Eprinomectin Extended-Release Injectable Parasiticide For Cattle

Environmental Impact Assessment

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ENVIRONMENTAL IMPACT ASSESSMENT FOR EPRINOMECTIN EXTENDED-RELEASE INJECTABLE PARASITICIDE FOR CATTLE

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ENVIRONMENTAL IMPACT ASSESSMENT FOR
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1. SUMMARY

This Environmental Impact Assessment (EIA) is for Eprinomectin Extended-Release Injectable Parasiticide for Cattle ("Eprinomectin ER")*, a formulation containing eprinomectin for the control of endoparasites of cattle. Eprinomectin ER is administered subcutaneously to non-lactating cattle. Each mL of Eprinomectin ER contains 50 mg of eprinomectin. Administration of 1 mL per 50-kg bodyweight (b.w.) corresponds to the recommended dose rate of 1 mg of eprinomectin per kg bodyweight.

The worst-case Predicted Soil Concentration (PEC_{soil}) value of 2.5 mcg/kg is well below the 100-mcg/kg level that requires further assessment. However, since eprinomectin is an ectoparasiticide and animals are treated on pasture, the potential for impact was further addressed in specific areas. None of the excipients should have any environmental impact and only the environmental impact of the active ingredient, eprinomectin, was assessed further.

The data presented in this EIA indicate that the use of Eprinomectin ER will not significantly impact terrestrial organisms, avians consuming insects in dung, aquatic or benthic organisms in streams adjacent to pastures, dung fauna, or dung pat degradation. Eprinomectin ER can be used safely without impact on the environment.

* Eprinomectin Extended-Release Injectable Parasiticide for Cattle ("Eprinomectin ER") was referred to as Eprinomectin Long-Acting Injection for Cattle ("Eprinomectin LAI") during development. Study titles and summaries referenced in this document employ the earlier nomenclature.

2. PHASE I ASSESSMENT

2.1. Drug residue in excreta

This product is an extended-release formulation, designed to release eprinomectin with an initial peak plasma level within the first few days after dosing followed by a low level of release and then a second peak plasma level at approximately 80-120 days. Because of the long duration of this product, only a single dose would be given during a grazing season. This extended-release formulation eliminates the need for additional anthelmintic treatments during the grazing season for first-year cattle (Forbes, 1993; Dorny, *et al.*, 2000).

Eprinomectin belongs to the chemical class of compounds known as avermectins. In cattle, nearly all administered avermectins are excreted primarily in the feces either as parent compound or as metabolites. For the purpose of this assessment it will be assumed that all of the residue will be excreted unchanged in the feces as eprinomectin.

Typically, only first-year grazing calves will be treated with Eprinomectin ER in spring to mid-summer when the spring-born calves are heavy enough to be treated. Treated cattle will be on pasture for the majority of the year and the majority of residues are expected to be excreted onto fields grazed by the treated calves.

A radioresidue depletion and metabolism study (PR&D 0049701) was conducted with tritium-radiolabeled eprinomectin and carbon-14-radiolabeled N-methyl pyrrolidone (NMP), an excipient, in the extended-release injectable formulation. As part of the study, the excretion of the radiolabeled residues was determined. Plasma samples were prepared from blood collected periodically throughout the study. The total tritium residues in the feces and urine were measured and demonstrated that the excretion of the eprinomectin-related residues was primarily in the feces, with the feces levels roughly following the profile of the tritium residues in the plasma. The total carbon-14 residues in the feces and urine were also measured and demonstrated that the excretion of the NMP-related residues was primarily in the urine, with the urine levels roughly following the profile of the carbon-14 residues in the plasma.

A plasma and feces residue study (PR&D 0058501) was conducted with cattle on pasture with Eprinomectin ER. Half of the cattle were dosed 91 days before the beginning of the grazing season (turnout); the other half at turnout. This dosing scheme was used to ensure that animals would be on pasture during the initial and final phases of the release of eprinomectin from the extended-release formulation. This way, levels of eprinomectin in feces would not be affected by a non-pasture diet, especially if the grazing season were shortened due to lack of sufficient pasture production late in the study. Plasma samples were prepared from blood collected periodically while the cattle were on pasture. The eprinomectin residues were also measured in the feces during the grazing season and the feces levels roughly followed the profile of the plasma levels. The arithmetic mean peak concentration of eprinomectin (determined as the B_{1a} component) was 528 ng/g of feces on Day 3 post-dose. The mean concentration of eprinomectin in feces decreased rapidly, falling below 100 ng/g between Days 7 and 14 and below 25 ng/g by Day 28. The mean level remained below 25 ng/g feces until a second peak was observed after Day 84. The second peak reached 73 - 74 ng/g on Days 98 and 105, then again decreased. After Day 189, eprinomectin levels in feces were near or below the limit of quantitation (3.60 ng/g) or the level of detection (1.80 ng/g).

2.2. Predicted Environmental Concentration (PEC) in soil from drug residues in feces excreted on fields

A worst-case approach assumes that all eprinomectin is excreted unchanged in the feces and is uniformly distributed in the upper 5 cm of soil (FDA, 2001; CVMP/VICH, 2000).

At the highest stocking density of 9.5 cattle/ha, each weighing 200-kg and on grass without cutting (EMEA/CVMP, 1997), the 9.5 calves/ha would receive a total dose of 1.9 g of eprinomectin (9.5 calves x 200-kg calves x 1 mg of eprinomectin/kg b.w.). Assuming all of the eprinomectin in the Eprinomectin ER output is excreted, is evenly distributed in the top 5 cm of soil by trampling, and that the soil density is 1500 kg/m^3 (FDA, 2001; CVMP/VICH, 2000; EMEA/CVMP, 1997), the concentration of residues in soil would be:

1.9 g/ha ÷ 7.5 x 10^5 kg of soil/ha = 2.5 x 10^{-6} g/kg

PEC_{soil} = 2.5 mcg/kg

The high stocking density (9.5 200-kg cattle/ha) used in the calculation of the maximum PEC_{soil} due to grazing animals (above) may not be sustainable. Dorny *et al.* (2000) found that the weight gain of six approximately 200-kg eprinomectin-treated cattle per hectare on pasture in Belgium was limited after 141 days due to overgrazing.

The residues of eprinomectin would degrade by photolysis on surfaces of dung pats and by microbes in soil and at soil/dung interfaces. The eprinomectin delivered by the Eprinomectin ER will degrade as the dung pats degrade. The rate of decomposition of dung pats, while not directly affected by the presence of eprinomectin residues, is extremely variable.

2.3. PEC values due to direct defecation into a stream

An exposure assessment of surface water through run-off is not considered necessary for compounds where the soil/water partition coefficient on an organic carbon basis (Koc) is >500 L/kg (Montforts, 1999). The Koc for eprinomectin is 3231 – 9208 in various agricultural soils (EPRINEX® Pour-On EA, 1996). Eprinomectin will be strongly adsorbed to dung and soil and therefore only low concentrations will be present in ground or pore water.

Avermectins bind tightly to soil and run-off to surface or percolation into ground water is unlikely (Nessel *et al.*, 1989). However, since treated calves will be on pasture, it is possible that calves will introduce eprinomectin directly into an aquatic environment by defecation into a stream. Using the scenario proposed in the EMEA/CVMP guidelines (1997) in which a 1-m wide stream, having a depth of 1 m, borders one side of a pasture 100 m x 100 m in size, it can be postulated that 1% of the time that the calves defecate, they defecate into the stream. A worst-case scenario assumes a maximal stock density of 9.5 200-kg calves/ha and that all of the drug residue is excreted as eprinomectin. Since the eprinomectin is excreted over 180 days or more, the highest one-day output of drug residues will be used as the worst-case. Based on study PR&D 0058501, the highest average concentration of eprinomectin in feces is 528 ng/g on Day 3 post dose. In that study, 12 male castrates (German Angus, German Angus × Fleckvieh, or German Angus cross), weight range 216 – 334

kg, were dosed. The total mass of feces excreted in 24 hours was determined on Days 7, 70, 140, and 210 after dosing. The amount of feces excreted per day ranged from 5.5 to 17.1 kg/animal. The overall mean on a bodyweight basis was 0.035 kg/kg b.w./day. Based on these data, the maximum excretion of eprinomectin in a single day by a 200-kg animal would be 3696 mcg/day (200 kg b.w./calf x 0.035 kg feces/kg b.w. x 528 mcg eprinomectin/kg feces).

Equations in EMEA/CVMP (1997) can then be used to calculate PEC_{water} (refined to take into account the binding of the eprinomectin to the sediment on the streambed). The concentration of drug residues in solution would be only 0.00044 mcg/L, as shown below.

Worst-case daily dose of eprinomectin into the stream

= 1% x 9.5 animals/ha x 3696 mcg eprinomectin/animal/day

= 351 mcg eprinomectin into stream/day

Volume of a 100 m long x 1 m wide x 1 m deep stream

= 100 m^3 = 100,000 L

Mass of water in the stream= $1x10^8$ g

Mass of sediment on 100 m long x 1 m wide x 0.05 m deep streambed

= 5 m^3 x 1500 kg/m^3 = 7.5 x 10^3 kg

Kd (soil/water partition coefficient) = (amount adsorbed/mass of soil) x (volume of water/amount in water)

= Koc (soil/water partition coefficient on an organic carbon basis) x foc (fraction of organic carbon in soil), i.e., Koc is defined as Kd / foc

For eprinomectin, values of Koc ranged from 3231 to 9208 (EPRINEX® Pour-On EA, 1996). Using a Koc of 3231 and a foc of 0.029, Kd = 94.

Substituting MA = amount adsorbed + amount in water, rearranging and solving for the amount in the water and dividing that by the mass of water yields:

PEC_{water} (refined for binding to sediment)

$$= \frac{MA}{\text{mass of water} + (\text{mass of sediment} \times Kd)}$$

$$= \frac{0.351 \text{ mg}}{10^8 \text{ g water} + (7.5 \times 10^6 \text{ g} \times 94)}$$

$$= 0.351 \text{ mg} / 8.05 \times 10^8 \text{ g}$$

$$= 4.4 \times 10^{-10} \text{ mg/L} = 0.00044 \text{ mcg/L}$$

The PEC in stream sediment is calculated analogously to the PEC in water. Solving the equations in EMEA/CVMP (1997) for $PEC_{sediment}$, the concentration of drug residues bound to sediment in the streambed would be 0.041 mcg/kg from the following calculations.

$$PEC_{sediment} = \frac{Kd \times MA}{\text{mass of water} + (\text{mass of sediment} \times Kd)}$$

$$= \frac{94 \times 0.351 \text{ mg}}{10^8 \text{ g water} + (7.5 \times 10^6 \text{ g} \times 94)}$$

$$= 33.0 \text{ mg} / 8.05 \times 10^8 \text{ g} = 4.1 \times 10^{-8} \text{ mg/g}$$

$$= 0.041 \text{ mcg/kg sediment}$$

2.4. Disposal of waste

Studies indicate that when eprinomectin comes in contact with the soil, it readily and tightly binds to the soil and becomes inactive over time. Drug containers and any residual contents should be disposed of safely (*e.g.*, by burying or incinerating or according to local regulations for waste disposal) as free eprinomectin may adversely affect fish and certain aquatic organisms.

Eprinomectin does not adversely affect dung beetle populations or their dispersal of dung. The rate of degradation of dung pats from cattle treated with the pour-on formulation has been shown to be the same as that from untreated cattle.

2.5. Phase I Summary

The worst-case PEC_{soil} value of 2.5 mcg/kg is well below the 100 mcg/kg level that requires further assessment (FDA, 2001; CVMP/VICH, 2000). However, since eprinomectin is an ectoparasiticide and animals are treated on pasture, Eprinomectin ER must be assessed further for its potential environmental impact (FDA, 2001; CVMP/VICH, 2000).

The excipients in Eprinomectin ER which are extruded with the active ingredient are N-methyl pyrrolidone (NMP), a biodegradable poly(lactide-co-glycolide) polymer, butylated hydroxytoluene, and glyceryl triacetate as the solvent. Carbon-14-labeled NMP was excreted in the urine of cattle in Study PR&D 0049701. As NMP is present at six times the level of eprinomectin in the extended-release formulation, its maximum PEC_{soil} value would be at most six times the maximum PEC_{soil} of eprinomectin, or only 15 mcg/kg; well below the trigger value requiring further assessment (FDA, 2001; CVMP/VICH, 2000). The other excipients are natural products or biodegradable materials that would be extensively metabolized prior to excretion. Hence none of the excipients should have any environmental impact and only the environmental impact of the active ingredient, eprinomectin, will be considered in Phase II.

Table I. Phase I Summary of PEC values from the use of Eprinomectin Extended-Release Injectable Parasiticide

PEC_{soil}	**Eprinomectin, mcg/kg**
from drug residues in dung excreted on fields (5 cm depth)	2.5
PEC_{water}	**mcg/L**
due to the direct defecation into a stream (refined to account for binding to sediment)	0.00044
$PEC_{sediment}$	**mcg/kg**
due to the direct defecation into a stream	0.041

3. PHASE II ASSESSMENT

A large number of fate and effect studies on eprinomectin, ivermectin and their natural-product precursor abamectin have been conducted. These studies are discussed in prior Environmental Assessments (EAs), for example, for IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle (EPRINEX® Pour-On EA, 1996) and for IVOMEC® SR Bolus (IVOMEC® SR Bolus EA, 1996); details of these studies are therefore not repeated here. This Phase II EIA will address in order; 1) impact on terrestrial organisms and avians, 2) impact on aquatic and benthic organisms, and 3) the impact on dung fauna and the subsequent effect on dung degradation from the defecation of treated cattle on pasture.

3.1. Impact on terrestrial organisms from drug residues in feces excreted on pasture or from the use of manure containing eprinomectin as fertilizer

As summarized above, assuming that all residues are eprinomectin, the highest resulting concentration of eprinomectin in the soil would be 2.5 mcg/kg from drug residues excreted by cattle on pasture, if deposited on the surface and left unplowed but penetrating 5 cm into the soil.

Several terrestrial effect studies have been performed and are discussed in prior Environmental Assessments (EPRINEX® Pour-On EA, 1996). In a study on the earthworm *Lumbricus terrestris*, a 28-day LC_{50} of >951 mg/kg dry soil and a no-mortality level of 295 mg/kg dry soil were determined. A no-observed effect level (NOEL) of <90.8 mg/kg dry soil (lowest level tested) was established, based on a dose-response-related loss in bodyweight among worms in all treatment groups. The predicted, chronic no-effect concentration (PNEC) is typically calculated by dividing the acute LC_{50} by 100 (EMEA/CVMP, 1997). The 28-day LC_{50} value was >951 mg/kg, but conservatively using 951 as the LC_{50}, then the calculated PNEC would be 9.5 mg/kg. This PNEC is well above the concentration of eprinomectin expected in soil as a result of the use of Eprinomectin ER, 2.5 mcg/kg. This PNEC is also well above the maximum mean concentration of eprinomectin in feces of cattle dosed with the Eprinomectin ER, 528 mcg/kg, on Day 3 post dose. The PEC/PNEC ratios for soil and Day-3 feces

are 0.00026 and 0.056, respectively. Further, only a small percentage of pats on pasture would be those excreted on Day 3; the remainder would have much lower levels of eprinomectin. No further assessment is indicated when the PEC/PNEC ratio is less than 1.

An additional study was conducted where earthworms were exposed to feces from cattle that had been treated with IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle. The incurred feces were the only food source for the worms and the loss of pat weights ensured the worms ingested the fecal pats. The study, PR&D 0058701, showed that earthworms, under natural conditions, were not negatively affected when eating feces from cattle that were treated with IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle at the recommended dose rate. The measured concentrations of eprinomectin (as eprinomectin B_{1a}) were 427, 152, 51.2 and 1.85 ng/g on a wet-weight basis on Days 2, 4, 7 and 14 after dosing, respectively. These concentrations corresponded to 3.34, 1.19, 0.40 and 0.01 mcg/g, respectively, on a dry matter basis. The numbers and weights of the earthworm *L. terrestris* were not affected by feeding on the feces excreted by cattle 0, 2, 4, 7 or 14 days after the cattle were dosed. In fact, the numbers of surviving worms and final mean weight of the worms after 28 days were greatest in the groups that included those fed on the feces from Days 2 and 4 after dosing, the time-points with the highest levels of eprinomectin. Survival was not significantly different ($p \geq 0.50$) in any of the post-dose groups relative to the control. Weight gain was not significantly different at $\alpha=0.05$ in any of the post-dose groups relative to the control. The naturally incurred levels of eprinomectin therefore did not affect the feeding, survival or weight gain of earthworms. No behavioral aspects were noted at the end of the study. The study PR&D 0058701 showed that earthworms, under natural conditions, are not negatively affected when eating feces from cattle that have been treated with IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle. The levels of eprinomectin observed in study PR&D 0058701 following treatment with IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle are similar to the levels of eprinomectin in the feces of cattle after treatment with Eprinomectin ER in study PR&D 0058501. Therefore, no effects on earthworm behavior, populations or weight gain are expected from the use of Eprinomectin ER.

With regard to soil microbes, eprinomectin was shown to have no significant antimicrobial effects against 26 microbial species (including bacteria and fungi) at concentrations as high as 1000 ppm in a standard, antimicrobial screen (EPRINEX® Pour-On EA, 1996). Incurred eprinomectin at 3.83 mcg/L of cows milk had no effect on the ability of bacteria in mesophilic starter, thermophilic starter, or thermophilic yogurt cultures to form fresh, soft, blue or hard cheeses or yogurt (Rehbein *et al.,* 2001). The related compounds abamectin and ivermectin also lack significant antibacterial or antifungal activity. Higher-tiered respiration and nitrification studies with ivermectin have demonstrated that at levels of residues at 30 mcg/kg, about 12 times higher than the maximum PEC_{soil}, there was no effect on soil respiration or nitrification (IVOMEC® SR Bolus EA, 1996). Therefore, there is no risk to soil microbes from the incorporation of feces from cattle treated with Eprinomectin ER into soil.

Phytotoxicity studies were carried out with eprinomectin (EPRINEX® Pour-On EA, 1996). Both seedling germination and seedling growth and root elongation studies were performed in sand. The lowest no-observed effect concentration (NOEC) value was 0.47 mg/kg for the parameter of root weight in the root elongation and seedling growth study. Since the NOEC is 188 times the maximum PEC_{soil} of 2.5 mcg/kg, no phytotoxic effects on plants are expected. A further reduction in toxicity of 2000-fold was observed in a phytotoxicity study conducted in soil instead of sand.

Birds may eat worms, insects, and seeds found in dung pats from cattle dosed with eprinomectin. Assuming an earthworm consumes 30% of its bodyweight each day in food, then a 100-mg earthworm would consume 30 mg of feces per day. If the feces contain the highest expected steady-state concentration of eprinomectin during treatment with Eprinomectin ER, 528 mcg/kg eprinomectin, then the earthworms would each contain 15.8 ng of eprinomectin. If an 89-g song thrush eats 22 g wet weight of food a day, and all of the song thrush's diet is earthworms, then the thrush will consume 220 worms and therefore 3.48 mcg of eprinomectin. This is equivalent to 0.039 mg/kg b.w. Since the acute oral LD_{50} values for the bobwhite quail and the mallard duck are 272 and 24 mg/kg bodyweight, respectively, the predicted no-effect dose would be 0.24 mg/kg, the LD_{50} from the more sensitive species (mallard duck) divided by 100

(EMEA/CVMP, 1997). The ratio of the predicted environmental dose to the predicted no-effect dose is therefore 0.039 ÷ 0.24 = 0.16. As this ratio is less than 1, there is no significant risk to avians from acute exposure due to the use of eprinomectin. There is a large margin of safety even for this worst-case scenario.

Further, the dietary exposure of the song thrush to 15.8 ng of eprinomectin per 100 mg earthworm, i.e., 0.158 mg/kg or 0.158 ppm in the diet, is also well below the chronic exposure (eight-day dietary) LD_{50} values for eprinomectin of 1813 and 447 mg/kg in the feed for the bobwhite quail and the mallard duck, respectively. The PNEC based on chronic studies is therefore 44.7 mg/kg, the LD_{50} from the more sensitive species (mallard duck) divided by 10 (EMEA/CVMP, 1997). The PEC/PNEC ratio is therefore 0.158 ÷ 44.7 = 0.0035. As this ratio is much less than 1, there is also no significant risk to avians from chronic dietary exposure due to the use of eprinomectin. This results in a daily dietary intake far below any level of concern.

3.2. Impact on aquatic and benthic organisms

An exposure assessment of surface water through run-off is not considered necessary for compounds where Koc is >500 L/kg (Montforts, 1999). The K_{oc} for eprinomectin is 3231 – 9208 in various agricultural soils.

PEC_{water} and $PEC_{sediment}$ in a stream environment due to direct defecation into a stream were calculated as 0.00044 mcg/L and 0.041 mcg/kg, respectively. The calculations assume worst-case stocking and worst-case (Day 3) eprinomectin levels in the feces.

Data on the aquatic toxicity of eprinomectin to *Daphnia magna* indicate that eprinomectin is toxic to this organism with a LC_{50} of 0.45 mcg/L and a NOEL of <0.37 mcg/L (lowest level tested). Dividing the LC_{50} by a 100-fold safety factor (EMEA/CVMP, 1997) yields a PNEC value of 0.0045 mcg/L. The PEC_{water}/PNEC is therefore 0.00044 mcg/L ÷ 0.0045 mcg/L = 0.098.

Fish and algae are less sensitive to eprinomectin than is *Daphnia magna* (EPRINEX® Pour-On EA, 1996), so no effects are predicted on higher trophic aquatic species either.

No data are available on the toxicity of eprinomectin toward benthic organisms, but data are available for the related compound ivermectin. The 10 day static acute toxicity test results for ivermectin for the larvae of the midge (*Chironomus tentans*), mayfly (*Hexagenia* sp.), and amphipod (*Hyalella azteca*) gave EC_{50} values of 0.12, 0.79, and 1.7 mg/kg, respectively. Using these values and applying a 100-fold safety factor to the acute EC_{50} of the most sensitive species, *Chironomus tentans*, gives a PNEC = 1.2 mcg/kg. The $PEC_{sediment}$/PNEC is therefore 0.041 mcg/kg ÷ 1.2 mcg/kg = 0.034.

The PEC_{water}/PNEC and $PEC_{sediment}$/PNEC values are both much less than 1, indicating no predicted effects on aquatic or benthic species. Moreover, these calculations likely over-estimate the PEC values for several reasons. The model assumes the highest grazing density and that all cattle in the field will be dosed with Eprinomectin ER. Photolysis will be a major route of elimination of unbound eprinomectin in the stream. The extent of photolytic degradation of eprinomectin is strongly dependent upon the stream environment (clear or muddy, exposed or shaded). Quantitative refinement of the PEC to account for photolysis has not been attempted but photolysis is expected to be rapid on the surface of the stream. Based on the small percentage of cattle eligible for treatment with Eprinomectin ER, the predicted use pattern, the tight soil binding of eprinomectin, its rapid photodegradation, and its degradation rate in soil, the PECs will be significantly below the values calculated above.

3.3. Effect on dung fauna and dung degradation

A large number of studies have been performed to investigate the effects of avermectins in cattle dung both on dung fauna and on dung degradation. Other EAs prepared for Merial's avermectin-based products for cattle address effects to dung fauna and on dung degradation. Among these, the EAs for IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle and IVOMEC® SR Bolus are the most appropriate to cite in relation to this current EIA for the Eprinomectin ER.

The EAs for IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle and IVOMEC® SR Bolus were prepared in the mid-1990s and they included reviews of the relevant literature on the effects of avermectins on dung-fauna and dung-degradation. The subsequent relevant studies and literature are reviewed for this EIA.

As previously discussed, the concentration of eprinomectin in feces versus time after dosing cattle with the Eprinomectin ER was determined in study PR&D 0058501. The results are plotted in Figure 1.

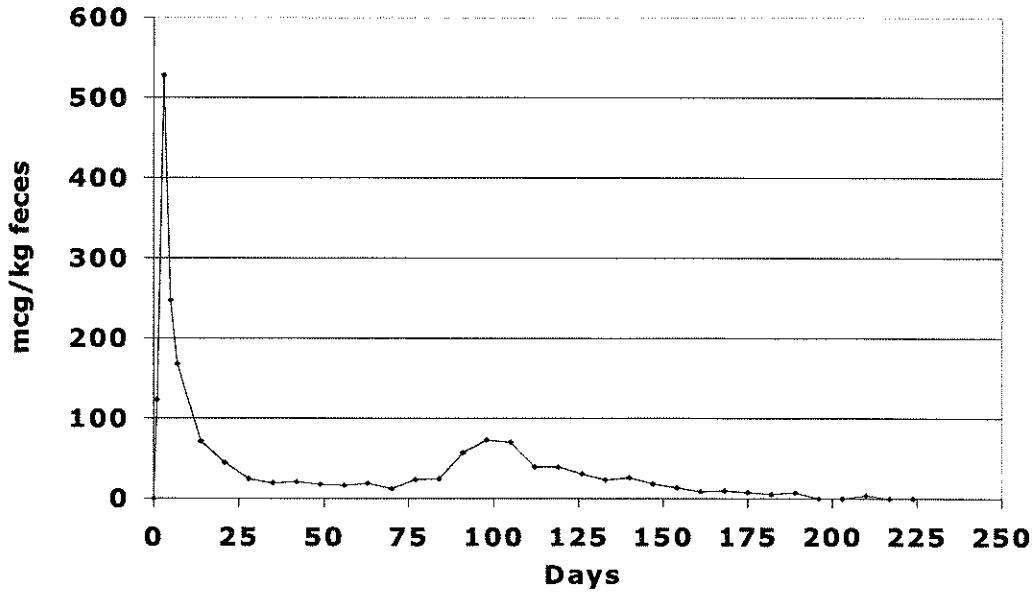

Figure 1. Mean levels of eprinomectin B_{1a} in feces of cattle dosed with Eprinomectin ER, study PR&D 0058501.

The highest level, 528 ng/g (mcg/kg) on a wet-weight basis, occurred in feces collected on Day 3 post-dose. The mean concentration of eprinomectin in feces decreased rapidly, falling below 100 ng/g between Days 7 and 14 and below 25 ng/g by Day 28. The mean level remained below 25 ng/g feces until a second peak was observed after Day 84. The second peak reached 73 - 74 ng/g on Days 98 and 105, then again decreased. After Day 189, mean eprinomectin levels in feces were near or below the limit of quantitation (3.60 ng/g) or the level of detection (1.80 ng/g) in all of the feces samples.

The total dose of eprinomectin from a single administration of the Eprinomectin ER (1 mg/kg b.w.) is equivalent to that from two treatments with IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle (500 mcg/kg b.w. each). The initial peak level of eprinomectin in the feces following a single administration of the Eprinomectin ER is of a similar magnitude as that following an administration of IVOMEC® EPRINEX® Pour-On for Beef and Dairy Cattle (EPRINEX® Pour-On EA, 1996). The second peak of eprinomectin in the feces following administration of the Eprinomectin ER is much less than that which would result from an administration of a second treatment with the pour-on formulation.

A study with *Musca autumnalis,* the face fly, a common dung fly, determined an EC_{50} of 41.64 mcg/kg and a NOEC of 26.62 mcg/kg based on the emergence of adult flies from larvae incubated in bovine dung spiked with eprinomectin (PR&D 0016601). Positive control (ivermectin), 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 was added to bovine feces to which first instar larvae of *M. autumnalis* were added. The impact of the eprinomectin and positive control on maturation to adults was assessed. The test was terminated 15 days after application of the larvae to the treated dung, 5 days after the last adult emerged from the untreated control vessels. Mean emergence from solvent and untreated controls was 66 and 65%, respectively; therefore, the definitive study was valid. Measured concentrations were 100.4, 50.63, 26.62, 12.58, and 7.03 mcg/kg wet-weight for eprinomectin. The concentration of eprinomectin in its radioactive control was 1.44 mcg/kg wet-weight. The EC_{50} for eprinomectin was calculated to be 41.64 mcg/kg (with 35.04 - 47.85 as the 95% C.I.) by applying the standard technique of maximum likelihood estimation to the probit model. The NOEC was 26.62 mcg/kg, calculated using Fisher's Exact Test. For the positive control, ivermectin, the measured concentrations were 118.65, 61.15, 29.67, 13.19, and 7.45 mcg/kg wet-weight. The concentration of ivermectin in its radioactive control was 0.46 mcg/kg wet-weight. The EC_{50} for ivermectin was calculated to be 78.38 mcg/kg (with 51.82 - 158.51 as the 95% C.I.), by the

probit model. The NOEC was 61.15 mcg/kg, by Fisher's Exact Test. Thus, the median effect level for eprinomectin appears to be slightly lower than the median effect level for ivermectin, as determined with *M. autumnalis*.

The toxicity of eprinomectin was determined using two species of dung beetles, *Onthophagus gazella* and *Euoniticellus intermedius* (EPRINEX® Pour-On EA, 1996). Control feces was homogenized and divided into 5-kg aliquots. One aliquot served as a non-treated control. Eprinomectin was added to the remaining aliquots in 5 mL of dimethylformamide. Treated fecal samples contained eprinomectin B_{1a} at 0.0 (vehicle-treated control), 7.0, 24, 64.7, 166, and 590 mcg/kg on a wet-weight basis. Fecal pats were placed on top of soil in plastic pails and three male-female pairs of *O. gazella* or *E. intermedius* beetles were placed in each of 6 pails per treatment for each species. There were no effects on adult beetles, as measured by lethality, i.e., number of live adults recovered or numbers of brood balls formed over the range of eprinomectin tested. No live progeny were recovered at the 166 or 590 mcg/kg levels. The NOEC, based on numbers of emerged progeny relative to pooled controls (untreated and solvent controls), was 64.7 mcg/kg for both species. A LC_{50}, based on the number of brood balls formed by the adults, could not be calculated.

Eprinomectin and ivermectin have similar larvicidal activity against the horn fly (*Haematobia irritans*), house fly (*Musca domestica*), and stable fly (*Stomoxys calcitrans*) (Floate *et al.*, 2001). Horn flies were most susceptible to the larvicidal action of fecal residues of endectocides. In laboratory bioassays, eprinomectin and ivermectin suppressed horn fly development in dung of cattle treated at least 4 weeks previously and suppressed house fly and stable fly development in dung of cattle treated 1-5 weeks previously. Eprinomectin exhibited larvicidal activity against the old world screw-worm fly (*Chrysomya bezziana*) for 3 to 7 days after dosing with the pour-on formulation whereas cattle treated with the IVOMEC® SR bolus may be protected from fly attack for 102 days after treatment (Wardhaugh *et al.*, 2001b).

The effects of eprinomectin residues on dung beetle (*Onthophagus taurus*) development and survival were investigated with the feces of cattle treated with

the pour-on formulation of eprinomectin (Wardhaugh *et al.*, 2001a). Eprinomectin residues did not affect survival of feeding adult beetles, but feces voided by cattle treated topically at 0.5 mg/kg b.w. were associated with high juvenile mortality during the first 1-2 weeks after treatment. Increased mortality also occurred among newly emerged beetles fed for 7 continuous days on feces collected 3 days after eprinomectin treatment and there was evidence of suppressed brood production among those that survived. However, when subsequently provided with feces from untreated cattle, surviving insects laid substantial numbers of eggs. Impairment of the reproductive capacity was thus only a temporary phenomenon. A model simulating the effects of drug residues on dung beetle populations suggests that in the absence of immigration a single treatment of eprinomectin could potentially reduce beetle activity in the next generation by 25-35%. Effects are likely to be greatest when treatment coincides with emergence of a new generation of beetles. In reality, dung beetles are highly mobile and the use of many anthelmintics is spread asynchronously throughout the year. Also, under field conditions, this suppression of brood production due to continuous feeding on feces with the maximal eprinomectin levels should not be a factor. Dung pats excreted on Day 3 after dosing of cattle with Eprinomectin ER would only be attractive to adult beetles for a few days. After that, arriving or newly emerging dung beetles would be attracted to fresh pats that would contain much lower levels of eprinomectin.

Since the target population of cattle for the Eprinomectin ER is similar to the target population for the IVOMEC® SR Bolus for Cattle, and since the insecticidal properties of eprinomectin and ivermectin are comparable (Floate *et al.*, 2001; PR&D 0016601), the residue levels of eprinomectin in the feces of cattle dosed with Eprinomectin ER can be compared to the residue levels of ivermectin in the feces of cattle dosed with the IVOMEC® SR Bolus to project effects on dung-dependent insects.

Herd *et al.* (1996) found 500 ± 200 mcg/kg ivermectin in the feces (wet-weight) of cattle, 14 and 49 days after dosing with an IVOMEC® SR Bolus. Alvinerie *et al.* (1998) measured the concentration of ivermectin in the feces up to 160 days post-dosing. Levels in feces peaked shortly after dosing at levels above 3000

mcg/kg, then decreased to between 2000 and 500 mcg/kg until after the bolus shut down at approximately 120-130 days. After this, the levels decrease rapidly. The levels determined by Alvinerie *et al.* (1998) are higher than those determined by Herd *et al.* (1996), which may be accounted for by differences between the cattle weights or diets in the two studies. In any case, the levels of ivermectin in the feces of cattle dosed with the IVOMEC® SR Bolus are much higher than those of eprinomectin in the feces of cattle dosed with Eprinomectin ER (see Figure 2). This is not surprising since the total dose of eprinomectin from Eprinomectin ER is 100 – 300 mg to cattle in the target population weight range of 100-300 kg, while the total dose of ivermectin from the IVOMEC® SR Bolus is 1.72 g (17.2 to 5.7 times higher). However, the duration of excretion of measurable residues is comparable following dosing with either formulation.

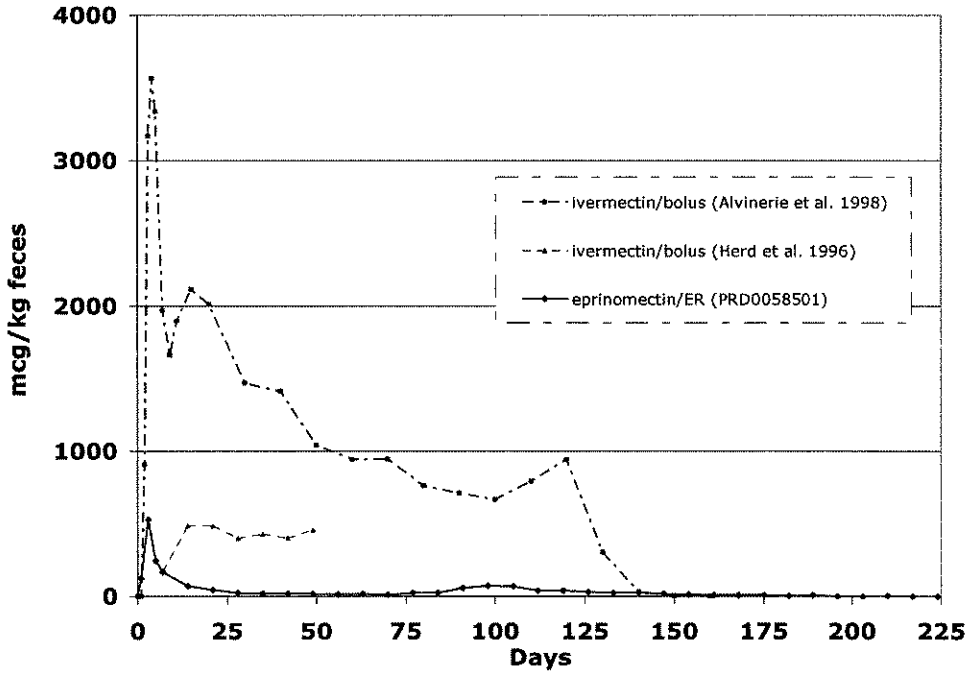

Figure 2. Levels of eprinomectin B_{1a} in feces of cattle dosed with Eprinomectin ER, study PR&D 0058501, compared with levels of ivermectin in feces of cattle dosed with the IVOMEC® SR Bolus.

Part of the Environmental Assessment of the IVOMEC® SR Bolus for Cattle (IVOMEC® SR Bolus EA, 1996) was a report on the Hazard Assessment of the effects of IVOMEC® SR Bolus use in pastured cattle on dung beetles. The 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® SR Bolus included the Hazard Assessment of other approved formulations of ivermectin. Since the target population of cattle for Eprinomectin ER and the IVOMEC® SR Bolus are similar, use of Eprinomectin ER is not expected to increase the number of animals dosed with avermectins, and the assessment already prepared for the IVOMEC® SR Bolus is appropriate for evaluating the effects on dung fauna and degradation following treatment of cattle with Eprinomectin ER.

In the Environmental Assessment of the IVOMEC® SR Bolus for Cattle, the projected seasonal use of all anthelmintics in pastured cattle by class, region of the U.S., and month was assessed. This assessment was prepared 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 their 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.

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. Only a fraction of the anthelmintic use would be from avermectins. 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.

The Hazard Assessment (IVOMEC® SR Bolus EA, 1996) 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 animal species.
- Although residues of ivermectin in dung may inhibit larval development, a high percentage of emergence can be expected from dung excreted before and 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 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 frequency. 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 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. This conclusion would also be appropriate for the use of Eprinomectin ER.

The Hazard Assessment for the IVOMEC® SR Bolus was reviewed by three independent dung beetle experts. 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 Assessment was presented to the FDA and a reviewer from the 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.

Results from field studies with avermectins have supported these conclusions. In a study where dung from 8 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 soil nematodes.

A field study conducted by Barth *et al.* (1994) 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. Even with three anthelmintic treatments in a year, the range and numbers of Coleoptera, soil nematodes, and earthworms were unaffected by either treatment. There were no treatment-related effects on numbers of earthworm species, on numbers of adult or juvenile earthworms, or on earthworm biomass associated with 63-day-old pats voided on Day 0 (the morning before first treatment) and on Days 3, 7, 14, or 28 after the first or third 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 soil 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.

With respect to dung degradation, no field studies have been conducted with Eprinomectin ER that investigated this parameter. However, in a 2-year field study with the IVOMEC® SR Bolus, Wratten, *et al.* (1993) found 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 pastures (i.e., no significant difference ($P > 0.05$) in the dry weights of cumulative standing dung), during either grazing season. Also, weights of ivermectin-containing and ivermectin-free pats decreased with time and the rate of decrease was not affected by treatment in either grazing season ($P > 0.05$). Likewise, pasture or soil qualities (monitoring the development of grazing avoidance patches among treatment groups and organic matter content of soil, respectively) did not differ among treatments for either year. This study confirms that dung degradation is not affected by the use of the bolus.

Similar results were found in a study conducted in Lauterbach, Germany (Barth *et al.*, 1993). 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 8 months. A slight delay in reduction in area of pats from the IVOMEC® SR Bolus-treated calves compared to that for pats from control calves was seen when the pats became older. However, the differences were not statistically significant at any time ($P>0.05$). 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. When photographs of the pats were examined by pat replicate, no marked difference between groups as to the degradation rate of pats was seen.

Because of the lower levels of eprinomectin residues in the feces of cattle dosed with Eprinomectin ER, as compared to levels of ivermectin in the feces of cattle dosed with the IVOMEC® SR Bolus, no effects on populations of dung fauna or on dung degradation are expected from the use of Eprinomectin ER.

In a review article, McKellar (1997) considered the ecotoxicological risk associated with benzimidazoles, levamisole, morantel, the avermectins, and milbemycins. Effects of endectocides on non-target species are summarized, with Diptera and Coleoptera found to be most sensitive. 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.

3.4. Phase II Summary

The impact on the environment from the use of Eprinomectin ER has been assessed with respect to the insecticidal properties of eprinomectin and the deposition of feces from grazing calves directly onto pasture and into streams. Potential effects were considered on terrestrial organisms and avians, in aquatic environments, and on dung fauna and dung pat degradation.

The data presented in this EIA indicate that the use of Eprinomectin ER will not significantly impact terrestrial organisms, avians consuming earthworms or insects in dung, aquatic- or benthic-organisms in streams adjacent to pastures, dung fauna, or dung pat degradation. Eprinomectin ER can be used safely without adverse impact on the environment.

4. REFERENCES

Alvinerie, M, J. F. Sutra, P. Galtier, A. Lifschitz, G. Virkel, J. Sallovitz, and C. Lanusse, 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., 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, 1994. Colonisation and degradation of dung pats after subcutaneous treatment of cattle with ivermectin or levamisole. Appl. Parasitol. 35: 277-293.

CVMP/VICH, 2000. VICH Topic GL6 (Ecotoxicity Phase I). Step 7. Guideline on environmental impact assessment (EIAs) for veterinary medicinal products – Phase I. CVMP/VICH/592/98-FINAL. The European Agency for the Evaluation of Medicinal Products.

Dorny, P., D. Demeulenaere, K. Smets, and J. Vercruysse, 2000. Control of gastrointesitnal nematodes in first season grazing calves by two strategic treatments with eprinomectin. Vet. Parasitol. 89: 277-286.

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.

FDA, 2001. Guidance for Industry 89. Environmental impact assessment (EIA's) for veterinary medicinal products (VMP's) – Phase I. VICH Topic GL6 Final Guidance.

Floate, K. D., R. W. Spooner, and D. D. Colwell, 2001. Larvicidal activity of endectocides against pest flies in the dung of treated cattle. Medical Vet. Entomol. 15: 117-120.

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

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.

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

Monforts, M. H. M. M, 1999. Environmental risk assessment for veterinary medicinal products. Part 1. Other than GMO-containing and immunological products. First update. RIVM report 601300 001. National Institute of Public Health and the Environment. Bilthoven, the Netherlands.

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.

Rehbein, S., M. Wiedemann, R. Ellner, S. Maier, and M. Pollmeier, 2001. The production of cheese and yogurt using milk from cows treated with the endectocide eprinomectin. Milchwissenschaft 56: 543-545.

Wardhaugh, K. G., B. C. Longstaff, and R. Morton, 2001a. A comparison of the development and survival of the dung beetle, *Onthophagus taurus* (Schreb.) when fed on the faeces of cattle treated with pour-on formulations of eprinomectin or moxidectin. Vet. Parasitol. 99: 155-168.

Wardhaugh, K. G., R. J. Mahon, and H. B. Ahmad, 2001b. Efficacy of macrocyclic lactones for the control of larvae of the Old World Screw-worm Fly (*Chrysomya bezziana*). Aust. Vet. J. Vol. 79, No 2: 120-124.

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.

5. CITED REPORTS

PR&D 0016601. Harwood, R. W. J. and J. A. Mackie, 1999. A Determination of the Acute Toxicity of Eprinomectin to *Musca autumnalis* (21 Day) in Comparison to Ivermectin.

EPRINEX® Pour-On EA, 1996. IVOMEC® EPRINEX® (eprinomectin) Pour-On for Beef and Dairy Cattle, Environmental Assessment, Merck & Co., Inc. 1996 (NADA 141-079).

IVOMEC® SR Bolus EA, 1996. IVOMEC® SR Bolus, Environmental Assessment, Merck & Co., Inc. 1996 (NADA 140-988 E0022).

PR&D 0049701. Wilkes, L., and C. Heird, 2003. A Study to Evaluate the Depletion of Radio-Residues in Cattle Treated with ^{3}H Eprinomectin at 1 mg/kg BW in a Long-Acting Injectable Formulation Containing ^{14}C Labeled N-Methyl Pyrrolidone.

PR&D 0058501. Pollmeier, M., and S. Rehbein, 2002. A Study To Evaluate The Residue Levels In Feces from Cattle Treated with Eprinomectin at 1 mg/kg bodyweight in a Long-Acting Injectable Formulation.

PR&D 0058701. Winter, R., S. Rehbein, and S. S. Yoon, 2002. Earthworm 28-Day Toxicity Test Using Faeces from Cattle Dosed with EPRINEX® Pour-On.

6. APPENDIX. STUDY SUMMARIES

PR&D 0016601. Harwood, R. W. J. and J. A. Mackie. 1999. A Determination of the Acute Toxicity of Eprinomectin to *Musca autumnalis* (21 Day) in Comparison to Ivermectin.

A study (PR&D 0016601) was conducted to determine the median effect concentration (EC_{50}) and the no-observed effect concentration (NOEC) of eprinomectin on the dung dwelling larvae of *Musca autumnalis,* the face fly, a common dung fly. Positive control (ivermectin), 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 was added to bovine feces to which first instar larvae of *M. autumnalis* were added. The impact of the eprinomectin and positive control on maturation to adults was assessed. The study was conducted within the Entomology Laboratories, Inveresk Research, Tranent, Scotland and was designed to meet the requirements of the EMEA/CVMP Note for Guidance: Environmental Risk Assessment for Veterinary Medicinal Products Other than GMO-Containing and Immunological Products, EMEA/CVMP/055/96-Final (EMEA/CVMP, 1997). This study was conducted in accordance with the OECD Principles of Good Laboratory Practice. The study was initiated on 22 October 1998 and the in-life stages were completed in July 1999.

For the definitive study, 25, one-day old larvae of *M. autumnalis* were added to test vessels containing 100 g of bovine dung. There were five replicates at each test material and positive control 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 vessels were placed into an incubator maintained at 30°C ± 2°C and a 16-hr light/ 8-hr dark cycle. Each vessel was covered with a piece of muslin secured with an elastic band. Emerged adults were counted and removed daily. The test was terminated 15 days after application of the larvae to the treated dung, 5 days after the last adult emerged from the untreated control vessels. Mean emergence from solvent and untreated controls was 66 and 65%, respectively; therefore, the definitive study was valid. Measured concentrations were 100.4, 50.63, 26.62, 12.58, and 7.03 mcg/kg wet-weight for eprinomectin. The concentration of eprinomectin in its radioactive control was 1.44 mcg/kg wet-weight. The EC_{50} for eprinomectin was calculated to be 41.64 mcg/kg (with 35.04 – 47.85 as the 95% C.I.) by applying the standard technique of maximum likelihood estimation to the probit model. The NOEC was 26.62 mcg/kg, calculated using Fisher's Exact Test. For the postitive control, ivermectin, the measured concentrations were 118.65, 61.15, 29.67, 13.19, and 7.45 mcg/kg wet-weight. The concentration of ivermectin in its radioactive control was 0.46 mcg/kg wet-weight. The EC_{50} for ivermectin was calculated to be 78.38 mcg/kg (with 51.82 – 158.51 as the 95% C.I.), by the probit model. The NOEC was 61.15 mcg/kg, by Fisher's Exact Test.

Thus, the median effect level for eprinomectin appears to be slightly lower than the median effect level for ivermectin, as determined with *M. autumnalis*.

PR&D 0049701. Wilkes, L., and C. Heird, 2003. A Study to Evaluate the Depletion of Radio-Residues in Cattle Treated with ^{3}H Eprinomectin at 1 mg/kg BW in a Long-Acting Injectable Formulation Containing ^{14}C Labeled N-Methyl Pyrrolidone.

This study was conducted with radiolabeled eprinomectin and N-methylpyrrolidone in a long-acting injectable (LAI) formulation with the following objectives: 1) to determine the total radioactive residues of ^{3}H eprinomectin (^{3}H-EP) and ^{14}C N-methylpyrrolidone (^{14}C-NMP) in edible tissue and plasma from cattle dosed once at 1 mg/kg body weight with eprinomectin and approximately 0.3 mL/ 50 kg bodyweight with NMP, 2) to confirm liver as the target tissue and eprinomectin B_{1a} as the marker residue, 3) to verify the metabolism profile for eprinomectin and confirm the ratio of marker residue to total residue, and 4) to measure the amount of radioactive residues of eprinomectin and NMP in excreta.

One steer and one heifer were randomly assigned to each of eight treatment groups. One group was designated as untreated controls; the rest were injected subcutaneously in the neck region with the long-acting injectable formulation. The control group was necropsied on Study Day 140; one treated group each on Study Days 5, 10, 50, 120, 150, and 180. One additional group was maintained as replacement cattle. Urine, feces and blood, to prepare plasma, were collected at scheduled intervals. Tissues were collected at necropsy. Total radioactive residues were determined for the tissues, plasma and excreta. Selected samples were extracted for metabolite profiling or for eprinomectin B_{1a} assays.

The majority of the ^{3}H-EP dose was excreted in the feces (70.7% for the steer and 85.0% for the heifer in the 180-day necropsy group). The corresponding values for urine were 1.7% and 2.0%. For ^{14}C-NMP, total recovery of the administered dose in the urine and feces was calculated on the totals for the first 14 days postdose. Urine contained 41.7 and 36.2% for the steer and heifer in the 180-day necropsy group, respectively. The corresponding values for feces were 11.2 and 14.9%.

PR&D 0058501. Pollmeier, M., and S. Rehbein, 2002. A Study To Evaluate The Residue Levels In Feces from Cattle Treated with Eprinomectin at 1 mg/kg bodyweight in a Long-Acting Injectable Formulation.

The residues of parent eprinomectin in feces and plasma from cattle dosed once at 1 mg/kg bodyweight with eprinomectin in a long acting injectable formulation were determined. Fourteen male castrate cattle, German Angus or German Angus crosses, were included in the study. Animals had plasma samples collected from blood during the acclimation phase and assayed to verify the lack of eprinomectin, abamectin or ivermectin residue by comparison with standards and to suggest the absence of moxidectin and doramectin by the absence of fluorescent HPLC peaks. Feces samples collected during the acclimation phase were assayed for eprinomectin B_{1a} residue. Cattle were approximately 5 to 13 months of age and weighed 216 to 334 kg on Day -2. The study was run in two separate phases including seven cattle each. On Day –2 of the first phase, one animal was randomly selected and assigned to Group 1. The remaining 6 cattle were allocated to Group 3. On Day –2 of the second phase, one animal was randomly selected and assigned to Group 2. The remaining 6 cattle were allocated to Group 4.

Treatments were: 1) & 2) Vehicle treated Control at 1 mL/50 kg bodyweight, 3) & 4) Eprinomectin LAI at 1 mL/50 kg bodyweight (nominally 1 mg/kg eprinomectin). Treatment doses were based on the Day -2 bodyweights rounded up to the next 0.1 mL above the calculated dose. Animals were weighed on Days –2 (Groups 1 to 4), 7, 70 (Groups 2 & 4), 140 (Groups 1 to 4) and 210 (Groups 1 & 3). Blood samples were collected and plasma recovered on Days –8/-7, 0 (prior to treatment and at approximately 10 hours post treatment), 1, 2, 3, 4, 5, 6, 7, 13, 17, 21, and weekly thereafter. Feces samples were collected on Days –1, 91 and then weekly for Groups 1 & 3; on Days –1, 1, 3, 5, 7 and then weekly for Groups 2 & 4. Plasma and feces samples were assayed for determination of eprinomectin B_{1a}. The average total mass of feces generated in 24 hours was measured on Days 140 and 210 in Groups 1 & 3 and on Days 7, 70 and 140 for Groups 2 & 4.

Eprinomectin B_{1a} peaked in plasma at 36.7 ng/mL on Day 2; a second peak was seen at 8.73 ng/mL on Day 98. Eprinomectin residues in feces followed a similar pattern. Peak levels were observed at 528 ng/g on Day 3 and at 74.2 ng/g on Day 105. Feces residues depleted to non-quantifiable levels by 196 days following treatment. The total mass of feces excreted per kg of bodyweight in 24 hours was 34.3 g (range from 28.8 to 42.3 g) on all collection days.

No extreme climatic conditions were encountered during the pasture season (recordings from 09 May 2001 until 31 October 2001). The average daily temperature was 16.1 °C (7.0-24.8°C), average relative humidity was 75% (50-98%) and the cumulative precipitation was 654 L/m².

No health problems related to treatment were observed.

PR&D 0058701. Winter, R., S. Rehbein, and S. S. Yoon, 2002. Earthworm 28-Day Toxicity Test Using Faeces from Cattle Dosed with EPRINEX® Pour-On.

The 28-day effect of feces from cattle dosed with EPRINEX® Pour-On using the earthworm species *Lumbricus terrestris* was determined.

The six cattle used for feces collection were male Braunvieh cattle, approximately 11 or 15 months old and weighing 145 to 329 kg. Analysis of plasma obtained prior to treatment confirmed that there were no cattle with detectable eprinomectin, abamectin, doramectin, ivermectin, or moxidectin residues.

A composite feces sample was prepared from feces collected rectally from three of six untreated, pastured cattle with a percentage moisture of ≤88%. After blending and determining the moisture content, portions of the composite feces were used to serve as the control feces. The six cattle were then dosed with EPRINEX® Pour-On per label instructions, i.e. 10 mL/50 kg bodyweight using the dispensing cap supplied with the product.

Feces were collected rectally from each of the cattle on days 2, 4, 7, and 14 after dosing and pooled by day of collection. Only feces samples with a percentage moisture of ≤88% were used, i.e. feces samples from three of the six cattle. After thorough mixing, each composite feces sample was assayed for its content of

moisture, organic matter, and eprinomectin. These samples were used in the earthworm toxicity test. Earthworms with well developed clitellum in artificial soil (2000 g medium per 10 earthworms; four replicates of 10 individually weighed earthworms each for the control group before dosing and for each composite sample from each collection day post-dose) were exposed to eprinomectin in artificial 150-g-dung pats, their only food source. The test duration was 28 days at 15 ± 2 °C with a 12 hour light (485 lux)/12 hour dark cycle. After 28 days the earthworms were removed from the test medium, counted, weighed and tested for behavioral or pathological signs.

Ten live earthworms, each without any behavioral or pathological signs, were recovered from all replicates except from Day 0 (before dose) sample, Repl. 1, Day 2 post-dose sample, Repl. 1, Day 7 post-dose sample, Repl. 1, and Day 14 post-dose sample, Repl. 3, where one earthworm each was missing, and except from Day 7 post-dose sample, Repl. 2, and the Day 14 post-dose sample, Repl. 1, where one earthworm each was found dead. The group percentage mortality was 2.5, 2.5, 0, 5, and 5 for earthworms exposed for 28 days to feces collected on day 0 (before dose) and on days 2, 4, 7, and 14 after dosing. All living earthworms appeared normal and had a well developed clitellum. A comparison of the arithmetic means of living earthworm weights recorded at the start and end of the earthworm toxicity test suggests that eprinomectin residues in feces samples used (0, 427, 152, 51.2, or 1.85 ng eprinomectin B_{1a}/g wet weight of feces collected on Day 0 (before dose) and on Days 2, 4, 7, or 14 post-dose) did not affect weight gain of earthworms. At end of the earthworm phase (Day 28) no fecal material was left for any replicate. It had been observed that the 150-g-fecal pats were already consumed after about 19 days of exposure to the earthworms.

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8. CERTIFICATION

The undersigned certifies that the information present 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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