose pupae were developed in the larvae of house flies exposed to ivermectin. IVOMEC Pour-On for Cattle was dissolved in ethanol and mixed with fly-rearing medium. Controls used medium only and medium fortified with ethanol. The ethanol was then removed by drying, and the control and ivermectin-fortified media was mixed with brewers yeast and water, and then seeded with fly eggs. The resultant fly pupae were collected, cleaned, and placed in containers with adult parasitoids for 24 hours. Fly

pupae were then removed and the pupation, emergence, male and female development time of the parasitoids, were recorded. F1 parasitoids were then placed with fresh fly pupae (raised in ethanol- and ivermectin-free medium) and the emergence of F2 parasitoids was measured using the same methodology. Fly pupae exposed to 0.25 – 1.5 mg/kg ivermectin produced about 63% fewer parasitoids than control fly larvae. An exception to the later effect was the lack of significant effect on parasitoid emergence from hosts exposed to 1.25 mg/kg ivermectin. More parasitoids (about 23%) emerged from fly larvae exposed to 0.01 mg/kg ivermectin. No effects on unemerged parasitoids (i.e. mortality), male and female development, fecundity, or sex ratios of offspring, were observed. The authors concluded that altered host quality was a mechanism by which fecal residues of ivermectin may affect insect activity in dung of treated cattle, but noted that the importance of this phenomenon under field conditions was undetermined. The presence of excipients (IVOMEC Pour-On contains 0.5% w/v ivermectin, 0.05% w/v triethylamine, and 20% Crodomol CAP in about 80% isopropanol) were not accounted for in controls.

2. Population-level

Forbes (1993) considered typical parasitic control programs, and global and regional use of avermectins in cattle and horses, to evaluate the potential environmental impact of ivermectin residues from treatment of animals on pasture. The author concludes that risk to the environment is limited by (1) the presence of untreated animals (2) lack of synchronicity of use (3) lack of synchronicity between treatments and dung insect breeding seasons (4) treatment of housed or penned animals, and (5) the finite time after treatment that effects may be experienced by sensitive insects. The absence of gross effects on dung degradation during 10 years of commercial use is offered as support for the analysis.

Lysyk and Colwell (1996) studied hornfly (*H. irritans*) control in pastured cattle by diazinon-impregnated ear tags, IVOMEC Pour-On, and a combination of both products, under conditions of constant fly challenge. Modeling indicated that diazinon ear tags provided >90% reduction in adult numbers for 50 days, and that control declined rapidly thereafter. IVOMEC Pour-On provided > 90% reduction in adult and larvae numbers for 5-15 days, > 50% reduction in adults for 18-26 days, and > 50% reduction in larvae for 19-24 days. The authors note that other studies generally found longer effects on adults, and similar duration of effects on larvae. The shorter duration of effects on adults in their work was attributed to the close proximity of untreated cattle, facilitating dispersal of adults into the treated herd and thereby facilitating population recovery.

A review by Wratten and Forbes (1996) on the environmental assessment of veterinary avermectins in temporate pastoral ecosystems concludes that there is no potential for serious effects on insect populations, food-webs, or dung degradation. Review topics include patterns of avermectin use, potential exposure of non-target insects, dung flora and fauna (breeding, effects of dung composition, competition, mobility, aggregation), dung decomposition, and data from micro-, meso- and field-scale studies on effects of avermectins. Micro-scale studies show larvae of corprophagous insects are generally more susceptible than adults (generally no effects on adults) and non-specific reductions in

reproductive capacity of some species have been found. Cyclorrhaphan (Diptera) larvae are more sensitive than Nematocera (Dipetera).

In a review article, McKellar *et al.* (1997) considered the ecotoxicological risk associated with benzimidazoles, levamisole, morantel, the avermectins, and milbemycins. Effects of endectocides on non-target species are summarized, with dipteran flies and coleoptera found to be most sensitive. No effects to species other than dung fauna were described. Of the products considered, avermectins and milbemycins were considered to present the greatest potential ecological risk, based on deleterious effects to non-target species, the amount of active excreted, as well as the temporal nature and stability of excreted residues. An increase in potential ecotoxicological risk is expected if sustained release devices are employed. The risk posed by avermectins and milbemycins on the environment was then considered. Estimates of the amount of feces containing drug residues produced by individual animals following normal annual treatment strategies reveal that a very large proportion of feces will not contain drug residue in most husbandry systems. Therefore, there is a large refugia for insect fauna and it was considered unlikely that any global or regional ecotoxicological impact could arise from use of avermectins or milbemycins.

Krüger and Scholtz (1998a,b) conducted field studies investigating structural effects on the dung insect community in South Africa from the combined stress of ivermectin use and drought (1992/93), and also from ivermectin use during a period of high rainfall (1993/94). The two annual trials differed in two other respects. In the 1993/94 study, high rainfall 2 months after treatment greatly reduced beetle activity and results for this period were not discussed. Additionally, control and treated groups were pastured in pairs of adjoining fields in 1993/94, whereas the 1992/93 study pastured treated and control groups in isolated fields. Both trials were conducted from December to March, during a period of peak beetle activity. A herd of 80 cows (some with calves) was randomly allocated into 4 groups and confined to two pairs of adjacent pastures. The two pairs of pastures were about 200 m apart and abutted other pastures with untreated cattle. Cattle in two pastures were treated with ivermectin (injectable formulation, 0.2 mg/kg) and the other two groups were untreated controls. Treatment of all cattle in one pasture was considered a worst-case scenario, as the management norm in South Africa is to treat only weaners. Artificial pats formed from another untreated cattle herd were placed in a transect across each paddock on the day before treatment, and at monthly intervals for 3 months thereafter. Ten fresh natural dung pats were also labeled in each pasture at monthly intervals for a period of 3 months after treatment. A total of 42, 422 specimens were collected from these samples in 1992/93, including about 81 beetle and 7 fly species. A total of 47, 611 specimens were collected in 1993/94, including about 91 beetle and 7 fly species. Shannon diversity indices, species richness and evenness of the dung insects in treated and control dung samples were measured, to indicate the structure of the community.

In the first trial (1992/93), pretreatment measurements showed that the pastures designated for treated cattle supported a significantly lower species richness and diversity than the pastures designated for control animals. The authors therefore emphasized that differences between treated and control pastures may simply be characteristics of the pastures, and effects from ivermectin residues could not be isolated from these intrinsic differences.

However, it appeared that ivermectin reduced the species diversity and evenness of the dung insect community 1 and 3 months after treatment. Results from natural and artificial pats collected 2 months after treatment were contradictory and showed few differences between communities in treated and control paddocks. The authors proposed that the transient recovery in natural pats at 2 months was due to emergence of beetles from the pre-treatment period. In another publication (Scholtz and Krüger, 1995) the same workers report results from a trial conducted at the same location, at the same time, following apparently the same protocol. However, different results are reported; significant differences in diversity and evenness were reported for the first month only in the 1995 article. Why these data differ, and why the differences are not discussed in either paper, is not clear. However, the different conclusions from apparently very similar (or perhaps in fact the same) study may be a consequence of interpreting the seemingly subtle effects. This subtlety is indicated by the following observations from the 1998 article (which contains more detailed data):

(1) Data varied significantly between natural and artificial pats.
(2) Data varied significantly in pretreatment samples.
(3) At a maximum, parameters differed by a maximum of 30% between treated and control groups.
(4) Despite slightly lower diversity and evenness, more individuals were found in the natural pats of treated compared with untreated animals.

In the second trial (1993/94), pre-treatment data showed very similar diversity and evenness in the dung insect community on pastures containing treated and untreated cattle in the previous years trials. Post-treatment data showed that larvae and pupae were reduced in treated dung compared with control dung 7 days after treatment, but that there were no significant differences in diversity or evenness 1 and 3 months after treatment. The authors proposed two principal factors were responsible for the overall lack of effects at 1 to 3 months in the second trial. Firstly, the community was not exposed to drought stress and secondly, the spatial scale of exposure was smaller (treatment groups in separate 80 ha pastures as opposed to the previous trial, where both treated groups were on adjoining pastures, totaling about160 ha).

The authors therefore concluded that the environmental impact of ivermectin is likely to be determined by several factors, including spatial scale of treatment (i.e. proximity of treated versus untreated cattle) and the prevailing climactic conditions/stressors. Diversity and evenness of dung fauna populations was reduced in pastures containing ivermectin-treated cattle for 1-2 months under drought conditions, but was unaffected under high rainfall conditions. Populations were unaffected by treatment during the previous season. These studies are key because they consider:

(1) Population effects, as opposed to pat-level effects.
(2) Acute (monthly) as well as chronic (two grazing seasons) effects.
(3) Pats (artificial and natural) produced by pastured, not grain-fed, cattle.
(4) Worst-case conditions (period of high beetle activity, all cattle in treated groups were treated, extreme climatic conditions).

Sherratt *et al.*, (1998) quantitatively estimated population effects on dung insects from avermectin residues using a tactical simulation model. Chemical fate (excretion profile and

degradation in dung), exposure (scale of avermectin use, time of attractiveness of dung to insect species and seasonal activity of insect species), and toxicity (direct effects on dung insect species) were integrated into the model. No account was taken of sublethal effects, or of density dependent compensatory effects by insect populations. The model provided an output of instantaneous (daily) and cumulative (proportion of the seasonal population) mortality from avermectin treatment. Model outputs varied considerably among the different simulations, but the majority of estimates under realistic farming practices predicted a maximum cumulative mortality of < 25% for the most sensitive modeled species, on a per farm basis.

3. Summary, effects and duration of effects on dung fauna

Combined, the pat and population-level studies conducted in the last decade support the general understanding that ivermectin is toxic to the larvae of many dung fauna, but that dung fauna populations recover quickly from any local perturbations. Both lethal and non-lethal effects have been observed; generally cyclorraphan larvae are more sensitive than nematoceran larvae, Diptera are more sensitive than Coleoptera, and adults are usually unaffected or far less sensitive than larvae. The pat-level effects studies indicate that the duration of effects is strongly influenced by the sensitivity of individual species, formulation, and route of administration. Population-level effects studies indicate that duration of effects is strongly influenced by the proximity of untreated animals, synchronicity between treatment and insect activity, and the presence of concomitant stressors (such as climate variations). The prolonged effects observed in some species by Floate (1998a) were likely contributed to by feed-effects. Data from studies measuring pat-level effects on specific species are summarized in the tables below.

Sensitivity of Fly Larval Development / Adult Emergence to Ivermectin Residues in Dung Pats of Cattle

Species	Days Post Dose, Dung With No Effect on Larval Development / Adult Emergence		Administration
*H. Irritans**	35-42	(Fincher, 1996)	Pour-On
	28	(Miller *et al.*, 1981)	Oral Capsule
	28	(Schmidt, 1983)	Intramuscular
	14	(Sommer *et al.*, 1992)	Pour-On
M. autumnalis	28	(section 2.D) [a]	Fortified feces
	14	(Sommer *et al.*, 1992)	Pour-on
M. domestica	21	(Wardhaugh *et al.*, 1996)	Subcutaneous
M. nevilli	42	(Krüger and Scholtz, 1995)	Subcutaneous
M. vetustisma	35	(Wardhaugh *et al.*, 1996)	Subcutaneous
	32	(Wardhaugh and Mahon 1998)	Subcutaneous
N. cornicina	13-14**	(Gover and Strong, 1995) [b]	Fortified feces
O. cornicina	14	(Sommer *et al.*, 1992)	Pour-on
	32	(Wardhaugh and Rodriguez-Menendez, 1988)	Subcutaneous

* IVOMEC Pour-On and IVOMEC EPRINEX Pour-On have label claims for control of *H. irritans* for 28 and 7 days, respectively

** Measured in the presence of excipitents, not accounted for in controls

[a] A laboratory study measured an NOEC = 61.2 mcg/kg. No emergence of adult *M. autumunalis* is expected from feces excreted between days 1 and about 13 –14, some emergence between days 14 and 28 and the same emergence rate as from control feces after 28 days, based on fecal elimination profiles after pour-on administration reported by Herd *et al.* (1996) and Sommer *et al.* (1992)(discussed in detail in section 2.D).

[b] Gover and Strong (1995) measured an LC_{50} = 139 mcg/kg in the presence of excipients. Sommer *et al.* (1992) found ivermectin levels fell below the detection limit, 7.5 mcg/kg (wet-weight basis, see section 2.D), by days 13-14 after pour-on administration. As this ivermectin concentration is more than 18 times less than the LC_{50}, effects are expected for <13-14 days.

Sensitivity of Beetle Larval Development / Adult Emergence to Ivermectin Residues in Dung of Cattle

Species	Days Post Dose, Dung With No Effect on Larval Development / Adult Emergence		Administration
O. gazella	17	(Sommer and Overgaard Nielsen, 1992)	Subcutaneous
	21	(Roncalli, 1989)[a]	Subcutaneous
	21	(Fincher, 1992)	Subcutaneous
	14-21	(Fincher, 1996)	Pour-On
Aphodius spp.	10	(Madsen *et al.*, 1990)	Subcutaneous
	13-14	(Sommer *et al.*, 1992)	Subcutaneous & Pour-On
	14	(Strong and Wall, 1994b)	Subcutaneous
E. intermedius	14	(Fincher, 1992)	Subcutaneous
	7-14	(Fincher, 1996)	Pour-On
	21	(Krüger and Scholtz, 1997) [b]	Subcutaneous
C. hispanus	16	(Wardhaugh and Rodriguez-Menendez,1988)	Subcutaneous
D. quinquedens	16	(94% emergence) (Sommer *et al.*, 1993)	Subcutaneous
E. alexi	21	(Krüger and Scholtz, 1997) [b]	Subcutaneous
E. fulvus	10	(Lumaret *et al.*, 1993) [b]	Subcutaneous

[a] Dose of 0.3 mg/kg

[b] A slight delay in development was observed relative to controls, but no inhibition or increased mortality.

B. Excretion profile of ivermectin and utilization of dung by dung fauna

Bernal *et al.* (1994) measured the concentration of ivermectin and the amino acid composition in the feces of cattle treated with ivermectin (subcutaneous administration at 0.2 mg/kg), and untreated controls, with time. The level of ivermectin in fresh feces reached a maximum concentration of approximately 400 mcg/kg on day 5 post-dosing and decreased rapidly to levels below the detection limit of the assay (10 mcg/kg) by day 12 post-dosing. Ivermectin was detected in feces exposed to field conditions up to 6-7 days post-dosing, but no ivermectin was detected in feces exposed to field conditions for 7 to 30 days. The amino acid profile of dung differed between treated and untreated groups, and it was proposed that this might influence the attractiveness of the dung to dung beetles. Cattle husbandry was not defined in this publication, although pasturing is implied from the description of dung degradation studies in the field. Also, little detail was provided on the protocols followed to determine the ivermectin levels in dung exposed to field conditions.

Barth *et al.* (1995) conducted five trials to investigate the effects of small changes in the water content of dung pats on dung fauna (Coleoptera, Diptera, nematodes and earthworms) and dung degradation. The number of Diptera and Coleoptera larvae and earthworms increased with increasing water content of the pat at the time of deposition, whereas the number of nematodes was unaffected by water content at deposition. Increased water content of pats at deposition significantly increased the rate of pat degradation, as determined by measurements of surface area, wet weight and organic matter content. The authors concluded that small differences in initial moisture content (1-2%), which might not be visible, may have a significant impact on the colonization and development of Diptera, Coleoptera, and earthworms, and may also impact degradation of the pat. These small changes in water content occur even between naturally voided and formed control pats (and can also occur due to differences in diet and gastrointestinal parasitic burden). The authors conclude that moisture content needs to be determined and properly paired control and experimental pats need to be compared in degradation studies.

Cook *et al.* (1996) investigated the effect of diet on the excretion profile of ivermectin in the feces of cattle treated with ivermectin (subcutaneous administration, 0.2 mg/kg). The concentration of ivermectin in the feces of grain-fed cattle was significantly higher (over 5 times) than in pasture-fed cattle. A Cmax of 360 mcg/kg and 90 mcg/kg was observed on day 6 and day 8 post-treatment, respectively, in the feces of grain-fed and pasture-fed cattle, respectively. Ivermectin levels dropped to below the detection limit by day 14 and day 15 post-dosing, in the feces of grain-fed and pasture-fed cattle, respectively. The authors proposed that the large feed effect was mainly due to differences in fecal volume, as grain-fed cattle excrete significantly smaller quantities of feces than pasture-fed cattle.

Dadour and Cook (1996) investigated the adult survival, brood mass production, emergence and progeny size of the dung beetle *Onthophagus binodis* inhabiting the dung of cattle maintained on different diets. One group of cattle were fed a grain ration of lupins (15%), oats (20%) and barley (65%), a second group were fed a grain ration of lupins (70%) and hay (30%) and a third group were pasture-fed. Dung from the grain-fed cattle contained higher levels of nitrogen, less water, and had a lower pH, than dung from the pasture-fed cattle.

Adult mortality and the emergence pattern of *O. binodis* was unaffected by differences in cattle diet. However brood mass production, F1 progeny survival and progeny size were all significantly lower in the dung of grain-fed cattle. Typical Australian feedlot diets can adversely effect the dung beetle *O. binodis*.

Herd *et al.* (1996) measured the plasma and fecal elimination profiles of ivermectin in four groups of young cows, treated either with an IVOMEC SR Bolus (1.72 mg/day for approximately 135 days), IVOMEC Pour-On (0.5 mg/kg), IVOMEC Injection (0.2 mg/kg), or untreated control. Blood and feces were collected 1 day before treatment and days 1, 2, 3, 7, 14, 21, 28, 35, 42 and 49 after treatment. Cattle were maintained in stalls on a roughage diet. The ivermectin fecal elimination profile (wet weight basis) of cattle treated with the IVOMEC SR Bolus displayed mean concentrations of about 500 mcg/kg (14 & 49 days). The elimination profile of cattle treated with IVOMEC Pour-On gave a sharp Cmax of 2.8 mg/kg 2 days after treatment and fell to 6 mcg/kg by day 28. The elimination profile of cattle treated with IVOMEC Injection gave a Cmax of 200 mcg/kg 3 days after treatment and fell to 10 mcg/kg by day 28. The authors discussed potential environmental impact from effects of drug residues to dung dependent insects, in relation to the excretion profile observed for the different formulations. They raise the concern that the higher Cmax observed for the pour-on, compared with the injectable formulation, may lead to increased effects on larvae from the former relative to the latter. However, the data show that, while the Cmax from the pour-on is higher, residues deplete more rapidly and are below the level observed for the injectable formulation by day 7 post-treatment. Therefore any effects arising from the pour-on would be less than any arising from the injectable formulation by day 7 post-dosing. In conclusion, the authors noted that the fecal elimination profile from the IVOMEC SR Bolus indicates this formulation had the greatest potential for environmental impact.

Alvinerie *et al.* (1998) measured the level of ivermectin in plasma and feces prior to, and at regular intervals after, treatment of calves with the IVOMEC SR Bolus. The maximum concentrations in plasma and feces were 28 ng/mL and 4.2 mcg/g, on day 15 and day 4 post-dosing, respectively. Steady-state concentrations of ivermectin in plasma and feces were 20 ng/mL and 1.18 mcg/g, respectively, and steady-state concentrations were maintained until 120 days post-dose. Levels in plasma and feces dropped dramatically after shut down of the bolus; plasma and fecal residues fell from approximately steady-state levels to 0.05 ng/mL and 0.00267 mcg/g at 160 days post-dose, respectively. The AUC of the fecal elimination profile indicated that 80 to 90 percent of the administered dose was excreted in the feces as ivermectin.

Floate and Gill (1998) compared data from pitfall trapping of dung beetles (Scarabaeidae) in two sites in southern Alberta from 1993 to 1995 with data from other sources. The species found at the two studied sites (Lethbridge Research Centre and the Animal Disease Research Centre, both near Lethbridge, Alberta) were similar to those reported elsewhere on pastures in temperate North America. Typically, pastures are dominated by European species of *Aphodius*. An exception was *Onthophagus nichicornis*, which comprised 50% of the beetles captured at the Animal Disease Research Centre in 1995 but was rarely or not observed in the USA. Individual species displayed two general seasonal patterns. *A. prodromus*, *O.*

nichicornis, *A. fimetarius*, and *A. distinctus* were bimodal, peaking in the spring and fall. *A. vittatus*, *A. ruricola*, *A. coloradensis*, *A. granarius*, *A. fossor* and *A. haemorrhoidalis* exhibited a single peak in spring to mid-summer. These two patterns of peak activity were considered to correspond to two different over-wintering strategies. Most of the species found were transcontinental, i.e. *A. distinctus*, *A. fimetarius*, *A. fossor*, *A. granarius*, A. *haemorrhoidalis*, *A. leopradus*, *A. pinguis*, *A. prodromus*, *A. vittatus* and *O. nuchicornis*. A minority of the species found were restricted to western Canada, i.e. *A. pinguellus*, *C. praticola* and *A. coloradensis*, with the latter apparently restricted to Alberta. The wide distribution of the majority of these species is at least partially attributed to their feeding behavior. *A. distinctus*, *A.eraticus*, *A. fimetarius*, *A. fossor*, *A. granarius*, A. *haemorrhoidalis* and *A. prodromus* do no show pronounced preferences for dung from any one animal species, except that they typically prefer cattle dung and rarely use deer dung. *A. vittatus* is a generalist and *A. pinguis* is a detritovore. *A. ruricola* and *A. leopardus* are associated with deer dung. Given the broad distributions of cattle and deer, the authors contend that the distribution of *Aphodius* species in Canada will not be restricted by the availability of suitable dung.

1. Summary, excretion profile of ivermectin and utilization of dung by dung fauna

New studies provide data on the fecal excretion profile of ivermectin after administration using pour-on, injectable, and SR Bolus formulations. Pour-on administration results in the highest peak in ivermectin residues (2.8 mg/kg on day 2, Herd *et al.*, 1996) but residues were quickly excreted and fell below levels observed after subcutaneous administration by day 7 post-dosing. Data from treatment of cattle by injectable formulations in housing and on pasture are summarized in the table below.

Cmax (mcg/kg)	Tmax (days post-dosing)	Husbandry	Study
400	5	pasture	Bernal *et al.*, 1994
360	6	housed	Cook *et al.*, 1996
90	8	pasture	Cook *et al.*, 1996
200	3	housed	Herd *et al.*, 1996

Herd *et al.* (1996) and Alvinerie *et al.* (1998) report steady-state concentrations of 0.5 and 1.18 mg/kg after administration of the IVOMEC SR Bolus, and the latter study reports a rapid drop in ivermectin levels in feces after shut down of the bolus, about 120 days after administration.

A non-pasture diet has been observed to increase (5-fold) ivermectin levels in feces and also to adversely effect dung beetles. Very small changes in water content of feces have been found to effect dung fauna and dung degradation. These studies emphasize the importance of using properly paired control and treated samples in studies on dung fauna and dung degradation, and employing husbandry practices as close as possible to actual use practices.

C. Soil organisms

Gunn and Sadd (1994) studied the effect of a commercial formulation of ivermectin, added directly to soil, on the earthworm *Eisenia fetida*. The formulation contained ivermectin at

0.08% w/v and a non-volatile organic solvent, an emulsifier, a stabilizer (benzyl alcohol) and buffer salts at approximately 21, 8, 3 and 1% w/v or v/v, respectively. Thus, their test system contained total excipients at approximately 372 times higher than the level of the ivermectin. A 14-day LC_{50} value of 15.8 mg/kg, a NOEC of 4 mg/kg based on weight change and a NOEC of 2 mg/kg based on cocoon formation, all on a dry-soil weight basis, were reported, in contrast to the data for non-formulated ivermectin tested using the U.S. FDA's recommended method (Halley, *et al.*, 1989). Repellency from soil containing 20 mg/kg of ivermectin was also noted. The authors attributed this repellency to ivermectin, but failed to note that benzyl alcohol was present at 680 mg/kg, an emulsifier was present at 1820 mg/kg and the non-volatile organic solvent was present at 4704 mg/kg in this sample. The authors also attributed the effects on cocoon formation at 4 mg/kg soil and above to the presence of ivermectin. Again, they did not consider the role of the non-volatile organic solvent, the emulsifier, the stabilizer or the buffer salts as contributing to or causing this effect. Gunn and Sadd did not include a solvent control. They assumed either (1) that the emulsifiers and stabilizers in the formulation had no effect on the observed toxicities or (2) that these emulsifiers and stabilizers would be present in the feces of treated animals to the same extent as in the formulation. Since the emulsifiers and stabilizers are present at much higher concentrations than the ivermectin in the tested formulation, the observed toxicities might have been due to the emulsifiers and stabilizers or to a synergy (altered solubility, enhanced bioavailability or enhanced uptake) between the compounds present. Contrary to their latter supposition, the emulsifiers and stabilizers are much more water soluble than ivermectin and would be eliminated in the urine or metabolized before being eliminated. Thus, neither of their assumptions is valid; the excipients would not be present in the feces of treated cattle and the values obtained represent the toxicity of a particular formulation, not of ivermectin. Inclusion of a solvent control sample would have allowed for an assessment whether these organic compounds and solvents are toxic or repellent, but would not have been valid for correcting the observed results since enhanced solubilization, bioavailability or synergy of effects could not be predicted from a solvent control. Therefore, Gunn and Sadd's results do not represent the toxicity of ivermectin. The toxicity data reported in the original EAs are the appropriate data to use not the data for the formulation.

Even so, it is also instructive to compare the effect levels determined for ivermectin (data from EAs) and the formulated, ivermectin-containing product (data from Gunn and Sadd, 1994) with the levels of ivermectin expected in soil fertilized with excreta from dosed cattle. The soil concentrations of ivermectin predicted in the original EAs for the subcutaneous injection and by the pour-on products are 0.00027 mg/kg and 0.00009 mg/kg, respectively. The concentrations in the soil in the EAs were not reduced by invoking any binding to soil that might render the ivermectin unavailable for uptake by earthworms. To compare these predicted environmental concentrations (PEC) with the predicted environmental no-effect concentration (PNEC), a safety factor of 100 is used with the acute toxicity data; that is LC_{50} divided by 100 (EMEA/CVMP, 1997). Thus, using the LC_{50} value of 315 mg/kg determined following the procedure (TAD 4.12) in the FDA's Technical Assistance Handbook (FDA, 1997), a PNEC of 3.15 mg/kg is predicted. A PNEC can also be predicted using chronic data and an extrapolation factor of 10 (EMEA/CVMP, 1997). This would lead to a PNEC of 1.2 mg/kg, using the observed NOEL from the sub-acute study with ivermectin (Halley *et al.*, 1989). Thus, a worst-case PEC/PNEC ratio of 2.3×10^{-4} (0.00027/1.2) is predicted. Since

this ratio is much less than 1, no chronic effects from ivermectin on earthworms are predicted and further studies are not warranted (EMEA/CVMP, 1997). Even using the data from Gunn and Sadd for the formulated product, PNEC values of about 0.2 mg/kg soil are predicted (using 15.8 mg/kg/100 or 2 mg/kg/10). Thus, the PEC/PNEC ratio for the formulated product is about 0.0014 (0.00027/0.2); again much less than 1. These calculations indicate that concentrations of ivermectin in soil are at least 3 orders of magnitude below those that might lead to reproductive effects in exposed earthworms. This is true even using the data from the study that assessed the effects caused by a formulation mixed into soil.

PEC/PNEC RATIOS FOR EARTHWORM TOXICITY FOR IVERMECTIN AND A FORMULATED PRODUCT CONTAINING IVERMECTIN [a]

Material Tested	LC50, mg/kg	NOEC, mg/kg	PNEC, mg/kg [d]	PEC/PNEC Ratio
Ivermectin [b]	315	12	1.2	0.00002
Formulation [c]	15.8	2	0.2	0.0014

[a] PEC = 0.00027 mg/kg soil, the soil concentration from the original EA for IVOMEC Injection for Cattle
[b] data from Halley, et al. (1989)
[c] data from Gunn and Sadd (1994)
[d] from LC50/100 or NOEC/10, see text

Furthermore, it is conservative to use a safety factor of 100, comprised of a factor of 10 to extrapolate from acute to chronic endpoints and another factor of 10 to account for potential species variability, to calculate a PNEC. The acute to chronic ratios (ACR) of effects for abamectin on *Daphnia*, mysid shrimp and rainbow trout are 6.5, 3.8 and 4.6 (Wislocki *et al.* 1989). Based on these ACR values, determined for two invertebrate and one vertebrate species, a similar ACR would be expected for earthworm species. Thus, use of a safety factor of 100 to calculate the earthworm PNEC values and the PEC/PNEC ratios for avermectins is additionally conservative. The margin of safety in the exposure of earthworms to residues of avermectins is therefore likely even greater than indicated in the table above.

In a field study, Barth *et al.* (1994a, discussed in Section 3. A. 1. above) found no effect on the range and numbers of earthworms associated with the pats of cattle treated with ivermectin (0.2 mg/kg) by subcutaneous administration 3, 8, and 13 weeks after turnout. There were no treatment-related effects measured at 63 days after deposition on earthworm biomass, numbers of earthworm species, or on numbers of adult or juvenile earthworms in soil underneath pats voided 0, 3, 7, 14 or 28 days after treatments. The lack of effects on the numbers of adult and juvenile earthworms or on biomass under typical use patterns confirms the predictions based on laboratory studies that ivermectin use has no chronic effects on earthworms and does not affect earthworm populations.

Comparison of Earthworms after 63 Days in Soil Beneath Naturally Voided Dung Pats of Cattle Treated Subcutaneously with Either Levamisole (LEV) or Ivermectin (IVM)[a]

Days post dose:	0		3		7		14		28	
Treatment:	LEV	IVM	LEV	IVM	LEV	IVM	LEV	IVM	LEV	IVM
No. of Adults	20	22	32	37	27	24	41	71	16	29
No. of Juveniles	24	47	54	38	28	30	19	87	34	56
No. of Species	6	6	5	5	8	6	8	7	8	9
Biomass, g	7.0	7.4	7.6	7.7	4.8	7.2	9.0	11.4	11.1	11.3

[a] Summarized from Barth *et al.* (1994a). Data from 5 pats per treatment at each time post dose.

McCracken and Foster (1993, also discussed in Section 3. A. 1.) included earthworms in the invertebrates studied in a multivariate analysis to examine the effects of ivermectin in artificial pats. The results of the analysis with respect to earthworms are not discussed by the authors and are not easily discerned by inspection of the data. However it appears that earthworms were present in a large proportion of, and prevalent in, treated and untreated pats, as well as in the soil beneath the pats. Wratten *et al.* (1993) found no effect on earthworm mean weights and numbers in the pats of cattle treated either with IVOMEC injection or IVOMEC SR Bolus, compared with pats from untreated cattle. The study was conducted over two grazing seasons in the UK, with cattle treated once during each grazing season (this study is described subsequently in more detail, in section 6). Earlier studies also investigated effects on earthworms. Wall and Strong (1987) observed similar numbers of earthworms in pats and underlying soil of treated (IVOMEC SR Bolus) and untreated cattle. Madsen *et al.* (1988) found no effect on earthworm activity or biomass over a period of 98 days in artificial pats in pots, formed from dung collected 24 hours after treatment with ivermectin (subcutaneous administration) relative to dung from untreated cattle. No effects on earthworms were found in field and laboratory studies of dung from cattle after treatment with ivermectin (Madsen *et al.*, 1990). A field study conducted by Barth *et al.* (1993) found treatment of cattle with the IVOMEC SR Bolus had no effect on soil-dwelling nematodes or earthworms.

1. Summary, soil organisms

New studies (i.e. 1993 – 2000) investigating the toxicity to soil organisms have focused on effects to earthworms. Effects on earthworms have been observed in the laboratory at very high levels from exposure to a formulation containing ivermectin, but not from exposure to the dung of treated cattle. Results of studies appearing in the published literature since the original EAs do not alter the conclusions in the original assessment.

D. Higher trophic levels

In a review article, McKellar *et al.* (1997, discussed in section 3.A) considered the ecotoxicological risk associated with avermectins. No evidence was presented or discussed to indicate effects to species other than dung fauna, or food chain effects on higher trophic levels. Considering the usage patterns of avermectins leads to the conclusion that there is a large refugia for insect fauna, and it is unlikely that any global or regional ecotoxicological impact could arise from use of avermectins. No impact on higher trophic levels is indicated.

McCracken (1993) considered risk to wildlife from use of avermectins, and presented two illustrative hypothetical scenarios for effects. The first scenario concerns rare dung fauna that may have limited ability to recolonize pasture locations. If such a species were sensitive to avermectin residues, then use of avermectins on pasture has the potential to impact populations. This potential concern can be addressed by considering the following: (1) The risk requires exposure of a rare sensitive species, yet no dung fauna are listed as endangered or threatened in the US. Furthermore, indigenous dung beetles in the US do not typically utilize cattle dung (preferring dung in forest environments, (Gordon 1983). Therefore only imported species of dung beetles would be exposed, which (by nature of their presence) are able colonizers. (2) The risk also requires a rare and sensitive species to be sufficiently distant from untreated dung, such that recolonization was affected. The pattern of use of ivermectin means that dung from untreated adult cattle is commonly available (in fact constitutes the majority of dung) on most farms using avermectins on pasture, and therefore recolonization would not be hampered by issues of isolation or mobility, even if only cattle dung were available.

The second scenario concerns pasture-birds suffering from a reduction in food supply. The potential for adverse effects in overwintering or young birds, which may rely more heavily on dung and soil fauna for food, are emphasized. These potential concerns are addressed by the following points. (1) Ivermectin is not typically used in cattle on pasture during the winter, therefore residues will not be present in dung available to overwintering birds. (2) Field studies have found that numbers of adult insects and earthworms are unaffected in pastures stocked entirely with treated animals (Barth *et al.*, 1994a; Krüger and Scholtz, 1998a). Therefore, even in areas of "worst-case" use of ivermectin, food supply (in the form of invertebrates) is not significantly affected. Furthermore, the scenario presented by McCracken requires that the supply of dung as insect food is a significant factor in the success of the wildlife species (as opposed to, for example, loss of habitat).

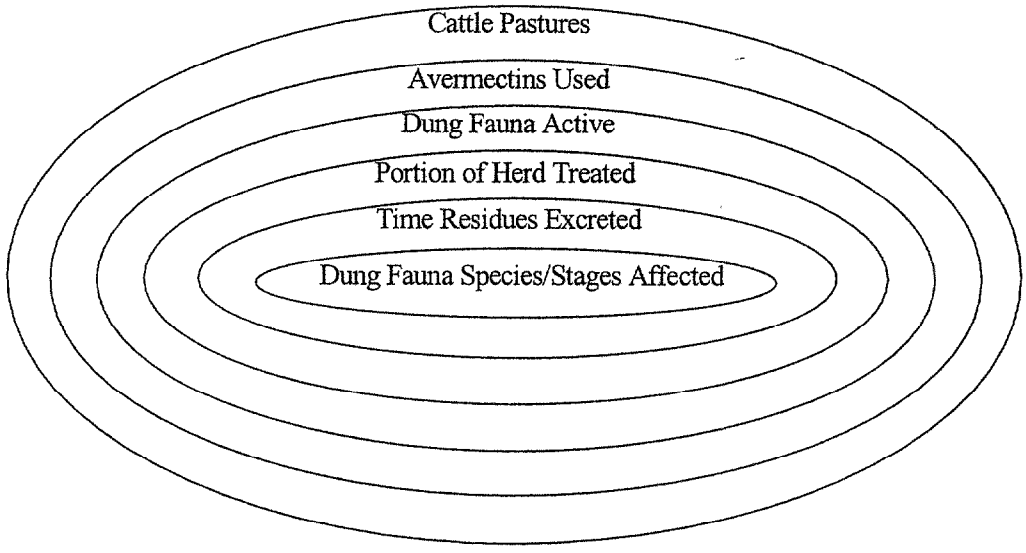
None of the potential concerns raised by McCracken (1993) are supported by observations or by data. An effect on insect populations is required to produce any putative impact on higher trophic levels, but whether pat-level effects are likely to impact insect populations was not considered. When these are considered, no significant effect on insect populations is indicated and therefore no impact on higher trophic levels are indicated. While the author states the scope of discussion includes effects on mammalian wildlife, potential effects are not addressed except to note that low mammalian toxicity means direct effects are unlikely.

Wratten and Forbes (1996) considered the potential for food-web effects on predators and parasites utilizing dung from animals treated with avermectins, but no data demonstrating a food-web effect was presented. Studies finding that predaceous beetles were not reduced in dung containing avermectins are referenced (Schmidt, 1983, Fincher, 1992; Barth *et al.*, 1994a,b) and two reports of effects on parasitoid wasps are noted (Schmidt 1983, Floate 1995). In a later laboratory study, Floate and Fox (1999) concluded that altered host quality, and not food supply, was the likely mechanism of effects on the parasitoid wasp. Wratten and Forbes (1996) note that the likely polyphagous and mobile nature of predators, coupled with the low proportion of pats likely to contain avermectins, indicates effects on predator populations will be negligible.

Floate (1998a, discussed previously in section 3.A) found that adult emergence of Diptera and Coleoptera larvae were sensitive to ivermectin residues in cattle dung in a species dependent manner. The author noted that reduced food supply might have contributed to effects on the adult emergence of some species with predaceous larvae, and on an eucoilid wasp, but that this putative effect could not be resolved from direct effects from ivermectin and this concern does not extend beyond dung fauna.

The potential for any population-level effects on dung fauna to impact on non-dung fauna species through food supply will likely be significantly affected by exposure of the dung fauna itself. Factors contributing to exposure of dung fauna (Forbes, 1993) are presented in the figure below;

Contributing factors in an exposure assessment of avermectins and dung fauna.



Floate (1998a) illustrates the importance of some of these exposure factors. The author notes that cattle are rarely treated on pasture with ivermectin in Alberta (typically treatment is given on entering feedlots) and concludes that lack of use on pasture alone (i.e. the first factor in the exposure assessment in the above figure) indicates there is no significant risk to the environment. Floate (1998a) also found that the insect population was reduced during

summer, such that the impact of ivermectin residues on dung insects could not be readily discerned. Treatment of cattle during this season of low dung fauna activity would therefore be unlikely to significantly impact dung fauna populations and consequently is unlikely to impact higher trophic levels. While the duration of effects observed in Floate's study (1998a) appear to combine effects from treatment with ivermectin with effects from change to a non-pasture diet, it is still apparent that effects are of finite duration, and that many species of dung fauna are unaffected.

1. Summary, higher trophic levels

Concerns for impact to higher trophic levels resulting from the use of avermectins in pastured cattle have been addressed in articles that incorporated data from field studies and the pattern of use of avermectins into risk assessments [McCracken (1993); Wratten and Forbes (1996)]. These assessments have concluded that no significant effects on insect populations are likely. No effect on higher trophic levels is therefore indicated.

E. Fungi

New data on the potential for ivermectin residues in dung to impact fungi is confined to one experiment in one preliminary paper, concerning the treatment of one heifer with IVOMEC Pour-On (husbandry undefined). Finnegan *et al.* (1997) found that the number of sporangia produced by *Pilobolus* fungus in feces from the treated heifer decreased in the order day 0 > pre-treatment > day 5 ≈ day 10 ≈ day 15, using fecal samples stored in the dark at 4 °C for a maximum of 15 days. The experiment was repeated 45 days later using the same fecal samples stored in the dark at 4 °C for 45-60 days. In the second replicate, the number of sporangia decreased in the order of day 0 > pre-treatment ≈ day 5 ≈ day 10 > day 15. The authors hypothesised that the lower sporangia production on days 5 to 15 (first replicate) and day 15 (second replicate), relative to pre-treatment feces, may indicate an anti-fungal effect from ivermectin, and also they note more data are required to clarify this. However, clarification does not appear to be necessary, as data is already presented that is inconsistent with the authors hypothesis.

(1) Sporangia production was significantly greater in day 0 feces (4 hours post-treatment) relative to pre-treatment feces in both replicates. Reduction in sporangia numbers therefore did not correspond with the excretion pattern of ivermectin. A greater reduction in sporangia numbers was observed between day 0 and day 5, 10 and 15 samples, than was observed between pre-treatment and day 5, 10 and 15 samples, in both replicates.
(2) Storage of feces in the dark at – 4 °C changed results, yet ivermectin is expected to be stable under these storage conditions.
(3) Storage of pre-treatment feces (in the dark at –4 °C) gave significantly different numbers of sporangia.

These observations indicate that effects were not likely related to the presence of ivermectin. Control experiments were all conducted using the same sample of pre-treatment feces, and were compared to feces samples collected at different times post-treatment. Sample variability with time (such as changing water or amino acid content or changing microbial

content) was therefore not accounted for by the control and may be responsible for the observed differences. No concern for effects from ivermectin residues on fungi inhabiting dung pats is indicated.

1. Summary, fungi

New data does not indicate risk to fungus from ivermectin residues. Previous studies investigating the antifungal activity of avermectin B_{1a} toward various filamentous fungi and yeasts (Burg and Stapely, 1989) found no significant antifungal activity in any species at a concentration of 1 mg/mL, a level about 1000-fold higher than the peak levels of ivermectin in the feces of pastured cattle dosed with IVOMEC products. No risk to fungi from use of avermectins in cattle is indicated.

F. Dung degradation

Floate (1998b) investigated whether ivermectin residues in dung attracted or repulsed coprophilous beetles, and found that neither effect could be discerned. If ivermectin residues had any attractive or repulsive properties, it was masked by other overriding factors (such as the effects of cattle diet, season, and species).

In a second publication, Floate (1998a, discussed in section 3.A and 3.D) investigated the effect of ivermectin residues on dung degradation. Large (1L) artificial pats, fortified with ivermectin (2 mg/kg) or unfortified, were placed on an ungrazed field under chicken wire in the spring. In other experiments described in the same article, artificial pats were prepared on sand in styrofoam plates before placement in the field. The authors do not report whether or not this same protocol was followed in the dung degradation study and therefore it is not clear if colonization by soil fauna, such as earthworms, might have been obstructed by a styrofoam plate. Degradation was measured as the portion of the pat degraded to a "sawdust" consistency. Two experiments on dung degradation were cancelled because heavy rain, foraging by birds, or weed growth disrupted the pats, before a third experiment was considered successful by the author. Floate's data on dung degradation therefore reflect conditions selected to discern effects from peak ivermectin residues on dung insects by:

(1) Employing one peak ivermectin concentration (expected to be present in feces for about 1 day post-dosing with IVOMEC Pour-On).
(2) Minimizing degradation via other major mechanisms (rain, foraging birds, trampling by cattle, and perhaps soil organisms such as earthworms (see above)).
(3) Measuring dung degradation using a parameter indicative of insect activity (i.e. observing a "sawdust" consistency) and not pat loss (such as changes in organic matter content or changes in dry weight).
(4) Disregarding experiments where degradation was influenced by factors other than dung-insects.

A study by Strong *et al.* (1996) compared the effects of fenbendazole and ivermectin residues on dung-colonizing Coleoptera and Diptera after administration by sustained-release bolus to cattle. Although this study did not investigate degradation of dung, the authors comment on

clear qualitative differences in pats from the different treatment groups (artificial pats in cages). Control pats and those from fenbendazole-treated cattle had a loose granular texture whereas pats from ivermectin-treated cattle were solid and compact. The study was conducted during a warm, dry summer (no rainfall for 33 days, average rainfall of 3 mm/day over 9 days) and it appeared that differences in larval insect activity contributed to differences in pat properties.

Barth *et al.* (1994a) found no difference in dung degradation (as determined by measurements of surface area, weight, organic matter content and monitored by photography) in pats deposited by cattle treated subcutaneously with ivermectin (0.2 mg/kg) and levamisole (5 mg/kg) 3, 8, and 13 weeks after turnout. Barth *et al.* (1995) also investigated the significance of water content on the colonization and degradation of cattle dung and found that small differences in initial moisture content (1-2%), may impact degradation of the pat. The authors conclude that moisture content needs to be determined, and properly paired control and experimental pats need to be compared, in dung degradation studies.

1. Summary, dung degradation

Data on dung degradation appearing in the literature over the period 1993-2000 are consistent with previous results. Delayed degradation has been observed in some studies utilizing protected or isolated artificial pats. However no impact has been observed in long-term field studies using natural pats, under conditions of high ivermectin use in several ecosystems (studies reviewed in EAs that were prepared for products containing ivermectin or eprinomectin). No impact on dung degradation under actual use conditions is indicated.

G. Conclusions of the review of key literature, 1993-2000

Review of the key literature establishes that new data on effects and duration of effects on dung fauna are consistent with earlier data. No effects on non-target organisms other than dung fauna are indicated, and field studies and risk assessments incorporating an exposure assessment indicate that dung insect populations recover quickly from any local perturbations. New data do not indicate impacts to soil organisms, higher trophic levels, fungi or on dung degradation, from the use of avermectins in cattle on pasture. The conclusions of previous EAs remain sound and appropriately consider risk in all environmental compartments and ecosystems of concern.

4. UPDATED ENVIRONMENTAL ASSESSMENT SUMMARY

The original conclusions in the Environmental Assessments of Merial's avermectin products for cattle are unchanged, namely that ivermectin and eprinomectin residues in feces from dosed cattle will have no detrimental impact on populations of dung-dependent insects or on dung degradation rates. These conclusions were supported by the seasonal patterns of cattle anthelmintic use in the United States, by the Hazard Assessments for effects on dung beetles of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle and the IVOMEC SR Bolus use in pastured cattle, and by field studies conducted with avermectin products. Since outside dung fauna experts, including one from the USDA, agreed with the conclusions of the Hazard Assessments, namely that impacts are not expected on populations of dung-dependent insects based on the anthelmintic and ectoparasiticide use patterns, no effects therefore would be expected on higher tropic species that feed or depend on dung fauna. This conclusion is also applicable for the use of the other avermectin-based products, such as IVOMEC Injection, IVOMEC Pour-On for Cattle and IVOMEC Cattle Paste, since the use of avermectin-based products was specifically included in the assessment for the IVOMEC SR Bolus and IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle.

Merial has prepared this update to the previously submitted EAs for IVOMEC products that updates and discusses the literature through the present. This updated assessment is also applicable for other avermectin-product formulations, including IVOMEC Injection and IVOMEC Cattle Paste. This updated assessment, in light of the environmental assessments previously conducted for IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle and IVOMEC SR Bolus and not just based on the results of laboratory scale studies reported in the literature, indicates that additional field studies are not necessary.

In summary, this updated assessment supports the conclusions from previous Environmental Assessments for Merial's avermectin-based products for cattle. No significant impacts on dung fauna populations, on dung degradation or on higher trophic levels are indicated. Laboratory studies indicate no chronic effects on earthworms are expected and this is confirmed in the literature. Also, field trials using feces from cattle treated with either the injectable or sustained-release bolus formulation show that although some species of dung-dependent insect populations are reduced for a duration after dosing, complete elimination of immature Coleoptera and Diptera are not observed. The total number of species, and in some cases the total numbers of insects including immatures, is not affected. This means it is extremely unlikely that any higher trophic species that depends solely upon larvae in dung for its food would be affected even within a short temporal period within a small spatial region.

Most of the articles cited in the review of key dung-fauna and dung-degradation literature since 1993 refer to laboratory and pat-level studies that do not address exposure on an ecosystem, population or community properties level. Such studies do not replace thorough risk assessments. Additionally, none of the data appearing in the literature considers the environmental effects with respect to cattle husbandry/management under commercial

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In summary, this updated assessment supports the conclusions from previous Environmental Assessments for Merial's avermectin-based products for cattle. No significant impacts on dung fauna populations, on dung degradation or on higher trophic levels are indicated. Laboratory studies indicate no chronic effects on earthworms are expected and this is confirmed in the literature. Also, field trials using feces from cattle treated with either the injectable or sustained-release bolus formulation show that although some species of dung-dependent insect populations are reduced for a duration after dosing, complete elimination of immature Coleoptera and Diptera are not observed. The total number of species, and in some cases the total numbers of insects including immatures, is not affected. This means it is extremely unlikely that any higher trophic species that depends solely upon larvae in dung for its food would be affected even within a short temporal period within a small spatial region.

Most of the articles cited in the review of key dung-fauna and dung-degradation literature since 1993 refer to laboratory and pat-level studies that do not address exposure on an ecosystem, population or community properties level. Such studies do not replace thorough risk assessments. Additionally, none of the data appearing in the literature considers the environmental effects with respect to cattle husbandry/management under commercial

conditions of use. Review of the key literature establishes that new data on effects and duration of effects on dung fauna are consistent with earlier data and with the mode of action of the avermectins. Population-level studies and assessments support previous conclusions that dung-insect populations recover quickly from any local perturbations. No effects on non-target organisms other than dung fauna are indicated; new data do not indicate impact to soil organisms, higher trophic levels, fungi or on dung degradation from the use of avermectins in cattle on pasture. Thus, no new, unaddressed concerns have been raised in a review of the recent literature.

The conclusions of previous EAs are sound, complete, and appropriately consider risk in all environmental compartments and ecosystems of concern. The use of Merial's avermectin products for cattle will not have a significant impact on the environment.

5. LABEL STATEMENT

An Environmental Safety statement appears on each label of Merial's avermectin-based products for cattle to inform users and to minimize any potential adverse aquatic impacts associated with the use and disposal of Merial's avermectin-based products. Each label statement for Merial's avermectin-based products will be modified to also indicate the potential for effects by members of this chemical class of compounds on dung-dependent insects. The modified statements will allow users to make informed decisions on the use of the products and to minimize any potential adverse impacts to aquatic and terrestrial organisms. The statement to be added to the product labels is presented below.

Proposed addition to Environmental Safety label statement for Merial's avermectin-based products for cattle:

Container Label

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

Package Insert

ENVIRONMENTAL SAFETY:

Studies indicate that when ivermectin comes in contact with soil it readily and tightly binds to the soil and becomes inactive. Free ivermectin may adversely affect fish and certain aquatic organisms. Do not contaminate water by direct application or by improper disposal of drug containers. Dispose of containers in an approved landfill or by incineration.
As with other avermectins, ivermectin is excreted in the dung of treated animals and can inhibit the reproduction and growth of pest and beneficial insects that use dung as a source of food and for reproduction. The magnitude and duration of such effects are species and life-cycle specific. When used according to label directions, the product is not expected to have an adverse impact on populations of dung-dependent insects.

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8. CERTIFICATION

The undersigned officials certify that the information presented is true, accurate and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

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6. REFERENCES

Alvinerie, M; Sutra, J. F.; Galtier, P.; Lifschitz, A.; Virkel, G.; Sallovitz, J. and Lanusse, C., 1998. Persistence of ivermectin in plasma and faeces following administration of a sustained release bolus to cattle. Research in Veterinary Science, 66: 57-61.

Barth, D., 1993. Importance of methodology in the interpretation of factors affecting degradation of dung. Vet. Parasitol. 48: 99-108.

Barth, D., E. M. Heinze-Mutz, R. A. Roncalli, D. Schlüter and S. J. Gross, 1993. The degradation of dung produced by cattle treated with an ivermectin slow-release bolus. Vet. Parasitol., 48: 215-227.

Barth, D., E. M. Heinze-Mutz, W. Langholff, R. A. Roncalli, and D. Schlüter, 1994a. Colonisation and degradation of dung pats after subcutaneous treatment of cattle with ivermectin or levamisole. Appl. Parasitol. 35: 277-293.

Barth, D., M. Karrer, E. M. Heinze-Mutz, and N. Elster, 1994b. Colonisation and degradation of dung: aspects of sampling, fecal composition and artificially formed pats. Environ. Entomol. 23: 571-578.

Barth, D., M. Karrer and E. M. Heinze-Mutz, 1995. Significance of moisture content of dung pats for colonisation and degradation of cattle dung. Appl. Parisitol., 36: 11-21.

Bernal, J. L., M. J. Nozal, M. del; Salas, E. Galante and J. P. Lumaret, 1994. HPLC determination of residual ivermectin in cattle dung following subcutaneous injection. Journal of Liquid Chromatography , 17: 11, 2429-2444.

Burg, R.W. and E.O. Stapley, 1989. Isolation and characterization of the producing organism. In, Ivermectin and Avermectin, W.C. Campbell, Ed., Springer-Verlag, New York, p 30.

Cook, D. F., I. R. Dadour, D. N. Ali, 1996. Effect of diet on the excretion profile of ivermectin in cattle faeces. International J. Parasitol., 26: 291-295.

Dadour, I. R. and Cook, D. F., 1996. Survival and reproduction in the Scarabaeine dung beetle *Onthophagus binodis* (Coleoptera: Scarabaeine) on dung produced by cattle on grain diets in feedlots. Environ. Entomol. 25: 1026-1031.

Doherty, W. M.; N. P. Stewart; R. M. Cobb and P. J. Keiran, 1994. *In-vitro* comparision of the larvicidal activity of moxidectin and abamectin against *Onthophagus gazella* (F.) (Coleoptera: Scarabaeidae) and *Haematobia irritans exigua* De Miejere (Diptera: Muscidae). J. Aust. Ent. Soc., 33: 71-74.

EMEA/CVMP, 1997. Note for guidance: environmental risk assessment for veterinary medicinal products other than GMO-containing and immunological products. EMEA/CVMP/055/96-Final. Committee for Veterinary Medicinal Products, the European Agency for the Evaluation of Medicinal Products.

Fincher, G. T., 1992. Injectable ivermectin for cattle: effects on some dung inhabiting insects. Environ. Entomol. 21: (4), 871-876.

Fincher, G. T., 1996. Ivermectin pour-on for cattle: effects on some dung inhabiting insects. Southwest. Entomol., 21: 4, 445-450.

Finnegan, P. M., D. P. Flanagan and M. J. Gormally, 1997. Preliminary investigations of *Aphodius* species activity in cattle faeces treated with ivermectin. Medicinal and Veterinary Entomology, 11: 139-142.

Floate, K. D., 1998a. Off-target effects of ivermectin on insects and dung degradation in southern Alberta, Canada. Bulletin of Entomological Research, 88: 25-35.

Floate, K. D., 1998b. Does a repellent effect contribute to reduced levels of insect activity in dung from cattle treated with ivermectin? Bulletin of Entomological Research, 88: 291-297.

Floate, K. D., and B. D. Gill, 1998. Seasonal activity of dung beetles (Coleoptera: Scarabaeidae) associated with cattle dung in southern Alberta and their geographic distribution in Canada. Canadian Entomologist,130: 131-151.

Floate, K. D. and A. S. Fox, 1999. Indirect effects of ivermectin residues across trophic levels: *Musca domestica* (Diptera: Muscidae) and *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). Bulletin of Entomological Research, 89: 225-229.

Forbes, A. B., 1993. A review of regional and temporal use of avermectins in cattle and horses worldwide. Vet. Parasitol., 48: 19-28.

Gayard, V; M. Alvinerie and P. L. Toutain, 1999. Comparison of pharmacokinetic profiles of doramectin and ivermectin pour-on formulations in cattle. Vet. Parasitol., 81: 47-55.

Gordon, R. D., 1983. Studies on the genus *Aphodius* of the United States and Canada (Coleoptera: Scarabaeidae). VII. Food and habitat; distribution; key to eastern species. Proc. Entomol. Soc. Wash. 85: 633-652.

Gover, J. and L. Strong, 1995. Effects of ingested ivermectin on body mass and excretion in the dung fly, *Neomyia cornicia*. Physiological Entomology, 20: 93-99.

Gover, J. and L. Strong, 1996. Determination of the toxicity of faeces of cattle treated with ivermectin sustained-release bolus and preference trials using a dung fly, *Neomyia cornicia*. Entomologia Experimentalis et Applicata, 81: 133-139.

Gunn, A. and J. W. Sadd. 1994. The effect of ivermectin on the survival, behaviour and cocoon production of the earthworm *Eisenia fetida*. Pedobiologia 38: 327-333.

Halley, B.A., T.A. Jacob and A.Y.H. Lu, 1989. The environmental impact of the use of ivermectin: environmental effects and fate. Chemosphere 18:1543-1563.

Herd, R., 1995. Endectocidal drugs: Ecological risks and counter-measures. Internat. J. Parasitol., 25: 875-885.

Herd, R. P., R. A. Sams and S. M. Ashcraft, 1996. Persistence of ivermectin in plasma and faeces following treatment of cows with ivermectin sustained-release, pour-on or injectable formulations. Internat. J. Parasitol., 26: 1087-1093.

Krüger, K., and C. H. Scholtz, 1995. The effect of ivermectin on the development and reproduction of the dung-breeding fly *Musca nevilli* Kleynhans (Diptera, Muscidae). Agric. Ecosyst. Environ., 53: 13-18.

Krüger, K and C. H. Scholtz, 1997. Lethal and sub-lethal effects of ivermectin on the dung-breeding beetles *Euoniticellus intermedius* (Reiche) and *Onitis alexis* Klug (Coleoptera, Scarabaeidae). Agric. Ecosyst. Environ., 61: 123-131.

Krüger, K and C. H. Scholtz, 1998a. Changes in the structure of dung insect communities after ivermectin usage in grassland ecosystem. I Impact of ivermectin under drought conditions. Acta Oecologica, 19: 425-438.

Krüger, K and C. H. Scholtz, 1998b. Changes in the structure of dung insect communities after ivermectin usage in grassland ecosystem. II Impact of ivermectin under highrainfall conditions. Acta Oecologica, 19: 439-451.

Lumerat, J. P., E. Galante, C. Lumbreras, J. Mena, M. Bertrand, J. L. Bernal, J. F. Cooper, N. Kadri and D. Crowe, 1993. Field effects of ivermectin residues on dung beetles. J. Appld. Ecol. 30: 428-436.

Lysyk, T. J. and D. D. Colwell, 1996. Duration of efficacy of diazinon ear tags and ivermectin pour-on for control of horn fly (Diptera: Muscidae). J. Econ. Entomol., 89(6): 1513-1520.

Madsen, M, J. Gronvold, P. Nansen, and P. Holter, 1988. Anthelmintics and degradation of dung. Acta. Vet. Scand., 29: 515-517.

Madsen, M., B.O. Nielsen, P. Holter, O.C. Pedersen, J.B. Jespersen, K.-M.V. Jensen, P. Nansen and J. Grønvold, 1990. Treating cattle with ivermectin: effects on the fauna and decomposition of dung pats. J. Appld. Ecol. 27: 1-15.

Mahon, R. J., K. G.Wardhaugh, A. C. M. van Gerwen and W. A. Whitby, 1993. Reproductive development and survival of *Lucilia cuprina* Weidmann when fed sheep dung containing ivermectin. Vet. Paristol., 48: 193-204.

McCracken, D. I., 1993. The potential for avermectins to impact wildlife. Vet. Parasitol., 48: 273-280.

McCracken, D. I. and G. N. Foster, 1993. The effect of ivermectin on the invertebrate fauna associated with cow dung. Environ. Toxicol. Chem. 12: 73-84.

McKeand, J., K. Bairden and A.-M. Ibarra-Silva. 1988. The degradation of bovine faecal pads containing ivermectin. Vet. Record 122: 587-588.

McKellar, Q. A. Ecotoxicology of anthelminitic compounds, 1997. Vet Parasitol., 72: 413-435.

Miller, J.A., S.E. Kunz, D.D. Oehler and R.W. Miller, 1981. Larvacidal activity of Merck MK-933, an Avermectin, against the horn fly, stable fly, face fly and house fly. J. Econ. Ent. 74: 608-611.

Nessel, R.J., D.H. Wallace, T.A. Wehner, W.E. Tait and L. Gomez, 1989. Environmental fate of ivermectin in a cattle feedlot. Chemosphere 18: 1531-1541.

Payne, L. D., Hicks, M. B. and Wehner, T. A., 1995. Determination of abamectin and/or ivermectin in cattle feces at low parts per billion levels using HPLC with fluorescence detection. J. Agric. Food Chem., 43: 1233-1237.

Roncalli, R. A., 1989. Environmental aspects of use of ivermectin and abamectin in livestock: effects on cattle dung fauna. In, W. C. Campbell, Ed., Ivermectin and Abamectin. Springer-Verlag, New York, pp. 173 - 181.

Schmidt, C.D., 1983. Activity of an avermectin against selected insects in aging manure. Environ. Entolmol. 12:455-457.

Scholtz, C. H. and K. Krüger, 1995. Effects of ivermectin residues in cattle dung on dung insect communities under extensive farming conditions in South Africa. Sym. Royal Entomol. Soc. London. 17: 465-471.

Sherratt, T. N., A. D. Macdougall, S. D. Wratten and A. B. Forbes, 1998. Models to assist the evaluation of the impact of avermectins on dung insect populations. Ecological Modelling, 110: 165-173.

Sommer, C., and B. Overgaard Nielsen, 1992. Larvae of the dung beetle *Onthophagus gazella* F. (Col., Scarabaeidae) exposed to lethal and sublethal ivermectin concentrations. J. Appld. Entomol. 114: 502-509.

Sommer, C., and B. Steffansen, 1993. Changes with time after treatment in the concentrations of ivermectin in fresh cow dung and in cow pats aged in the field. Vet. Parasitol. 48: 67-73.

Sommer, C., B. Steffansen, B. Overgaard Nielsen, J. Grønvold, K.-M. Vagn Jensen, J. Brøchner Jespersen, J. Springborg, and P. Nansen, 1992. Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: concentrations and impact on dung fauna. Bull. Entomol. Res. 82: 257-264.

Sommer, C., J. Grønwold, P. Holter, M. Madsen and P. Nansen, 1993. Dung burial activity and development of ivermectin exposed *Diastellopalpus quinquedens* in a field experiment. Entomol. Exp. Appl. 66: 83-89.

Strong, L. and T.A. Brown, 1987. Avermectins in insect control and biology: a review. Bull. Ent. Res. 77: 357-389.

Strong, L. and S. James, 1993. Some effects of ivermectin on the yellow dung fly, *Scatophaga stercoraria*. Vet. Parasitol., 48: 181-191.

Strong, L. and R. Wall, 1988. Ivermectin in cattle treatment: nonspecific effects on pastureland ecology. Aspects Appld. Biol. 17: 231-238.

Strong, L and R. Wall, 1994a. Ecological impacts of the avermectins: recent developments. Pesticide Outlook, 13-16.

Strong, L. and R. Wall, 1994b. Effects of ivermectin and moxidectin on the insects of cattle dung. Bull. Ent. Res., 84: 403-409.

Strong, L; R. Wall; A Woolford and D. Djeddour, 1996. The effect of faecally excreted ivermectin and fenbendazole on the insect colonization of cattle dung following the oral administration of sustained-release boluses. Vet. parasitol., 62, 253-266.

Wall, R. and L. Strong, 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. Nature, 327: 418-421.

Wallace, D. H., Holste, J. E., Roncalli, R. and Gross, S. J., 1991. The degradation of dung pats from ivermectin-treated cattle under field conditions. In: Proceedings of the American Association of Veterinary Parasitologists Annual Meeting, Seattle, WA, U.S.A.

Wardhaugh, K. G., P. Holter, W. A. Whitby and K. Shelley, 1996. Effects of drug residues in the faeces of cattle treated with injectable formulations of ivermectin and moxidectin on larvae of the bush fly, *Musca vetustissima* and the house fly, *Musca domestica*. Aust. Vet J. 74:370-374.

Wardhaugh, K. G., and R. J. Mahon, 1998. Comparative effects of abamectin and two formulations of ivermectin on the survival of larvae of a dung-breeding fly. Aust. Vet J. 76:270-272.

Wardhaugh, K.G. and H. Rodriguez-Menendez, 1988. The effects of the antiparasitic drug, ivermectin, on the development and survival of the drug-breeding fly, Onthelia cornicina (F.) and the scarabaeine dung beetles, Copris hispanus L., Bubas bubalus (Oliver) and Onitis belial. F. J. Appld. Ent. 106: 381-389.

Wislocki, P. G., L. S. Grosso and R. A. Dybas, 1989. Environmental aspects of abamectin use in crop protection. In: W. C. Campbell (Editor), Ivermectin and Abamectin. Springer-Verlag, New York, pp. 182-200.

Wratten, S. D. and A. B. Forbes, 1996. Environmental assessment of veterinary avermectins in temperate pastoral ecosystems. Ann. appl. Biol. 128: 329-348.

Wratten, S. D., M. Mead-Briggs, G. Gettinby, G. Ericsson and D. G. Baggott. 1993. An evaluation of the potential effects of ivermectin on the decomposition of cattle dung pats. Vet. Rec. 133: 365-371.