Date of Approval: September 26, 2011

CORRECTED FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-327

LONGRANGE (eprinomectin) Extended-Release -
Injectable Parasiticide

"for the treatment and control of internal and external parasites of cattle on pasture with persistent effectiveness"

Sponsored by:

Merial Ltd.

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I. GENERAL INFORMATION:

A. File Number:	NADA 141-327
B. Sponsor:	Merial Ltd. 3239 Satellite Blvd. Bldg 500 Duluth, GA 30096-4640 Drug Labeler Code: 050604
C. Proprietary Name:	LONGRANGE
D. Established Name:	Eprinomectin
E. Pharmacological Category:	Antiparasitic
F. Dosage Form:	Injectable solution
G. Amount of Active Ingredients:	5% w/v eprinomectin (50 mg eprinomectin per mL)
H. How Supplied:	50 mL rubber-capped glass bottles
I. How Dispensed:	Prescription (Rx)
J. Dosage:	1 mg eprinomectin per kg body weight (1 mL/110 lb body weight)
K. Route of Administration:	Subcutaneous injection
L. Species/Class:	Cattle/pastured

M. Indications: For the treatment and control of the following parasites:

Gastrointestinal Roundworms
Cooperia oncophora – Adults and L_4
Cooperia punctata – Adults and L_4
Cooperia surnabada – Adults and L_4
Haemonchus placei – Adults
Oesophagostomum radiatum – Adults
Ostertagia lyrata – Adults
Ostertagia ostertagi – Adults, L_4, and inhibited L_4
Trichostrongylus axei – Adults and L_4
Trichostrongylus colubriformis - Adults

Lungworms
Dictyocaulus viviparus – Adults

Grubs
Hypoderma bovis

Mites
Sarcoptes scabiei var. *bovis*

Persistent Activity
LONGRANGE (eprinomectin) has been proven to effectively protect cattle from reinfection with the following parasites for the indicated amounts of time following treatment:

Parasites	Duration of Persistent Effectiveness
Gastrointestinal Roundworms	
Cooperia oncophora	100 days
Cooperia punctata	100 days
Haemonchus placei	120 days
Oesophagostomum radiatum	120 days
Ostertagia lyrata	120 days
Ostertagia ostertagi	120 days
Trichostrongylus axei	100 days
Lungworms	
Dictyocaulus viviparus	150 days

II. EFFECTIVENESS:

A. Dosage Characterization:

A dose titration study (PR&D 0011301) evaluated five formulations of Eprinomectin extended-release injectable (ERI) for the control of endoparasites in cattle at doses of 0.5, 0.75, and 1.0 mg of eprinomectin per kg body weight. Included in this study were 48 male-castrate Holstein cattle, approximately 4 to 6 months old and weighing 126 to 223 kg on Day -4. All treatments were given once on Day 0 by subcutaneous injection at 1 mL/110 lb (50 kg) body weight. On Day 120, cattle were inoculated orally with infective third-stage nematode larvae of *Ostertagia ostertagi*, *Trichostrongylus axei*, *Cooperia oncophora/surnabada*, *Haemonchus placei*, *Nematodirus helvetianus*, and *Dictyocaulus viviparus*. Cattle were necropsied for worm recoveries, and liver and injection site tissues were collected for eprinomectin assay by replicate on Days 148 and 149.

Cattle treated with all doses of Eprinomectin ERI had significantly ($p<0.05$) fewer of the following parasites than the control animals: *Cooperia oncophora*, *Cooperia surnabada*, *Dictyocaulus viviparus*, *Haemonchus* spp. (identified to genus only), *Nematodirus helvetianus*, *Ostertagia ostertagi*, and *Trichostrongylus axei*. Percent effectiveness was ≥ 95% for all parasites. However, only the cattle treated with 1.0 mg eprinomectin/kg body weight with the formulation containing 5% poly-lactide-co-glycolic-acid (PLGA) had 100% effectiveness, therefore this formulation and dose were selected for development.

B. Substantial Evidence for Endoparasite Indications:

Eleven dose confirmation studies were performed, evaluating a 1 mg eprinomectin/kg body weight dose (1 mL/110 lb) and using natural and artificially induced infections. Studies were conducted using common protocols in multiple geographic locations throughout the United States and Europe. All protocols were reviewed and accepted by CVM. Effectiveness against 4th stage larvae (L4) was evaluated in three studies using artificially induced infections. Effectiveness in adults was evaluated in eight studies using both natural and artificially induced infections. A claim could only be granted in an artificial infection study if the isolate for a given species of parasite was 10 years old or less.

DATA ANALYSIS: In all effectiveness studies, the Wilcoxon rank sum test was used to compare the distribution of speciated parasite counts for the treated group to that of the control group. A two-sided test was used at $\alpha=0.05$. Speciated parasite counts for each animal were transformed to the natural logarithm of (count + 1) for analysis and calculation of geometric means. Effectiveness was calculated as $100[(C-T)/C]$, where C is the geometric mean for the control group and T is the geometric mean for the treated group. An indication was granted if there was a minimum of two studies (with at least one of the studies conducted in the United States) that fulfilled the following requirements: an adequate level of

infection in 6 control animals, a statistically significant difference between treated and control animals at $P<0.05$, and 90% or greater effectiveness using geometric means for each species of parasite. If there were more than 2 studies with an adequate level of infection for a species, then the geometric means of the percent effectiveness against that species of parasite from each study was added together and divided by the number of studies with that species of parasite. If this average was greater than or equal to 90%, then the claim was granted.

A treatment and control claim is granted for *Cooperia oncophora* (Adults and L_4), *Cooperia punctata* (Adults and L_4), *Cooperia surnabada* (Adults and L_4), *Haemonchus placei* (Adults), *Oesophagostomum radiatum* (Adults), *Ostertagia lyrata* (Adults), *Ostertagia ostertagi* (Adults, L_4, and inhibited L_4), *Trichostrongylus axei* (Adults and L_4), *Trichostrongylus colubriformis* (Adults), *Dictyocaulus viviparus* (Adults), *Hypoderma bovis*, and *Sarcoptes scabiei* var. *bovis*. The individual studies are summarized below.

B.1 Study Number PR&D 0052101

1) Type of Study: Dose confirmation study in cattle with artificially-induced nematode infections.

2) Investigator: A. Marchiondo, Ph.D., G.C. Royer, D.V.M.
Merial, Missouri Research Center
Fulton, MO -

3) General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment of fourth stage nematode larvae in cattle.

b. Animals: Sixteen male Holstein calves, approximately 5.5 months of age and weighing 148.0 to 167.4 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two groups.

c. Housing: Individual pens

d. Infection: The inoculation schedule was designed so that nematodes were fourth stage larvae (L4) on Day 0.

Table IIB.1.1: Inocula

Species	Number of infective	Day of inoculation	Age of Strain (Yrs)
Nematodirus helvetianus	5,124	-14	5
Bunostomum phlebotomum	1,092	-14	4
Oesophagostomum radiatum	1,014	-14	4
Haemonchus placei	5,072	-7	5
Ostertagia ostertagi	10,020	-7	5
Trichostrongylus axei	15,020	-7	5
Cooperia oncophora, and Cooperia surnabada	10,144	-7	5
Cooperia punctata	10,130	-7	4
Trichostrongylus colubriformis	10,054	-5	5
Dictyocaulus viviparus	1,014	-5	4

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body.

i. Test Duration: All cattle were necropsied either 21 or 22 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection in at least six control animals is summarized in the following table:

Table IIB.1.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	6	377.6	0.0	100.0
Cooperia punctata	8	2422.8	0.0	100.0
Cooperia surnabada	7	245.3	0.0	100.0
Ostertagia ostertagi	7	801.7	0.0	100.0
Trichostrongylus axei	8	1135.3	0.0	100.0

5) - Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.2 Study Number PR&D 0052102

1) - Type of Study: Dose confirmation study in cattle with artificially-induced nematode infections.

2) Investigator: - S. Rehbein, Dr. med. vet. habil., DipEVPC
Merial GmbH, Kathrinenhof Research Center
Rhordorf, Germany

3) - General Design:

a. - Purpose: This study was designed to confirm the effective dose for the treatment of fourth stage nematode larvae in cattle.

b. - Animals: Sixteen male Braunvieh calves, approximately 5.5 months of age and weighing 109 to 159 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. - Housing: Individually stanchioned

d. - Infection: The inoculation schedule was designed so that nematodes were fourth stage larvae (L4) on Day 0.

Table IIB.2.1: Inocula

Species	Number of Infective Larvae	Day of Inoculation	Age of Strain (Yrs)
Bunostomum phlebotomum	1,500	-14	3
Nematodirus helvetianus	4,000	-14	4
Oesophagostomum radiatum	1,500	-14	unknown
Cooperia oncophora/surnabada	10,000	-8	4
Cooperia punctata	10,000	-8	4
Haemonchus contortus	5000	-8	4
Oesophagostomum venulosum	1,500	-8	4
Ostertagia leptospicularis	10,000	-8	5
Trichostrongylus axei	10,000	-8	4
Cooperia curticei	10,000	-5	4
Dictyocaulus viviparus	1,500	-5	4
Ostertagia ostertagi	10,000	-5	4
Ostertagia spp. (sheep)	8,000	-5	2
Strongyloides papillosus	200,000	-5	<1
Trichostrongylus colubriformis	10,000	-5	4

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 100 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight. -

i. Test Duration: All cattle were necropsied either 21 or 22 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection in at least six controls is summarized in the following table:

Table IIB.2.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	8	2625.8	1.1	99.9
Cooperia punctata	8	6982.0	0.0	100.0
Cooperia surnabada	8	443.3	1.1	99.7
Dictyocaulus viviparus	8	282.3	0.0	100.0
Haemonchus contortus	8	696.1	0.0	100.0
Nematodirus helvetianus	8	228.1	0.0	100.0
Oesophagostomum radiatum	8	521.3	0.0	100.0
Ostertagia leptospicularis	8	4264.2	0.0	100.0
Ostertagia ostertagi	8	4769.0	0.0	100.0
Strongyloides papillosus	8	943.2	1.0	99.9
Trichostrongylus axei	8	2719.8	0.0	100.0
Trichostrongylus colubriformis	7	166.4	0.0	100.0

5) - Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.3 Study Number PR&D 0052103

1) Type of Study: Dose confirmation study in cattle with artificially-induced nematode infections.

2) Investigator: - S.R. Pitt, BvetMed, Ph.D., MRCVS -
Merial, Highfield Research Center -
Hertford, Hertfordshire, UK -

3) General Design:

a. - Purpose: This study was designed to confirm the effective dose for the treatment of fourth stage nematode larvae in cattle.

b. Animals: Sixteen male castrate continental-cross breed calves, approximately 4 to 6 months of age and weighing 122.5 to 152.5 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Individually penned

d. Infection: The inoculation schedule was designed so that nematodes were fourth stage larvae (L4) on Day 0.

Table IIB.3.1: Inocula

Species	Number of infective larvae	Day of inoculation	Age of Strain (Yrs)
Bunostomum phlebotomum	1,500	-13	3.5
Nematodirus helvetianus	5,000	-12	4
Oesophagostomum radiatum	1,500	-12	10-20
Cooperia oncophora	11,000	-7	6
Haemonchus contortus	5,000	-7	4
Ostertagia ostertagi	11,000	-7	6
Trichostrongylus axei	10,000	-7	4
Dictyocaulus viviparus	1,500	-5	> 20

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 21 or 22 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection is summarized in the following table:

Table IIB.3.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Dictyocaulus viviparus	7	244.1	0.0	100.0
Ostertagia ostertagi	7	5894.6	0.0	100.0
Cooperia oncophora	7	5753.9	0.0	100.0
Cooperia surnabada	7	460.6	0.0	100.0
Nematodirus helvetianus	6	72.7	0.0	100.0
Oesophagostomum radiatum	7	170.5	0.0	100.0

5) - Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.4 Study Number PR&D 0052001

1) - Type of Study: Dose confirmation study in cattle with artificially-induced nematode infections.

2) Investigator: - A. Marchiondo, Ph.D., G.C. Royer, D.V.M. -
Merial, Missouri Research Center -
Fulton, MO -

3) - General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment of adult nematodes in cattle.

b. Animals: Sixteen Holstein calves, approximately 5.5 months of age and weighing 157.6 to 177.4 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Individually penned

d. Infection: Cattle were inoculated according to the following schedule:

Table IIB.4.1: Inocula

Species	Number of infective larvae	Day of inoculation	Age of Strain (Yrs)
Bunostomum phlebotomum	1,024	-56	4
Oesophagostomum radiatum	1,004	-49	4
Haemonchus placei	5,130	-35	5
Cooperia	10,087	-28	5
Cooperia punctata	10,023	-28	4
Dictyocaulus viviparus	1,017	-28	4
Nematodirus helvetianus	5,046	-28	5
Ostertagia ostertagi	10,123	-28	5
Trichostrongylus axei	15,143	-28	5
Trichostrongylus colubriformis	10,048	-28	4

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 14 or 15 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection is summarized in the following table:

Table IIB.4.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes				
Parasite	**Number of Infected Control Animals**	**Vehicle (Control)**	**Eprinomectin**	**Effectiveness (%)**
		Geometric Mean		
Cooperia oncophora	7	669.2	1.6	99.8
Cooperia punctata	7	261.1	0.6	99.8
Cooperia surnabada	7	141.9	0.6	99.6
Dictyocaulus viviparus	8	21.0	0.0	100.0
Haemonchus placei	8	394.9	0.0	100.0
Oesophagostomum radiatum	8	51.2	0.0	100.0
Ostertagia ostertagi	8	3367.3	0.3	99.9
Trichostrongylus axei	8	1767.7	0.0	100.0
Trichostrongylus colubriformis	6	40.5	0.0	100.0

5) Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.5 Study Number PR&D 0052002

1) - Type of Study: Dose confirmation study in cattle with artificially-induced nematode infections.

2) Investigator: S. Rehbein, Dr. med. vet. habil., DipEVPC
Merial GmbH, Kathrinenhof Research Center
Rohrdorf, Germany -

3) - General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment of adult nematodes in cattle.

b. Animals: Sixteen Braunvieh calves, approximately 5 months of age and weighing 124.5 to 186.5 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to separate treatment groups.

c. Housing: Individually stanchioned

d. Infection: Cattle were inoculated according to the following schedule:

Table IIB.5.1: Inocula

Species	Number of L3/Eggs	Day of Inoculation	Age of Strain (Yrs)
Bunostomum phlebotomum	1,500	-56	4
Chabertia ovina	1,500	-56	4
Trichuris spp.	300	-56	<1
Oesophagostomum radiatum	1,500	-49	Unknown
Cooperia punctata	10,000	-28	5
Dictyocaulus viviparus	1,500	-28	4
Haemonchus contortus	5,000	-28	4
Nematodirus helvetianus	4,000	-28	4
Oesophagostomum venulosum	1,500	-28	4
Ostertagia leptospicularis	10,000	-28	6
Cooperia curticei	10,000	-21	4
Cooperia	10,000	-21	5
Ostertagia ostertagi	10,000	-21	4
Ostertagia spp. (sheep)	8,000	-21	2
Trichostrongylus axei	10,000	-21	5
Trichostrongylus capricola	10,000	-21	5
Trichostrongylus colubriformis	10,000	-21	5
Strongyloides papillosus	200,000	-12	<1

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 14 or 15 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection is summarized in the following table:

Table IIB.5.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes				
Parasite	**Number of Infected Control Animals**	**Vehicle (Control)**	**Eprinomectin**	**Effectiveness (%)**
		Geometric Mean		
Bunostomum phlebotomum	8	345.6	0.0	100.0
Cooperia oncophora	8	3518.5	2.0	99.9
Cooperia punctata	8	4215.9	2.6	99.9
Cooperia surnabada	8	574.1	1.1	99.8
Dictyocaulus viviparus	8	198.0	0.0	100.0
Haemonchus contortus	8	1002.8	0.0	100.0
Nematodirus helvetianus	8	309.6	0.0	100.0
Oesophagostomum radiatum	8	590.2	0.0	100.0
Ostertagia leptospicularis	8	3262.6	0.0	100.0
Ostertagia ostertagi	8	3160.5	0.0	100.0
Ostertagia spp. L4 inhibited	8	900.0	0.0	100.0
Strongyloides papillosus	8	6651.8	111.8	98.3
Trichostrongylus axei	8	1971.7	0.0	100.0
Trichostrongylus colubriformis	8	231.9	0.0	100.0

5) Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.6 Study Number PR&D 0052003

1) Type of Study: Dose confirmation study in cattle with artificially-induced nematode infections.

2) Investigator: - D.G. Baggott, BVSc, Ph.D., MRCVS -
Merial, Highfield Research Center -
Hertford, Hertfordshire, UK -

3) -General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment with adult nematodes in cattle.

b. Animals: Sixteen Limousin (or Limousin cross) calves, approximately 4 to 7 months of age and weighing 129 to 153.5 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Individually stanchioned

d. Infection: Cattle were inoculated according to the following schedule:

Table IIB.6.1: Inocula

Species	Number of Infective Larvae	Day of Inoculation	Age of Strain (Yrs)
Bunostomum phlebotomum	1,700	-55	3.5
Oesophagostomum radiatum	1,500	-47	10-20
Cooperia oncophora/surnabada	11,000	-28	6
Dictyocaulus viviparus	1,500	-28	>20
Haemonchus contortus	5,000	-28	4
Nematodirus helvetianus	6,000	-28	4
Ostertagia ostertagi/lyrata	11,000	-28	6
Trichostrongylus axei	10,000	-28	4

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of - the shoulder. -

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 - mL per 110 lb (50 kg) body weight. -

i. Test Duration: All cattle were necropsied either 14 or 15 days post-treatment. -

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection is summarized in the following table:

Table IIB.6.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes				
Parasite	**Number of Infected Control Animals**	**Vehicle (Control)**	**Eprinomectin**	**Effectiveness (%)**
		Geometric Mean		
Cooperia oncophora	8	8117.1	1.9	99.9
Cooperia surnabada	8	717.1	0.6	99.9
Dictyocaulus viviparus	8	112.8	0.0	100.0
Nematodirus helvetianus	7	170.7	0.0	100.0
Oesophagostomum radiatum	8	436.8	0.0	100.0
Ostertagia ostertagi	8	5896.7	0.3	99.9
Ostertagia lyrata	6	26.4	0.3	98.7

5) - Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.7 Study Number PR&D 0045601

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigator: - D. Reddick, M. Sc., B.Sc. -
Moredun Scientific Ltd. -
Penucuik, Midlothian, Scotland, UK -

3) - General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment of nematodes in cattle.

b. Animals: Twenty male Holstein Friesian, Friesian, or Friesian-cross cattle, between 11 and 15 months of age and weighing 195 to 315 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Pasture

d. Infection: Cattle had naturally-acquired nematode infections.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 14 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection is summarized in the following table:

Table IIB.7: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	10	1475.8	68.1	95.4
Cooperia surnabada	7	78.8	3.7	95.4
Ostertagia ostertagi	10	7029.9	1.4	99.9
O. ostertagi L4 inhibited	10	201123.9	1422.2	99.3
Trichostrongylus axei	8	141.1	0.0	100.0
Trichostrongylus colubriformis	8	162.6	0.0	100.0

5) Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.8 Study Number PR&D 0045602

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigator: T.A. Yazwinski, Ph.D.
University of Arkansas
Fayetteville, AR

3) General Design:

a. - Purpose: This study was designed to confirm the effective dose for the treatment of nematodes in cattle.

b. Animals: Twenty male castrate Holstein cattle, approximately 6 months of age and weighing 79 to 182 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Pasture

d. Infection: Cattle had naturally-acquired nematode infections.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 14 or 15 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection is summarized in the following table:

Table IIB.8: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes				
Parasite	**Number of Infected Control Animals**	**Vehicle (Control)**	**Eprinomectin**	**Effectiveness (%)**
		Geometric Mean		
Cooperia oncophora	10	7685.8	208.4	97.3
Cooperia punctata	10	557.6	1.7	99.7
Cooperia surnabada	10	533.7	27.8	94.8
Haemonchus placei	10	652.9	0.4	99.9
Ostertagia lyrata	10	138.5	0.3	99.8
Ostertagia ostertagi	10	4746.9	2.0	99.9
Ostertagia spp. L4 inhibited	10	2182.0	0.5	99.9

5) - Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.9 Study Number PR&D 0045603

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigators: - S. Rehbein, Dr. med. vet. habil., DipEVPC - Merial GmbH, Kathrinenhof Research Center - Rohrdorf, Germany -

3) - General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment of nematodes in cattle.

b. Animals: Twenty intact male Holstein-Friesian cattle, approximately 12 months of age and weighing 353 to 491 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to separate treatment groups.

c. Housing: Pasture

d. Infection: Cattle had naturally-acquired nematode infections.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 14 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection is summarized in the following table:

Table IIB.9: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Means		
Cooperia oncophora	10	808.8	4.4	99.5
Dictyocaulus viviparus	9	30.3	0.0	100.0
Ostertagia ostertagi	10	1650.5	0.0	100.0
O. ostertagi L4 inhibited	10	10803.4	907.7	91.6

5) Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.10 Study Number PR&D 0045604

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigator: J.C. Williams, Ph.D.
Louisiana State University
Baton Rouge, LA

3) General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment of nematodes in cattle.

b. Animals: Twenty crossbred beef cattle (15 females, 3 male castrates and 2 males), approximately 9 to 14 months of age and weighing 137 to 224 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Pasture

d. Infection: Cattle had naturally-acquired nematode infections.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 14 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite genus species with an adequate level of infection is summarized in the following table:

Table IIB.10: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes				
Parasite	**Number of Infected Control Animals**	**Vehicle (Control)**	**Eprinomectin**	**Effectiveness (%)**
		Geometric Mean		
Cooperia pectinata	9	799.5	0.0	100.0
Cooperia punctata	10	4221.9	0.0	100.0
Oesophagostomum radiatum	10	185.2	0.0	100.0
Ostertagia ostertagi	10	9236.0	0.6	99.9
O. ostertagi L4 inhibited	10	30773.2	5726.1	81.4
Trichostrongylus axei	10	3930.7	0.0	100.0

5) Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

B.11 Study Number PR&D 0045606

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigator: - J.C. Hunter, III, M.S., Ph.D. -
Merial, Missouri Research Center -
Fulton, MO -

3) General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment of nematodes in cattle.

b. Animals: Twenty crossbred Angus cattle (12 male castrates and 8 females), approximately 9 to 15 months of age and weighing 142 to 239 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to separate treatment groups.

c. Housing: Pasture

d. Infection: Cattle had naturally-acquired nematode infections.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. -Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 14 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite genus species with an adequate level of infection is summarized in the following table:

Table IIB.11: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia punctata	9	336.6	0.4	99.9
Oesophagostomum	10	101.2	0.0	100.0
Ostertagia ostertagi	10	30158.4	0.8	99.9
Ostertagia lyrata	9	702.4	0.0	100.0
Ostertagia spp. L4 inhibited	10	5665.5	9.8	99.8
Trichostrongylus axei	10	53639.6	1.0	99.9

5) - Adverse Reactions: There were no drug related health problems or adverse drug events observed during the study.

C. Substantial Evidence for Endoparasite Persistent Activity

Fourteen studies were conducted to evaluate the persistent effect of Eprinomectin ERI against artificial and natural nematode infections. Studies were conducted using common protocols in multiple geographic locations throughout North America and Europe. Seven studies were conducted to confirm the persistent

effect against infections with third-stage larvae (L3) and/or eggs of gastrointestinal and pulmonary nematodes of cattle induced 100 or 120 days after treatment. Four studies were conducted to confirm the persistent effect against natural infections with third-stage larvae (L3) and/or eggs of gastrointestinal and pulmonary nematodes of cattle for 120 days after treatment. One study was conducted to confirm the persistent effect against both natural and induced infections; cattle grazed on infected pasture for 120 days and then received an inoculation with infective third-stage larvae (L3) at Day 120. Two studies were conducted to confirm the persistent effect against infections with third-stage larvae (L3) of gastrointestinal and pulmonary nematodes of cattle induced 150 days after treatment. A claim could only be granted in an artificial infection study if the isolate for a given genus species of parasite was 10 years old or less. In addition, a persistent effect claim could only be granted for those parasite species for which a treatment and control claim was already established.

DATA ANALYSIS: In all effectiveness studies, the Wilcoxon rank sum test was used to compare the distribution of speciated parasite counts for the treated group to that of the control group. A two-sided test was used at $\alpha=0.05$. Speciated parasite counts for each animal were transformed to the natural logarithm of (count + 1) for analysis and calculation of geometric means. Effectiveness was calculated as 100[(C-T)/C], where C is the geometric mean for the control group and T is the geometric mean for the treated group. An indication was granted if there was a minimum of two studies (with at least one of the studies conducted in the United States) that fulfilled the following requirements: an adequate level of infection in 6 control animals, a statistically significant difference between treated and control animals at $P<0.05$, and 90% effectiveness using geometric means for each genus species of parasite and at each persistent effect period. If there were more than two studies, then the reported percent against a genus species of parasite was the arithmetic mean of the percent effectiveness for all studies with that genus species of parasite. If this average was greater than or equal to 90%, then the claim was granted. If this average was less than 90%, the claim was not granted.

A persistency claim is granted for *Dictyocaulus viviparus* for 150 days after treatment, for *Haemonchus placei, Oesophagostomum radiatum, Ostertagia lyrata,* and *Ostertagia ostertagi* for 120 days after treatment and for *Cooperia oncophora, Cooperia punctata,* and *Trichostrongylus axei* for 100 days after treatment based on the above criteria. The fourteen studies are summarized as follows.

C1. Study Number PR&D 0073701

1) Type of Study: Dose confirmation study in cattle with induced gastrointestinal roundworm and lungworm infections.

2) Investigators: R.E. Plue, D.V.M, M.S.
G.C. Royer, D.V.M.
Merial, Missouri Research Center
Fulton, MO

J.E. Holste, D.V.M.
Holste Biological and Pharmaceutical Services
Columbia, MO

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 100 and 120 days post-treatment.

b. Animals: Thirty Holstein male calves, aged approximately 4 months and weighing 87 to 121 kg, were ranked by decreasing body weights and allocated consecutively to 10 replicates of three animals each. Animals within each replicate were randomly assigned to one of three treatment groups.

c. Housing: These cattle were maintained in pens by treatment group.

d. Infection: Each animal was incolulated *per os* with infective larvae

Cooperia punctata. The age of the nematode strains used in this experiment were 6 years old, 6 years old, 6 years old, and 5 years old, respectively.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 148 or 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection at 100 and 120 days is summarized in the following table:

Table IIC.1: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	n	Control	Day 120 (Eprinomectin ERI on Day 0)[1]		Day 100 (Eprinomectin ERI on Day 20)[2]	
		GM	GM	Effectiveness	GM	Effectiveness
Cooperia oncophora	9	1850.9	38.4	97.9%	2.5	99.9%
Cooperia punctata	10	8005.7	45.9	99.4%	2.1	99.9%
Cooperia surnabada	9	671.9	3.4	99.5%	1.0	99.9%
Trichostrongylus axei	10	2596.9	408.1	84.3%	3.8	99.9%

[1] Animals were treated once with Eprinomectin ERI on Day 0 and artificially infected with nematode larvae (L3) on Day 120.
[2] Animals were treated once with Eprinomectin ERI on Day 20 and artificially infected with nematode larvae (L3) on Day 120.

5) Adverse Reactions: No adverse reactions to treatment were noted.

C2. Study Number PR&D 0073702

1) Type of Study: Dose confirmation study in cattle with induced gastrointestinal roundworm and lungworm infections.

2) Investigator: T.A. Yazwinski, Ph.D.
University of Arkansas
Fayetteville, AR

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 100 and 120 days post-treatment.

b. Animals: Thirty female Angus calves, approximately 5 months of age and weighing 168 to 257 kg, were ranked by decreasing body weights and allocated consecutively to 10 replicates of three animals each. Animals within each replicate were randomly assigned to one of three treatment groups.

c. Housing: These cattle were maintained in pens by treatment group.

d. Infection: Each animal was inoculated *per os* with infective larvae of *Trichostrongylus axei*, *Cooperia oncophora*, and *Cooperia*

punctata. A mixed culture was used for inoculation, which also included *Ostertagia ostertagi*, *Haemonchus placei*, and *Oesophagostomum radiatum* larvae. All nematode strains used in this experiment were 1 to 3 months old.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 148 or 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 100 and 120 days is summarized in the following table:

Table IIC.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	n	Control	Day 120 (Eprinomectin ERI on Day 0)[1]		Day 100 (Eprinomectin ERI on Day 20)[2]	
		GM	GM	Effectiveness	GM	Effectiveness
Cooperia oncophora	9	226.8	2.0	99.1%	6.8	97.0%
Cooperia punctata	10	1143.2	2.2	99.8%	4.9	99.6%
Ostertagia ostertagi	10	205.0	0.4	99.8%	0.6	99.7%
Trichostrongylus axei	10	159.3	0.8	99.5%	0.5	99.7%

[1] Animals were treated once with Eprinomectin ERI on Day 0 and artificially infected with nematode larvae (L3) on Day 120.
[2] Animals were treated once with Eprinomectin ERI on Day 20 and artificially infected with nematode larvae (L3) on Day 120.

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.3 Study Number PR&D 0047301

1) Type of Study: Dose confirmation study in cattle with induced gastrointestinal roundworm and lungworm infections.

2) Investigators: S.R. Pitt, BVetMed, Ph.D., MRCVS
B.J. Timms, MPhil., MSc., CBiol, MIBiol
Merial, Highfield Research Centre
Hertford, Hertfordshire, UK

S. Rehbein, Dr. med. vet. habil., DipEVPC
Merial GmbH, Kathrinenhof Research Center
Rohrdorf, Germany

3) General Design

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 120 days post-treatment.

b. Animals: Twenty male castrate Limousin and Limousin cross calves, approximately 5 to 7 months of age and weighing 154 to 191 kg, were ranked by decreasing body weights and allocated consecutively to 10 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: These cattle were maintained in pens by treatment group.

d. Infection: Experimentally induced nematode infections were used. Each animal was inoculated on Day 120 with infective third-stage larvae (L3) from nematode species listed below:

Table IIC.3.1: Inocula

Species	Number of infective larvae	Age of Strain (Yrs)
Cooperia oncophora/surnabada	10,700	7
Dictyocaulus viviparus	1,257	>20
Haemonchus contortus	6,830	5
Nematodirus helvetianus	5,010	5
Oesophagostomum radiatum	1,403	20
Ostertagia ostertagi/lyrata	10,760	7
Trichostrongylus axei	2,925	5

e. Dosage Form: Eprinomectin ERI -

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 148 or 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.3.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	9	2440.3	6.6	99.7
Cooperia surnabada	7	95.3	3.7	96.1
Dictyocaulus viviparus	9	77.4	0.0	100.0
Nematodirus helvetianus	9	965.8	8.7	99.1
Ostertagia ostertagi	9	3983.6	2.8	99.9

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.4 Study Number PR&D 0047302

1) Type of Study: Dose confirmation study in cattle with induced gastrointestinal roundworm and lungworm infections.

2) Investigator: - S. Rehbein, Dr. med. vet. habil., DipEVPC -
Merial GmbH, Kathrinenhof Research Center -
Rohrdorf, Germany -

3) - General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 120 days post-treatment.

b. Animals: Twenty male Pinzgauer calves, approximately 5 to 6 months of age and weighing 152 to 210 kg, were ranked by decreasing body weights and allocated consecutively to 10 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: These cattle were maintained in pens by treatment group.

d. Infection: Experimentally induced nematode infections were used. Each animal was inoculated on Day 120 with infective third-stage larvae (L3) or eggs (*Trichuris* spp. [ovine]) from the nematode species listed below:

Table IIC.4.1: Inocula

Species	Number of infective larvae/eggs	Age of Strain (Yrs)
Bunostomum phlebotomum	1,500	4
C. curticei	10,000	4
C. punctata	10,000	5
Cooperia oncophora/surnabada	10,000	5
Haemonchus contortus	5,000	4
Nematodirus helvetianus	4,000	4
Ostertagia leptospicularis	10,000	6
Oesophagostomum radiatum	1,500	Unknown
Oesophagostomum venulosum	1,500	4
Ostertagia ostertagi	10,000	4
Ostertagia spp. (ovine)	10,000	2
Strongyloides papillosus	200,000	<1
Trichostrongylus colubriformis	10,000	4
Trichostrongylus axei	10,000	5
Trichuris spp. *(ovine)*	1,000	<1

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 148 or 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.4.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Bunostomum phlebotomum	10	173.2	0.4	99.8
Cooperia oncophora	9	733.1	219.0	70.1
Cooperia punctata	10	2854.8	870.8	69.5
Cooperia surnabada	9	164.1	43.5	73.5
Dictyocaulus viviparus	10	202.2	0.6	99.7
Nematodirus helvetianus	10	587.5	89.1	84.8
Oesophagostomum	8	45.4	0.5	99.0
Ostertagia leptospicularis	10	5553.7	39.6	99.3
Ostertagia ostertagi	10	7433.4	42.2	99.4
Trichostrongylus axei	10	1382.8	349.3	74.7

5) Adverse Reactions: No adverse reactions to treatment were noted. -

C5. Study Number PR&D 0047303

1) Type of Study: Dose confirmation study in cattle with induced - gastrointestinal roundworm and lungworm infections. -

2) Investigators: R.E. Plue, D.V.M., M.S.
G.C. Royer, D.V.M.
Merial, Missouri Research Center
Fulton, MO

J.E. Holste, D.V.M.
Holste Biological and Pharmaceutical Services
Columbia, MO

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 120 days post-treatment.

b. Animals: Twenty Holstein male calves, approximately 5.5 months of age and weighing 153 to 194 kg, were ranked by decreasing body weights and allocated consecutively to 10 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: These cattle were maintained in pens by treatment group.

d. Infection: Experimentally induced nematode infections were used. Each animal was inoculated on Day 120 with infective third-stage larvae (L3) from the nematode species listed below.

Table IIC.5.1: Inocula

Species	Number of infective larvae	Age of Strain (Yrs)

Bunostomum phlebotomum	1,545	5
Cooperia punctata	15,037	5
Cooperia oncophora/surnabada	15,111	6
Dictyocaulus viviparus	1,999	5
Haemonchus placei	7,048	6
Nematodirus helvetianus	6,029	6
Oesophagostomum radiatum	2,003	5
Ostertagia ostertagi	20,016	6
Trichostrongylus colubriformis	20,137	6
Trichostrongylus axei	15,061	6

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight to provide 0 mg eprinomectin/kg body weight.

i. Test Duration: All cattle were necropsied either 148 or 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.5.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia punctata	9	1708.2	3.7	99.8
Dictyocaulus viviparus	9	220.7	0.0	100.0
Haemonchus placei	9	650.3	49.2	92.4

Oesophagostomum radiatum	9	101.1	0.4	99.6
Ostertagia ostertagi	9	7251.7	7.3	99.9
Trichostrongylus axei	9	415.1	141.9	65.8

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.6 Study Number PR&D 0128701

1) Type of Study Dose confirmation study in cattle with induced gastrointestinal roundworm infections.

2) Investigator: M. Visser, Biol.
Merial GmbH, Kathrinenhof Research Center
Rohrdorf, Germany

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 120 days post-treatment.

b. Animals: Sixteen Fleckvieh (Simmental) male calves, between 5 and 6 months of age and weighing 164 to 197.5 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to separate groups.

c. Housing: Individually stanchioned.

d. Infection: Experimentally induced nematode infections were used. Each animal was inoculated on Day 120 with infective third-stage larvae (L3) from the nematode species listed below:

Table IIC.6.1: Inocula

Species	Number of infective larvae	Age of Strain (Years)
Cooperia oncophora/surnabada	10,000	9
Cooperia punctata	10,000	9
Nematodirus battus	6,125	9
Ostertagia ostertagi/lyrata	10,000	9
Trichostrongylus axei	10,000	9

e. Dosage Form: Eprinomectin ERI

f. - Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. - Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. - Controls: Saline was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. - Test Duration: All cattle were necropsied 148 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.6.2: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Saline (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	8	3519.6	597.4	83.0
Cooperia punctata	8	3697.3	808.4	78.1
Cooperia surnabada	8	537.1	70.2	86.9
Nematodirus battus	6	120.8	104.3	13.6
Ostertagia ostertagi	8	6176.6	180.7	97.1
Trichostrongylus axei	8	1848.5	566.3	69.4

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.7 Study Number PR&D 0160101

1) Type of Study: Dose confirmation study in cattle with induced gastrointestinal roundworm infections.

2) Study Director: S. Rehbein, Dr. med. vet. habil., DipEVPC
Merial GmbH, Kathrinenhof Research Center
Rohrdorf, Germany

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 120 days post-treatment.

b. Animals: Thirty-six Fleckvieh (Simmental) male calves, between 4 and 6 months of age and weighing 147 to 196 kg, were ranked by decreasing body weights and allocated consecutively to 12

replicates of three animals each. Animals within each replicate were randomly assigned to one of three treatment groups. Only results from the control group and the 1.0 mg eprinomectin/kg body weight dose group are reported here.

c. Housing: Individiually stanchioned.

d. Infection: Experimentally induced nematode infections were used. Each animal was inoculated on Day 120 with infective third-stage larve (L3) from the nematode species listed below:

Table C.7.1:

Species	Number of infective larvae	Age of Strain (Years)
Cooperia oncophora/surnabada	~10,000	2
Cooperia punctata	~10,000	2
Nematodirus helvetianus	~3,000	2
Ostertagia ostertagi/lyrata	~10,000	2
Trichostrongylus axei	~10,000	2
Trichostrongylus colubriformis	~10,000	2

e. Dosage form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 50 kg body weight to provide 1.0 mg/kg body weight.

h. Controls: Saline was administered at 1.0 mL per 50 kg body weight to provide 0 mg eprinomectin/kg body weight.

f. Test Duration: All cattle were necropsied 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

g. Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in a following table:

Table IIC.7.2: Persistent Effectiveness of Eprinomectin ERI Against Nematodes				
Parasite	**Number Of Adequately Infected Control Animals**	**Saline (Control)**	**Eprinomectin**	**Effectiveness (%)**
		Geometric Mean		
Cooperia oncophora	9	896.5	2006.7	<0
Cooperia punctata	11	1788.2	1267.5	29.1
Cooperia surnabada	8	331.4	233.4	29.6
Nematodirus helvetianus	8	257.7	201.2	21.9
Ostertagia lyrata	7	35.3	1.1	96.8
Ostertagia ostertagi	12	3912.9	102.4	97.4
Trichostrongylus axei	11	1392.7	1430.0	<0
Trichostrongylus colubriformis	7	182.4	194.8	<0

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.8 Study Number PR&D 0073201

1) Type of Study: Dose confirmation study in cattle with natural and induced gastrointestinal roundworm and lungworm infections.

2) Investigator: A. Marchiondo, Ph.D.
B.N. Kunkle, D.V.M., Ph.D.
Missouri Research Center
Fulton, MO

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI in preventing nematode infections for 120 days post-treatment.

b. Animals: Seventy-five beef breed calves, approximately 9 to 11 months of age and weighing 190 to 276 kg, were ranked by decreasing body weights and allocated consecutively to 15 replicates of 5 animals each. Animals within each replicate were randomly assigned to separate treatment groups. Only results from the control group and the 1.0 mg eprinomectin/kg body weight dose group are reported here.

c. Housing: Pasture

d. Infection: In addition to continuous grazing on nematode-infested pasture, experimentally-induced nematode infections were given. Each animal was inoculated on Day 120 with approximately 10,000 infective third stage nematode larvae (L3) of *Trichostrongylus axei*, 15,000 L3 of *Cooperia oncophora/surnabada*, and 15,000 L3 of *Cooperia punctata*. All nematode strains used in this experiment were 5.5 years old.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 147 to 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.8: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia punctata	8	26.2	1.1	95.8
Oesophagostomum radiatum	12	20.6	0.0	100.0
Ostertagia ostertagi	15	823.4	0.4	99.9
Trichostrongylus axei	15	1369.6	0.0	100.0

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.9 Study Number PR&D 0047201

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigators: B.J. Timms, MPhil., MSc., CBiol, MIBiol
Merial, Highfield Research Centre
Hertford, Hertfordshire, UK

S. Rehbein, Dr. med. vet. habil., DipEVPC
Merial GmbH, Kathrinenhof Research Center
Rohrdorf, Germany

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI in preventing nematode infections for 120 days post-treatment.

b. Animals: Thirty female Limousin or Limousin cross (with one Salers cross) calves, 4 to 6 months of age and weighing 107.5 to 199.0 kg, were ranked by decreasing body weights and allocated consecutively to 15 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Pasture

d. Infection: Natural parasitic nematode infections were acquired by grazing for 120 days post-treatment on a pasture with a history of nematode infestation.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 147 to 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.9: Therapeutic Efficacy of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	14	1288.1	0.8	99.9
Cooperia surnabada	12	107.3	0.0	100.0
Ostertagia lyrata	14	121.0	0.0	100.0
Ostertagia ostertagi	15	12537.1	0.2	99.9
Nematodirus helvetianus	7	24.0	0.8	96.5

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.10 Study Number PR&D 0047202

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigator: - S. Rehbein, Dr. med. vet. habil., DipEVPC
Merial GmbH, Kathrinenhof Research Center
Rohrdorf, Germany

3) General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI in preventing nematode infections for 120 days post-treatment.

b. Animals: Thirty male castrated Pinzgauer calves, approximately 6 months of age and weighing 117 to 178 kg, were ranked by decreasing body weights and allocated consecutively to 15

replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Pasture

d. Infection: Natural parasitic nematode infections were acquired by grazing for 120 days post-treatment on a pasture with a history of nematode infestation.

e. Dosage Form: Eprinomectin ERII

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 148 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.10: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	13	2108.9	0.4	99.9
Cooperia punctata	14	34182.9	3.8	99.9
Cooperia surnabada	11	349.8	0.4	99.9
Dictyocaulus viviparus	13	9.8	0.0	100.0
Oesophagostomum radiatum	14	65.8	0.0	100.0
Ostertagia leptospicularis	13	698.1	0.5	99.9

Ostertagia ostertagi	14	4960.8	0.5	99.9
O. ostertagi inhibited L4	14	14703.1	3.6	99.9
Trichostrongylus axei	14	3124.9	5.2	99.8

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.11 Study Number PR&D 0047203

1) Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) - Investigator: T.A. Yazwinski, Ph.D.
University of Arkansas
Fayetteville, AR

3) - General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI in preventing nematode infections for 120 days post-treatment.

b. Animals: Thirty mixed stocker breed calves, approximately 6 months of age and weighing 144 to 183 kg, were ranked by decreasing body weights and allocated consecutively to 15 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Pasture.

d. Infection: Natural parasitic nematode infections were acquired by grazing for 120 days post-treatment on a pasture with a history of nematode infestation.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied 148 to 150 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness days against parasite species with an adequate level of infection at 120 is summarized in the following table:

Table IIC.11: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia punctata	15	1784.5	280.2	84.3
Haemonchus placei	15	616.0	7.2	98.8
Oesophagostomum radiatum	12	69.9	1.0	98.5
Ostertagia lyrata	15	139.1	0.8	99.5
Ostertagia ostertagi	15	3380.5	7.9	99.8
Trichostrongylus axei	15	108.3	0.2	99.8

5) Adverse Reactions: No adverse reactions to treatment were noted.

C.12 Study Number PR&D 0047204

1) - Type of Study: Dose confirmation study in cattle with naturally-acquired nematode infections.

2) Investigator: - E.G. Johnson. D.V.M. -
Johnson Research -
Parma, ID -

3) - General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI in preventing nematode infections for 120 days post-treatment.

b. Animals: Thirty Holstein male-castrates, approximately 5 months of age and weighing 192 to 240 kg, were ranked by decreasing

body weights and allocated consecutively to 15 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Pasture

d. Infection: Natural parasitic nematode infections were acquired by grazing for 120 days post-treatment on a pasture with a history of nematode infestation.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: All cattle were necropsied either 148 or 149 days post-treatment.

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 120 days is summarized in the following table:

Table IIC.12: Therapeutic Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Vehicle (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Cooperia oncophora	14	458.5	0.2	>99.9
Cooperia surnabada	8	12.8	0.2	98.6
Ostertagia ostertagi	15	1773.6	0.5	>99.9
Trichuris ovis	12	45.2	2.4	94.8

5) - Adverse Reactions: No adverse reactions to treatment were noted.

C.13 Study Number PR&D 0135201

1) - Type of Study: Dose confirmation study in cattle with induced - gastrointestinal roundworm and lungworm infections. -

2) Investigator: Gregory C. Royer, D.V.M. -
Merial, Missouri Research Center -

3) - General Design:

a. - Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 150 days post-treatment.

b. - Animals: Sixteen Angus/Angus cross calves (8 male castrates and 8 females), approximately 6 to 7 months of age and weighing 148 to 183 kg, were ranked by decreasing body weights by sex and allocated consecutively, within sex, to 8 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. - Housing: Cattle were maintained in pens by treatment group.

d. - Infection: Experimentally induced nematode infections were used. Each animal was orally inoculated on Day 150 with infective third-stage larvae (L3) from the nematode species listed below. *Bunostomum phlebotomum* was inoculated topically into the ear canal.

Table IIC.13.1: Inoculation Schedule

Species	Number of infective larvae	Age of Strain (Yrs)
Bunostomum phlebotomum	1,033	9.5
Dictyocaulus viviparus	1,998	9.75
Oesophagostomum radiatum	1,874	9.5
Ostertagia ostertagi/lyrata	15,008	0.5

e. Dosage Form: Eprinomectin ERI

f. - Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. - Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. - Controls: Saline was administered at 1.0 mL per 110 lb (50 kg) body.

i. - Test Duration: All cattle were necropsied either 175 or 176 days post-treatment.

j. - Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 150 days is summarized in the following table:

Table IIC.13.2: Persistent Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number of Infected Control Animals	Saline (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Dictyocaulus viviparus	6	78.8	0.0	100.0
Ostertagia ostertagi	6	3472.0	859.8	75.2

5) - Adverse Reactions: No adverse reactions to treatment were noted.

C.14 Study Number PR&D 0135301

1) - Type of Study: Dose confirmation study in cattle with induced gastrointestinal roundworm and lungworm infections.

2) Investigator: - M. Visser, Biol.
Merial GmbH, Katherinenhof Research Center
Rohrdorf, Germany

3) - General Design:

a. Purpose: This study was designed to determine the effectiveness of Eprinomectin ERI against nematode infections induced at 150 days post-treatment.

b. Animals: Twenty-four Braunvieh (Brown Swiss) male calves, approximately 6 months of age and weighing 128 to 168 kg, were ranked by decreasing body weights and allocated consecutively to eight replicates of three animals each. Animals within each replicate were randomly assigned to one of three treatment groups. A description and the results for Treatment Groups 1 and 2 (Saline and Eprinomectin ERI, respectively) are reported here. The results for Treatment Group 3 (positive control) are not reported here.

c. Housing: Individually stanchioned.

d. Infection: Experimentally induced nematode infections were used. Each animal was inoculated on Day 150 with infective third-stage larvae (L3) from the nematode species listed below. *Bunostomum phlebotomum* was inoculated topically into the ear canal.

Table IIC.14.1: Inocula

Species	Number of infective larvae	Age of Strain (Years)
Bunostomum phlebotomum	1,280	<1
Dictyocaulus viviparus	1,350	<1
Haemonchus contortus	5,200	<1
Oesophagostomum radiatum	1,200	<1
Ostertagia ostertagi/lyrata	12,800	<1

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Controls: Saline was administered at 1.0 mL per 110 lb (50 kg) body.

i. Test Duration: All cattle were necropsied 178 days post-treatment -

j. Pertinent Measurements/Observations: Nematodes recovered from animals at necropsy were counted and identified.

4) - Results: Effectiveness against parasite species with an adequate level of infection at 150 days is summarized in the following table:

Table IIC.14.2: Persistent Effectiveness of Eprinomectin ERI Against Nematodes

Parasite	Number Of Infected Control Animals	Saline (Control)	Eprinomectin	Effectiveness (%)
		Geometric Mean		
Bunostomum phlebotomum	8	578.7	25.3	95.6
Dictyocaulus viviparus	8	209.9	4.7	97.7
Haemonchus contortus	8	937.3	78.5	91.6
Oesophagostomum radiatum	8	269.3	12.0	95.5
Ostertagia ostertagi	8	8112.1	1548.2	80.9

5) - Adverse Reactions: No adverse reactions to treatment were noted.

D. Substantial Evidence for Ectoparasite Indications

GRUBS – Two studies were conducted to evaluate the effectiveness of Eprinomectin ERI against naturally occurring infestations of all parasitic larval stages (L1, L2, L3) of *Hypoderma bovis*. Studies were conducted using common protocols in Germany and Wisconsin, USA. All protocols were reviewed and accepted by CVM. In each study, 30 animals were selected based on a positive ELISA test for *Hypoderma* spp. exposure.

MITES – Three studies were conducted to evaluate the effectiveness of Eprinomectin ERI against artificial infestations with *Sarcoptes scabiei*. Three European Union locations were accepted by CVM as appropriate sites for these studies.

DATA ANALYSIS: In all effectiveness studies, the Wilcoxon rank sum test was used to compare the distribution of speciated parasite counts for the treated group to that of the control group. A two-sided test was used at $\alpha=0.05$. Speciated parasite counts for each animal were transformed to the natural logarithm of (count + 1) for analysis and calculation of geometric means. Effectiveness was calculated as 100[(C-T)/C], where C is the geometric mean for the control group and T is the geometric mean for the treated group. An indication was granted if there was a minimum of two studies having the following: an adequate level of infestation in 6 control animals, a statistically significant difference between treated and control animals at $P<0.05$, and 90% effectiveness using geometric means for each genus species of parasite. If there were more than 2 studies, then the reported percent effectiveness against a genus species of parasite was the arithmetic mean of the percent effectiveness for all studies with that genus species of parasite. If this average was greater than or equal to 90%, then the claim was granted. For the mite studies, lesion scores were also analyzed using the Wilcoxon rank sum test.

A claim is granted for *Hypoderma bovis* and *Sarcoptes scabiei* based on the above criteria. The five studies are summarized as follows:

D.1 Study Number PR&D 0047402

1) Type of Study: Dose confirmation study in cattle with naturally-acquired *Hypoderma bovis* infestations.

2) Investigator: - S. Rehbein, Dr. med. vet. Habil., DipEVPC -
Merial GmbH -
Kathrinenhof Research Center -
Rohrdorf, Germany -

3)General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment against all parasitic larval stages of *Hypoderma bovis* (L1, L2, L3).

b. Animals: Twenty-seven Rotbunte (26 females, one male), one Schwarzbunte female, and two Angus cross female cattle were used in this study. They were approximately 6 to 25 months of age and weighed 190 to 482 kg. They were ranked by decreasing ELISA antibody titers and allocated consecutively to 9 replicates of three animals each. Animals within each replicate were randomly assigned to one of three treatment groups. One group of cattle was treated with eprinomectin ERI when the *Hypoderma* larvae were expected to be in the L1 stage of development (Group 2) and a second group of cattle (Group 3) was

treated when the *Hypoderma* spp. larvae were in the L2/L3 stage of development.

c. Housing: Individually stanchioned

d. Infestation: Cattle were from a naturally infested herd.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once to Group 2 on Day 0 and once to Group 3 on Day 119 when sufficient warbles were present at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Control: Vehicle containing no eprinomectin was administered (to Group 1) at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: 195 days.

j. Pertinent Measurements/Observations: Starting on Day 119, when the *Hypoderma* larvae were mature enough to be collected and identified, the cattle were inspected weekly until larvae were confirmed to no longer emerge (Day 195). In each treatment group, numbers of *Hypoderma* larvae emerging from animals were counted.

4) Results: The geometric means for the control and eprinomectin-treated groups were 12.3, 0, and 0, respectively. Thus the therapeutic effectiveness of eprinomectin against *Hypoderma bovis* larvae is 100%.

5) Adverse Reactions: No adverse reactions to treatment were noted.

D2. Study Number PR&D 0047403

1) Type of Study: Dose confirmation study in cattle with naturally-acquired *Hypoderma bovis* infestations.

2) Investigator: L. L. Smith, D.V.M.
L. Smith Farms
Readstown, WI

3) General Design:

a. Purpose: This study was designed to confirm the effective dose for treatment against all parasitic larval stages of *Hypoderma bovis* (L1, L2, L3).

b. Animals: Thirty mixed beef breeds cattle, in a combination of 22 male castrates and 8 females, were included in this study. The animals were approximately 5 to 9 months of age and weighed 200 to 390 kg. They were ranked by decreasing ELISA antibody titers and allocated consecutively to 10 replicates of three animals each. Animals within each replicate were randomly assigned to one of three treatment groups. One group of cattle was treated with eprinomectin ERI when the *Hypoderma* larvae were expected to be in the L1 stage of development (Group 2) and a second group of cattle (Group 3) was treated when the *Hypoderma* spp. larvae were in the L2/L3 stage of development.

c. Housing: All animals were housed by group in indoor/outdoor pens in an open front building.

d. Infestation: Cattle were from a naturally infested herd.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once to Group 2 on Day 0 and once to Group 3 on Day 140 when sufficient warbles were present at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Control: Vehicle containing no eprinomectin was administered -

(to Group 1) at 1.0 mL per 110 lb (50 kg) body weight. -

i. Test Duration: 189 days.

j. Pertinent Measurements/Observations: Starting on Day 147, when the *Hypoderma* larvae were mature enough to be collected and identified, the cattle were inspected weekly until larvae were confirmed to no longer emerge (Day 189). In each treatment group, numbers of *Hypoderma* larvae emerging from animals were counted.

4) Results: The geometric means for the control and eprinomectin-treated groups were 1.8, 0, and 0, respectively. Thus the therapeutic effectiveness of eprinomectin against *Hypoderma bovis* larvae is 100%.

5) Adverse Reactions: No adverse reactions to treatment were noted.

D.3 Study Number PR&D 0104601

1) Type of Study: Dose confirmation study in cattle with artificially-induced *Sarcoptes scabiei* infestations.

2) Investigator: M.Visser, Biol.,
Merial GmbH,
Kathrinenhof Research Center
Rohrdorf, Germany -

3)General Design:

a. Purpose: This study was designed to confirm the effective dose for treatment against infestations with *Sarcoptes scabiei* in cattle.

b. Animals: Sixteen male Fleckvieh (Simmental) calves were used in this study. They were approximately 7 months of age and weighed 210 to 248 kg. They were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Individually stanchioned

d. Infestation: Infestations were induced with 6800 *Sarcoptes scabiei* var. *bovis* and 4200 *S. scabiei* var *bovis* on Days -56 and -50, respectively.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Control: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: 56 days.

j. Pertinent Measurements/Observations: On Day -1, scrapings were taken to determine live mite counts, and lesions were scored on a five point scale (0 to 4; see Table IID.3.2 legend for description of scoring). This

process was repeated on Day 7 and at weekly intervals until study termination on Day 56.

4) Results:

Table IID.3.1: Summary of Live Mite Counts			
Day	**Vehicle (Control) GM[1]**	**Eprinomectin ERI GM**	**Efficacy (%)**
-1	311.0	281.4	-
7	398.0	0.0	100.0
14	421.3	0.0	100.0
21	487.3	0.0	100.0
28	519.1	0.0	100.0
35	547.4	0.0	100.0
42	563.7	0.0	100.0
49	545.2	0.0	100.0
56	562.8	0.0	100.0

[1] Geometric mean, based on transformation to ln(count +1): 8 animals per treatment

Table IID.3.2: Summary of Lesion Scores[1]			
Day	**Vehicle (Control)**	**Eprinomectin ERI**	**Prob[2]**
1	3 (**8**) -	3(**8**)	ns[3]
7	3(**7**) and 4(**1**) -	3(**8**)	ns
14	3(**6**) and 4(**2**) -	1(**1**) and 3(**7**)	ns
21	3(**6**) and 4(**2**) -	1(**8**)	<0.05
28	3(**6**) and 4(**2**) -	1(**8**)	<0.05
35	3(**4**) and 4(**4**) -	1(**8**)	<0.05
42	3(**5**) and 4(**3**) -	1(**8**)	<0.05
49	3(**5**) and 4(**3**) -	0(**2**) and 1(**6**)	<0.05
56	3(**1**) and 4(**7**) -	0(**7**) and 1(**1**)	<0.05

[1] 0=healthy skin **Bold** numbers are the number of animals with the lesion score.
1=healing lesion, crusts lifted detached easily but hair growth not complete
2=active lesion, extent of less than the palm of the hand
3=active lesion, extent of more than the palm of the hand
4=active lesion, extent of more than the half of the body of the animal

[2] - Probability values from Wilcoxon rank sum test comparing Eprinomectin ERI to Vehicle (Control).

[3] Not significant at α=0.05.

5) Adverse Reactions: No adverse reactions to treatment were noted.

D.4 Study Number PR&D 0104602

1) Type of Study: Dose confirmation study in cattle with artificially-induced *Sarcoptes scabiei* infestations.

2) Investigator: M. Löwenstein, Dr. med. vet., DipEVPC -
Veterinärmedizinische Universität Wien, -
Wien, Austria -

3) General Design:

a. Purpose: This study was designed to confirm the effective dose for the treatment against infestations with *Sarcoptes scabiei* in cattle.

b. Animals: Sixteen female Fleckvieh (Simmental) calves were used in this study. They were approximately 5 to7 months of age and weighed 150 to 221 kg. They were ranked by decreasing body weights and allocated consecutively to eight replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Individually stanchioned

d. Infestation: Infestations were induced with 1600 *Sarcoptes scabiei* var. *bovis* and 2800 *S. scabiei* var *bovis* on Days -57 and -48, respectively.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Control: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: 56 days.

j. Pertinent Measurements/Observations: On Day -1, scrapings were taken to determine live mite counts, and lesions were scored on a five point scale (0 to 4; see Table IID.4.2 legend for description of scoring). This process was repeated on Day 7 and at weekly intervals until study termination on Day 56.

4) Results:

Table IID.4.1: Summary of Live Mite Counts			
Day	**Vehicle (Control) GM[1]**	**Eprinomectin (ERI) GM**	**Efficacy %**
1	64.6	69.9	-
7	120.3	2.9	97.6
14	124.3	0.6	99.5
21	177.7	0.2	99.9
28	151.6	0.0	100.0
35	218.0	0.0	100.0
42	197.6	0.0	100.0
49	254.6	0.0	100.0
56	501.5	0.0	100.0

[1] Geometric mean, based on transformation to In(count+1): 8 animals per treatment.

Table IID.4.2: Summary of Lesion Scores[1]			
Day	**Vehicle (Control)**	**Eprinomectin ERI**	**Prob[2]**
-1	3 (**8**)	3(**8**)	ns[3]
7	3 (**8**)	3(**8**)	ns
14	3 (**8**)	3(**5**) and 2(**3**)	ns
21	3 (**8**)	0(**2**),1(**4**), and 2(**2**)	<0.05
28	3 (**8**)	0(**5**),1(**1**), and 2(**2**)	<0.05
35	3 (**8**)	0(**6**) and 1(**2**)	<0.05
42	3(**7**) and 2(**1**)	0(**6**) and 1(**2**)	<0.05
49	3(**7**) and 2(**1**)	0(**6**) and 1(**2**)	<0.05
56	3(**7**) and 2(**1**)	0(**7**) and 1(**1**)	<0.05

[1] Raw data lesion score was translated to numeric scores for the purpose of data analysis. Original scoring system can be found in the raw data.
0=healthy skin
1=healing lesion, crusts lifted detached easily but hair growth not complete
2=active lesion, extent of less than the palm of the hand
3=active lesion, extent of more than the palm of the hand
4=active lesion, extent of more than the half of the body of the animal

[2] Probability values from Wilcoxon rank sum test comparing Eprinomectin ERI to Vehicle (Control).

[3] Not significant at a=0.05.

Bold numbers in parenthesis are the number of animals with the lesion score.

5) Adverse Reactions: No adverse reactions to treatment were noted.

D5. Study Number PR&D 0052201

1) Type of Study: Dose confirmation study in cattle with artificially-induced *Sarcoptes scabiei* infestations.

2) Investigator: S. Rehbein, Dr. med. Vet. Habil., DipEVPC
Merial GmbH,
Kathrinenhof Research Center
Rohrdorf, Germany

3) General Design:

a. Purpose: This study was designed to confirm the effective dose for treatment against infestations with *Sarcoptes scabiei* in cattle.

b. Animals: Twelve male Braunvieh calves were used in this study. They were approximately 6.5 months of age and weighed 149 to 174 kg. They were ranked by decreasing body weights and allocated

consecutively to 6 replicates of two animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

c. Housing: Individually stanchioned

d. Infestation: Infestations were induced with 7300 *Sarcoptes scabiei* var. *bovis* and 6400 *S. scabiei* var *bovis* on Days -56 and -49, respectively.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Control: Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: 56 days.

j. Pertinent Measurements/Observations: On Day -1, scrapings were taken to determine live mite counts, and lesions were scored on a five point scale (0 to 4; see Table IID.5.2 legend for description of scoring). This process was repeated on Day 7 and at weekly intervals until study termination on Day 56.

4) Results:

Table IID.5.1: Summary of Live Mite Counts			
Day	**Vehicle (Control) GM[1]**	**Eprinomectin ERI GM**	**Efficacy (%)**
-1	276.2	235.5 -	14.7
7	303.9	0.6 -	99.8
14	385.1	0.1 -	99.9
21	336.9	0.0 -	100
28	341.3	0.0 -	100
36	323.7	0.0 -	100
42	254.9	0.0 -	100
49	255.1	0.0 -	100
56	204.8	0.0 -	100

[1] Geometric mean, based on transformation to In(count+1): 6 animals per treatment.

Table IID.5.2: Summary of Lesion Scores[1]			
Day	**Vehicle (Control)**	**Eprinomectin ERI**	**Prob[2]**
-1	3 (**6**) -	3(**6**)	ns[3]
7	3(**6**) -	3(**6**)	ns[3]
14	3(**4**) and 4(**2**) -	1(**5**) and 3(**1**)	<0.05
21	3(**3**) and 4(**3**) -	1(**6**)	<0.05
28	3(**2**) and 4(**4**) -	1(**6**)	<0.05
35	3(**2**) and 4(**4**) -	1(**6**)	<0.05
42	3(**2**) and 4(**4**) -	1(**6**)	<0.05
49	3(**2**) and 4(**4**) -	1(**6**)	<0.05
56	3(**1**) and 4(**5**) -	1(**6**)	<0.05

[1] - Raw data lesion score was translated to numeric scores for the purpose of data analysis. Original scoring system can be found in the raw data.
0=healthy skin
1=healing lesion, crusts lifted detached easily but hair growth not complete
2=active lesion, extent of less than the palm of the hand
3=active lesion, extent of more than the palm of the hand
4=active lesion, extent of more than the half of the body of the animal

[2] Probability values from Wilcoxon rank sum test comparing Eprinomectin ERI to Vehicle (Control).

[3] Not significant at a=0.05.

Bold numbers in parenthesis are the number of animals with the lesion score.

5) Adverse Reactions: No adverse reactions to treatment were noted.

E. Clinical Field Studies

Seven studies were conducted under field conditions to confirm the effectiveness of Eprinomectin ERI against naturally-acquired nematode infections in cattle. Animals were selected from commercial herds across the Midwest and Northwest United States in which gastrointestinal parasitism had been confirmed by the presence of nematode eggs in the feces. In each study, 68 (67 in one study) animals were selected and randomly assigned to a saline- or eprinomectin-treated group (25% and 75% of test animals, respectively). Fecal egg counts were conducted on samples collected from each animal before treatment, at the time of treatment, and at regular intervals throughout the study. Positive fecal samples were cultured en masse to determine the nematode genera composition. Body weights were recorded throughout the study. Animals were observed daily for health problems. Since these trials were conducted following a similar protocol, the results of all seven trials are summarized in a single section.

DATA ANALYSIS: In all clinical field studies, the Wilcoxon rank sum test was used to compare the distribution of fecal egg (strongylid) counts for the treated group to that of the control group. A two-sided test was used at α=0.05. Fecal counts for each animal were transformed to the natural logarithm(count + 1) for analysis and calculation of geometric means. Effectiveness was calculated as 100[(C-T)/C], where C is the geometric mean for the control group and T is the geometric mean for the treated group. These studies were used as supportive data in determining effectiveness of the drug.

1) Type of Study: Clinical Field Study

2) Investigators:

PR&D 0011504	J.C.Williams, Ph.D. Louisiana State University -Baton Rouge, LA
PR&D 0011505	T.A. Yazwinski, Ph.D. University of Arkansas -Fayetteville, AR
PR&D 0011506	E.G. Johnson, D.V.M. Johnson Research -Parma, ID
PR&D 0011507	A. Marchionido, Ph.D., G.C. Royer, D.V.M. Merial, Missouri Research Center -Fulton, MO
PR&D 0011508	B.E. Stromberg, Ph.D. University of Minnesota

St. Paul, MN

PR&D 0011509 L.L. Smith, D.V.M., M.S., B.S.
Smith R&D
Lodi, WI
(Study site – Readstown, WI)

PR&D 0011510 E.G. Johnson, D.V.M.
Johnson Research
Parma, ID
(Study site = Blachy, OR)

3) General Design:

a. Purpose: To confirm the effectiveness and safety of the recommended dose of Eprinomectin ERI solution when used under pasture conditions for the treatment and control of endoparasites in cattle.

b. Animals: At each study site approximately 51 cattle were treated with Eprinomectin ERI and approximately 17 cattle were treated with vehicle. They were ranked within sex by decreasing body weights and allocated consecutively to seventeen replicates of four animals each. Animals within each replicate were randomly assigned to one of two treatment groups.

Table IIE.1: Animal Summary for Clinical Field Studies

Study PR&D	Control Cattle #	Treated Cattle #	Weight (kgs)	Breed
0011504	17	51	208 - 299	Angus-cross
0011505	17	51	148 - 209	Beef-cross
0011506	17	50	197 - 335	Beef cross
0011507	17	51	133 - 239	Angus/Angus-cross
0011508	17	51	141 - 268	Angus-cross
0011509	17	51	163 - 274	Beef-cross
0011510	17	51	144 - 284	Beef-cross

c. Housing: The cattle were maintained on pasture throughout the studies.

d. Infection: Cattle had naturally acquired nematode infections from grazing on infested pasture.

e. Dosage Form: Eprinomectin ERI

f. Route of Administration: Single subcutaneous injection in the front of the shoulder.

g. Dose: Group 2 - Eprinomectin ERI was administered once on Day 0 at 1.0 mL per 110 lb (50 kg) body weight to provide 1.0 mg/kg body weight.

h. Control: Group 1 - Vehicle containing no eprinomectin was administered at 1.0 mL per 110 lb (50 kg) body weight.

i. Test Duration: 120 days.

j. Pertinent Measurements/Observations: Fecal samples were collected (and body weights recorded) within a week prior to treatment (Day 0) to confirm nematode infection and then every 28 days after treatment (from Day 0 to Day 120). Fecal samples were analyzed to demonstrate continued effectiveness of treatment and for coproculture to determine the nematode genera present. Body weights were monitored throughout the study.

4) Results: Strongylid egg count data and effectiveness is summarized in Table IIE.2.

Table IIE.2: Therapeutic Effectiveness of Eprinomectin ERI (E-ERI) Against Nematodes in Clinical Field Studies

Study PR&D	Treat-ment	Strongylid eggs per gram counts (GM[1])					
		Days before treatment		Days after treatment			
		-7 to –3	0	28	56	84	120
0011504	Control	281	140	28	22	14	19
	E-ERI	148	82	<1	<1	<1	<1
	Effectiveness	-	-	98%	98%	>99%	>99%
0011505	Control	60	79	61	191	359	220
	E-ERI	68	65	<1	1	2	3
	Effectiveness	-	-	99%	>99%	>99%	98%
0011506	Control	75	59	19	14	11	2
	E-ERI	76	61	0	0	0	0
	Effectiveness	-	-	100%	100%	100%	100%
0011507	Control	51	96	42	18	34	21
	E-ERI	77	112	2	2	<1	<1
	Effectiveness	-	-	96%	88%	>99%	>99%
0011508	Control	28	54	12	11	8	8
	E-ERI	27	23	<1	<1	<1	<1
	Effectiveness	-	-	96%	93%	99%	99%
0011509	Control	27	NA	6	3	6	4
	E-ERI	27	NA	<1	<1	<1	0
	Effectiveness	-	-	97%	94%	99%	>99%
0011510	Control	51	59	84	58	19	17
	E-ERI	35	44	3	4	2	1
	Effectiveness	-	-	96%	94%	91%	95%

[1] Geometric mean, based on transformation to ln(count+1)

5) Adverse Reactions: No adverse reactions to treatment were noted in any of the studies.

III. TARGET ANIMAL SAFETY:

The clinical effects of eprinomectin pour-on administered topically at 10X (5.0 mg/kg) the recommended therapeutic level of 0.5 mg/kg were assessed in the original approval of IVOMEC EPRINEX Pour-On for Beef and Dairy Cattle (see FOI Summary of NADA 141-079, approval dated April 16, 1997). The only significant clinical adverse effect observed was mydriasis on Days 4 to 5 in one of six cattle. The clinical effects of eprinomectin extended-release injectable were assessed in a toxicity study when the drug was administered as a single subcutaneous dose to cattle at 1X, 3X, and 5X (1.0, 3.0, and 5.0 mg/kg) the recommended therapeutic level of 1.0 mg/kg. Two reproductive safety studies were conducted to evaluate the safety of eprinomectin extended-release injectable when administered at 3X the use level to beef cows at all stages of breeding or pregnancy. Reproductive safety was not evaluated in bulls. The toxicity study and the reproductive safety studies are summarized below.

A. Toxicity Study:

1) Type of Study: Evaluation of the safety of Eprinomectin ERI administered to cattle at elevated dose levels and observed for 120 days.

2) Study Director: Raymond Plue, D.V.M., M.S. and John E. Holste, D.V.M.
Missouri Research Center
Fulton, MO

3) - General Design:

a. Purpose: To determine the safety of Eprinomectin ERI in cattle when administered once subcutaneously at 1X, 3X, or 5X the proposed dose (1.0 mg/kg). Cattle were observed for 120 days.

b. Animals: 24 Angus cattle (12 females and 12 intact males), 6 to 7 months of age, weighing 165 to 228 kg

4) Housing: Individual pens

5) Dosage Form: Eprinomectin ERI

e. Route of Administration: Drug administration was by subcutaneous injection in the neck in front of the shoulder. No more than 10 mL was administered per injection site. The 1X dose was administered as one injection, the 3X dose was administered as three injections, and the 5X dose as five injections.

f. Doses: 1 mL/110 lb (50 kg) body weight (1X the use level of 1.0 mg/kg), 3.0 mL/110 lb (50 kg) body weight (3X the use level of 1.0 mg/kg), 5.0 mL/110 lb (50 kg) body weight (5X the use level of 1.0 mg/kg) administered once on Day 0.

g. Controls: Vehicle was administered by subcutaneous injection at 1 mL/110 lb (50 kg) body weight.

h. Test Duration: The cattle were necropsied 120 days after treatment with eprinomectin extended-release injectable.

i. Pertinent Parameters Measured: Physical examinations were conducted on Days -7, -1, 0 (between 4 and 8 hours post-treatment), 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and just prior necropsy (Days 120, 121, or 122). Blood samples were collected at regular intervals for eprinomectin assay, hematology, and blood chemistry examination. Daily feed consumption was measured from Day -7 to Day 120. Water consumption was evaluated by determining the hydration status of each animal at every physical examination. Animals were necropsied on Days 120, 121, or 122. Tissues samples were collected from all animals. Histopathological examination was done on tissue samples from the control and 5X groups.

j. Statistical Analysis: Analyses of clinical pathology and physical examination variables were performed using an analysis of covariance for repeated measures design. The factors included treatment, sampling day, sex, and all interactions among these three factors as fixed effects and replicate nested within sex, and its interactions with sampling day nested within sex, as random effects.

4) Results: When comparing the treated groups to the control group, there were no clinically or biologically significant differences in hematology values, clinical pathology values, or physical examination findings. Animals in the 3X and 5X dose groups had a statistically significant reduction in average weight gain (on average, approximately 25 kg or 50 lbs), when compared to the 1X dose group and the control group (control vs. 3X, $p = 0.0941$; control vs. 5X, $p = 0.0682$).

No treatment-related systemic lesions were observed in animals administered Eprinomectin extended-release injectable. Injection site reactions were limited to occasional edema, swelling without pain, hyperemia, or skin necrosis. Small focal granulomas in the subcutis were present at some of the injection sites at the end of the study.

5) Conclusions: Subcutaneous administration of Eprinomectin extended-release injectable at up to 5X the recommended therapeutic dose level was well tolerated by cattle.

B. Reproductive Safety in Female Cattle of Breeding Age:

1) Type of Study: Evaluation of the reproductive safety of Eprinomectin ERI in cattle of breeding age at 3X the use level prior to and at approximately 9 and 21 days after breeding.

2) Study Director: Bruce N. Kunkle, D.V.M., M.S., Ph.D. -
Missouri Research Center -
Fulton, MO -

3) General Design:

a. Purpose: To determine the safety of Eprinomectin extended-release injectable in female cattle of breeding age.

b. Animals: 136 Angus and Angus Cross cattle, 13 to 15 months of age, weighing 320 to 475 kg.

c. Housing: Cows of a given replicate kept in the same pasture up to at least 10 to 14 days prior to the estimated due date of calving or at the time of the first indication that calving would begin.

d. Dosage Form: Eprinomectin ERI

e. Route of Administration: Drug administration was by subcutaneous injection in the neck in front of the right and/or left shoulder. No more than 10 mL was administered per injection site.

f. Doses: 3.0 mL/110 lb (50 kg) body weight (3X the use level of 1.0 mg/kg) given at 0, 22, or 34 days after the initiation of the study.

Table IIIB.3: Treatment and Control Groups					
Trt. Group	**Drug**	**Dose**	**Route**	**Treatment Days**	**Total # Animals**
1	Saline (Control)	3.0 mL/50 kg body weight	SC	0, 22, or 34	34
2	Eprinomectin 5% w/v Extended-Release Injectable	3.0 mL/50 kg body weight	SC	0	34
3	Eprinomectin 5% w/v Extended-Release Injectable	3.0 mL/50 kg body weight	SC	22	34
4	Eprinomectin 5% w/v Extended-Release Injectable	3.0 mL/50 kg body weight	SC	34	34

g. Controls: Saline was administered by subcutaneous injection at 3.0 mL/110 lb (50 kg) body weight.

h. Test Duration: 328 days from the first treatment day through the 30-day post–parturition clinical exams of cows and calves

i. Statistical Methodology: The primary variables for analysis were conception rates, calving rates, abortion rates, calving failure rates, calf body weights, calf average daily body weight gain, and presence of congenital anomalies. Secondary variables examined were cow body condition scores, cow body weight change on Day 90, 180, and post-parturition, and calving ease/dystocia. Discrete variables were examined for differences between treatment groups using Fisher's exact test or the Wilcoxon rank sum test. Continuous variables were analyzed for treatment differences using analysis of variance for a mixed model design with treatment as a fixed effect and block as a random effect.

j. Results: There were no statistically significant differences between the saline-treated group and eprinomectin-treated group (regardless of time administered) for conception rates, calving rates, abortion rates, calving failure rates, assessment of maturity, presence of congenital anomalies, calf body weights (Day 0, Day 30, and average daily weight gain), and cow body condition scores. For cows, weight gain from Day -2 to post-parturition was significantly higher in animals where eprinomectin was administered on Days 22 or 34. The distribution of calving ease/dystocia scores were different in animals where eprinomectin was administered on Day 0 when compared to saline treated animals. This difference was not clinically significant.

k. Conclusions: Subcutaneous administration of Eprinomectin ERI for cattle at 3X the recommended therapeutic dose level was well tolerated by cattle of breeding age.

C. Reproductive Safety in Pregnant Cattle:

1) Type of Study: Evaluation of the reproductive safety of Eprinomectin extended-release injectable when administered to pregnant cattle at 3X the use level during early, mid, and late gestation.

2) Study Director: Luiz Carvalho, D.V.M., MSc.
Merial Saúde Animal Ltda.
Uruguaiana Research Center
Brazil

3) General Design:

a. Purpose: To determine the safety of Eprinomectin ERI in pregnant cattle.

b. Animals: 120 female cattle, 4 to 8 years of age, weighing 342 to 570 kg, and having a gestational age of about 50 days (+/- 10 days) at the initiation of the study.

c. Housing: Cows of a given replicate were kept in the same paddock or pasture.

d. Dosage Form: Eprinomectin ERI

e. Route of Administration: Drug administration was by subcutaneous injection in the neck in front of and/or behind the right and/or left shoulder using sterile hypodermic syringes fitted with 16 gauge x 1 1/2 inch needles. No more than 10 mL was administered per injection site.

f. Doses: 3.0 mL/110 lb (50 kg) body weight (3x use level of 1.0 mg/kg)

Table IIIC.3: Treatment and Control Groups					
Trt. Group	**Drug**	**Dose**	**Route**	**Treatment Days**	**Total # Animals**
1	Saline (Control)	3.0 mL/50 kg body weight	SC	0, 90, and 180	30
2	Eprinomectin ERI for Cattle	3.0 mL/50 kg body weight	SC	~50 days of gestation (Day 0)	30
3	Eprinomectin ERI for Cattle	3.0 mL/50 kg body weight	SC	~140 days of gestation (Day 90)	30
4	Eprinomectin ERI for Cattle	3.0 mL/50 kg body weight	SC	~230 days of gestation (Day 180)	30

g. Controls: Saline was administered by subcutaneous injection at 3.0 mL/110 lb (50 kg) body weight.

h. Test Duration: Middle of first trimester through the 30 day post-parturition clinical examinations of cows and calves.

i. Statistical Methodology: The primary variables for analysis were calving rates, abortion rates, calving failure rates, calf body weights, calf average daily body weight gain, and presence of congenital anomalies. Secondary variables examined were cow body condition scores, cow body weight change on Day 90, 180, and post-parturition, and calving ease/dystocia. Discrete variables were examined for differences between treatment groups using Fisher's exact test or the Wilcoxon rank sum test. Continuous variables were analyzed for treatment differences using analysis of variance for a mixed model design with treatment as a fixed effect and block as a random effect.

j. Results: There were no statistically significant differences between the saline-treated group and eprinomectin-treated group (regardless of time administered) for calving rates, abortion rates, calving failure rates, presence of congenital anomalies, and calf body weights. There were some statistical differences noted for cow body condition scores, calving ease/dystocia scores, and cow body weight changes (body weight on Day 180 to cow body weight on Day -1). With body condition scores, the distribution of scores was statistically different for animals administered eprinomectin early and late gestation when compared to the scores of the control animals. For calving ease/dystocia scores, the distribution of scores for animals administered eprinomectin in early gestation was statistically different from the scores of control animals. The differences

in body condition score and calving ease/dystocia scores were not considered clinically significant. Cow body weight gains were significantly higher in animals administered eprinomectin early and late gestation when compared to weight gains of control animals.

k. Conclusions: Subcutaneous administration of Eprinomectin ERI for cattle at 3X the recommended therapeutic dose level was well tolerated by pregnant cattle.

IV. HUMAN FOOD SAFETY:

A. Toxicology:

The FOI Summaries for the original approval of NADA 141-079 dated April 16, 1997, and a supplemental approval dated August 9, 1998, contain summaries of toxicology studies for the human food safety of IVOMEC EPRINEX (eprinomectin) in support of approval of this NADA.

The current FOI summary describes the additional toxicology studies supporting the human food safety of Eprinomectin Extended-Release Injectable (ERI) for injection site residues and the excipient, N-methyl-2-pyrrolidone (NMP).

Toxicology of Eprinomectin (Active Ingredient):

1. Summary of Toxicology Studies

Eprinomectin: Acute Oral Toxicity (Non-Lethality) Study in Rats.

Report Number: 0067801
Report Date: November 2, 2001
Study Director: Ali S. Faqi
Study Location: IIT Research Institute, Chicago, IL

Experimental Design and Conclusion: Eprinomectin (Lot No. 006JBB) in aqueous methyl cellulose was administered once by oral gavage to 10 male Sprague-Dawley rats per group at 0, 8, 10, 13, 16, 20, and 25 mg/kg body weight (bw). Body weight, weight gain, physical signs, hindlimb/forelimb grip strength, foot splay and right reflex were measured for all groups up to 24 hours following dosing. Gross necropsies were performed on all animals and histopathology was conducted on animals in the vehicle and highest dose groups. No significant effect of treatment on body weight was observed. Weight gain means showed a decreasing trend in the 13, 16, 20, and 25 mg/kg bw dose groups; however, the decrease was statistically significant only in the high dose (25 mg/kg bw) group compared to the control. One animal in the 20 mg/kg bw dose group was found dead approximately 24 hours post dosing. All groups had some incidence of diarrhea without a dose-

related trend. Clinical observations in the 13, 16, 20, and 25 mg/kg bw dose groups included hyper-irritability, drags on abdomen, red around mouth/face, dyspnea (not observed at 16 mg/kg bw), and hyperactivity. Additional observations at 16, 20, and 25 mg/kg bw include alopecia, tremors, hunched posture (not observed at 20 mg/kg bw), wet inguinal fur, redness around the eyes, and discolored inguinal fur. The high dose group also had convulsions, slight reflex reaction, hyperactivity, and cyanosis. Foot splay was significantly decreased in the 10, 16, 20, and 25 mg/kg bw dose groups at 2 hours post dosing. Foot splay was significantly reduced in the high dose group (25 mg/kg bw) at 24 hours post dosing. One animal in the 20 mg/kg bw group and one animal in the 25 mg/kg bw group were reported to have kidney dilatation and pigmentation, respectively, while one 8 mg/kg bw animal had an enlarged pigmented mandibular lymph node. No treatment-related effects on gross pathological observations or histopathology were observed. The no-observed-effect-level (NOEL) was determined to be 8 mg/kg bw based on decreased foot splay at doses of 10 mg/kg bw and higher.

2. Determination of No Observed Effect Level (NOEL) for chronic exposure and the NOEL for acute exposure.

The no-observed-effect level (NOEL) for chronic exposure of eprinomectin has been established from a 53-week oral toxicity study in dogs at 1.0 mg/kg bw/day, as published in the FOI Summary for NADA 141-079, dated April 16, 1997. The NOEL for acute exposure of eprinomectin was 8 mg/kg bw/day based on decreased foot splay at doses of 10 mg/kg bw/day and higher.

3. Acceptable Daily Intake (ADI) and Acceptable Single-Dose Intake (ASDI)

The acceptable daily intake (ADI) for eprinomectin is 0.01 mg/kg bw/day based on the NOEL of 1.0 mg/kg bw/day and a safety factor of 100, and was published in the FOI Summary for NADA 141-079, dated April 16, 1997. Four percent (4%) of the ADI is allocated for milk and the remaining 96% is allocated for the edible tissues resulting in the following ADI assignments:

0.0004 mg/kg bw/day for milk and
0.0096 mg/kg bw/day for the edible tissues.

For eprinomectin residues at an injection site, an acceptable single-dose intake (ASDI) of 0.8 mg/kg bw is calculated based on a NOEL of 8 mg/kg bw from the acute oral toxicity study in rats and a 10-fold safety factor, as follows:

$$ASDI = \frac{8\ mg/kg\ bw}{10} = 0.8\ mg/kg\ bw$$

4. Safe Concentrations for Total Residues (edible tissues and injection sites)

Safe concentrations for total eprinomectin-related residues resulting from chronic exposure are 16 ppb in milk, 1.92 ppm in muscle, 5.76 ppm in liver, 11.52 ppm in kidney and 11.52 ppm in fat, as published in the FOI summary for NADA 141-079, dated April 16, 1997.

Assuming a 60 kg average human body weight and a food consumption factor of 0.3 kg for muscle, a safe concentration of 160 ppm for total residues of eprinomectin at injection site tissues resulting from acute exposure is calculated as:

$$\textit{Safe Concentration at the injection site} = \frac{0.8\ mg/kg\ bw \times 60\ kg\ bw}{0.3\ kg}$$
$$= 160\ mg/kg \text{ or } 160\ ppm$$

<u>Toxicology of N-methyl-2-pyrrolidone (Excipient)</u>:

1. Summary of Toxicology Studies

Studies reported in the literature conducted on rodents have demonstrated that the excipient, N-methyl-2-pyrrolidone (NMP), increases the incidence of hepatocellular carcinomas in mice, but not in rats. The following studies were conducted to investigate the human food safety issues of the new excipient, NMP, used in Eprinomectin Extended-Release Injectable Solution.

a. *In Vitro* Mechanistic Studies

Studies have been conducted to assess the mode of action for the carcinogenicity of the excipient, NMP, in order to establish its safety in human food. The *in vitro* and *in vivo* studies, described below, address the carcinogenic potential of NMP.

i. Peroxisome proliferation potential of N-methylpyrrolidone (NMP)

Report Number: PR&D 0128501
Report Date: July 11, 2005 Study Director: John P. Vanden Heuvel
Study Location: Indigo Biosciences, L.L.C., State College, PA

Experimental Design and Conclusion: To determine the mode of action for NMP to promote hepatic carcinoma incidence in the mouse, an *in vitro* cell culture study was performed. Mouse 3T3-L1 fibroblasts were transfected with the mouse or rat Peroxisome

Proliferator-Activated Receptor alpha (PPARα) fused to a luciferase reporter gene. Cells were treated for 24 hours with NMP dissolved in DMSO at concentrations ranging from 0.08 to 5000 μM. NMP was shown to cause mild activation of the mouse PPARα at concentrations of 2500 and 5000 μM. NMP was unable to activate the rat PPARα at doses up to 1000 μM. The study demonstrated that NMP is a weak mouse PPARα agonist and is not a PPARα agonist in the rat.

ii. Summary of exploratory PPAR-transactivation assay in COS-7 cells

Study Number: TT 05-4546-01 and TT 05-4546-03
Memorandum Date: December 12, 2005
Report Date: not provided
Study Director: Timothy E. Johnson
Study Location: Merck

Experimental Design and Conclusion: To determine the mode of action for NMP to cause carcinogenic effects in rodents, an *in vitro* cell culture study was performed. African Green Monkey kidney cells were co-transfected with the rat PPARα and a luciferase reporter gene (MMTV-luc). Cells were treated for 24 hours with NMP, dissolved in DMSO, with concentrations ranging from 0.25 to 10 mM. The ability of NMP to cause receptor activation was determined by luciferase activity and toxicity was determined by measurement of lactate dehydrogenase activity at 24 and 48 hours. NMP did not result in an elevation of lactate dehydrogenase above the control, indicating that NMP was not toxic to the cell culture at the concentrations tested. NMP did not activate the rat PPARα receptor in the COS-7 reporter gene assay, demonstrating that NMP is not a PPARα agonist under the conditions of the assay.

iii. Mutagenicity Test on GMP M-Pyrol (93519-90) in the *Salmonella*/mammalian-Microsome Reverse Mutation Assay (Ames Test) with a Confirmatory Assay

Report Number: A-25620
Start Date: January 14, 1991
Termination date: February 28, 1991
Study Director: Timothy E. Lawlor, M.A.
Study Location: Hazelton Laboratories America, Inc.,
Kensington, MD

Experimental Design and Conclusion: The tester strains used were the *Salmonella* Typhimurium histidine auxotrophs TA98, TA100,

TA1535, TA1537, and TA1538. The doses tested in the mutagenicity assay were selected based upon the results of a dose range finding study using tester strain TA100 and ten dose levels of test article ranging from 6.67 to 5000 µg per plate, one plate per dose, both in the presence and absence of S9. The assay was conducted using three plates per dose level both in the presence and absence of S9. Six doses of test article were tested, from 100 to 5000 µg per plate both in the presence and absence of S9. This assay evaluates the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in the genome of specific *Salmonella* Typhimurium tester strains both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor-induced rat liver (S9). In the initial mutagenicity assay, all data were acceptable and no positive increases in the number of histidine revertants were observed with any of the tester strains either in the presence or absence of S9. In the confirmatory assay, all data were acceptable and no positive increases in the number of histidine revertants per plate were observed with any of the tester strains either in the presence or absence of S9. NMP was not mutagenic under the conditions of this assay.

iv. Mutagenicity Test on N-methyl-2-pyrrolidone in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

Study Number**:** 10519-0-447 -
Study Dates: August 22 to October 8, 1988 -
Study Location: Hazelton Laboratories America, Inc.
Kensington, MD -

Experimental Design and Conclusion: In the *In Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis (UDS) Assay, NMP did not induce significant increases in UDS. Freshly prepared rat hepatocytes were exposed to NMP at concentrations ranging from 0.5 µg/mL to 5000 µg/mL in the presence of 5 mCi/mL ^{3}HTdr (20 Ci/mmole). Treatment at 5000 µg/mL was not analyzed for nuclear labeling due to high toxicity. Treatments from 250 µg/mL to 4000 µg/mL, which covered a good range of toxicity (97.6 to 75.3% survival), were selected for analysis. The test material was soluble in media at all concentrations tested. No UDS was observed in the treated rat primary hepatocytes. In conclusion, NMP was considered negative in the Rat Primary Hepatocyte UDS assay.

v.Mutagenicity Test on N-methyl-2-pyrrolidone in the CHO/ HGPRT Forward Mutation Assay

Study Number: 10194-0-435
Report Date: June 23, 1988
Study Location: Hazelton Laboratories America, Inc.
Kensington, MD

Experimental Design and Conclusion: The objective of this *in vitro* assay was to evaluate the ability of NMP to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese Hamster Ovary (CHO) cells in the presence and absence of metabolic activation. NMP was soluble in F12 culture medium at 50 mg/mL. Preliminary range finding cytotoxicity testing found the test article to be non-toxic at all dose levels tested from 0.005 mg/mL to 5.0 mg/mL in the presence and absence of S9 metabolic activation test conditions. For each test condition, six dose levels that ranged from 0.5 mg/mL to 5.0 mg/mL were used in the mutation assays. The test article was not toxic at any dose level in the mutation assays. Mutant frequencies of all cultures treated with test material varied randomly with dose within the range of negative control mutant frequencies. In the S9 metabolic activation mutation assay, one of the six treatment conditions achieved statistical significance. The culture that achieved statistical significance had a mutant frequency within the acceptable range for background mutant frequencies. The statistical significance was apparently due to normal assay variation. Without S9 metabolic activation, none of the six dose levels had mutant frequencies that achieved statistical significance. Therefore, NMP was considered negative for inducing forward mutation at the HGPRT locus in CHO cells in both the presence and absence of the S9 metabolic activation conditions of the assay.

b. *In Vivo* Mechanistic Study

N-methylpyrrolidone (NMP): One-Week Oral (Gavage) Toxicity Study in Mice

Report Number: RPTSN05-351 (PR&D 0135001)
Report Date: December 15, 2005 -
Study Director: John Findlay -
Study Location: Experimur, Chicago, IL -

Experimental Design and Conclusion: NMP in demineralized water was administered by oral gavage in the drinking water for 7 consecutive days to 6 male and 6 female B6C3F1 mice at 0, 1000, or 3000 mg/kg bw/day

(analysis was performed on 5 animals per sex per group). Body weight, body weight gain, water consumption, and liver and brain weights were obtained. Livers were further examined by histopathology and biochemical analysis. Treatment with NMP resulted in no mortality and no clinical observations. No treatment-related effects by NMP on body weight, weight gain, or water consumption were observed. NMP did not affect peroxisomal β-oxidation activity or expression levels of biomarkers of PPAR activation (ACOX, bifunctional protein or thiolase). NMP caused a 10-fold induction of Cytochrome P450 (Cyp) 4a mRNA levels in male, but not female mice; however, this induction was approximately 300-fold less than positive controls. The induction of mRNA did not result in increases in protein levels of Cyp4a. NMP also resulted in modest increases in hepatic Cyp2b protein and mRNA levels, while no increases in Cyp1a1, Cyp1a2, or Cyp3a11 were observed. The high dose group of NMP revealed centrilobular hepatocyte cytoplasmic clumping by histology and a 12% increase in liver weight in only the male high dose group. The NOEL for this acute study was 1000 mg/kg bw/day based on proliferative effects at 3000 mg/kg bw/day.

c. Sub-Chronic Toxicity Studies

i. 90-Day Subchronic Toxicity Study in Rats and Mice Fed N-Methyl-2-Pyrrolidone (NMP) Including Neurotoxicity Evaluation in Rats. Malley, L.A., Kennedy, G.L., Elliott, G.S., Slone, T.W., Mellert, W., Deckardt, K., Gembardt, C., Hildebrand, B., Parod, R.J., McCarthy, T.J., and Griffiths, J.C. Drug and Chemical Toxicology, 22 (3), 455-480 (1999).

Report Number: 60C0225
Report Date: November 13, 1995
Study Director: W. Mellert
Study Location: BASF Aktiengesellschaft, Ludwigshafen, Germany

Experimental Design and Conclusion: NMP was administered by diet for 90 consecutive days to groups of 10 male and 10 female B6C3F1 mice at 0, 167, 417, or 1250 mg/kg bw/day equivalent (it was reported as 0, 320, 820, 2550 mg/kg bw/day, respectively, but lacking actual food consumption data, the concentration in ppm was divided by six to approximate the daily dose in mg drug/kg bw/day calculated by CVM). Body weight, body weight gain, ophthalmologic examination, clinical chemistry, hematology, organ weights, histopathological examination, and clinical observations were obtained. A satellite study with 10 male and 10 female mice was performed in a 28-day study, as well as, a 90-day study in Crl:CD®BR rats, but is not reported here because the data was not used to make CVM's NMP safety

evaluation. No compound related effects on mortality, hematologic parameters, body weight, or food consumption were reported. Changes in urine color, but not kidney function were observed at the doses of 417 and 1250 mg/kg bw/day. Changes in cholesterol, triglycerides, calcium, and alkaline phosphatase at 28 days, but not following 90 days of NMP treatment were reported. Liver weights were elevated in males fed 417 mg/kg bw/day and increased in both male and female mice at 1250 mg/kg bw/day for 90 days compared to controls. Hepatocellular hypertrophy was noted by histological examination for both male and female mice at the 417 and 1250 mg/kg bw/day doses. The NOEL for this study was 167 mg/kg bw/day based on the increased liver weight and increased incidences of centrilobular hepatocellular hypertrophy observed at 417 mg/kg bw/day.

ii. Evaluation of the Safety of N-methyl-2-pyrrolidone in Wistar-Derived Rats Following 90-Day Administration in the Diet

Report Number: 5026
Report Date: December 9, 1976
Study Location: Food and Drug Research Laboratories, Inc.
Waverly, NJ

Experimental Design and Conclusion: Two hundred (200) weanling FDRL-Wistar rats (25/sex/dose level) were administered with 0 (control), 800, 2000, and 5000 ppm of NMP in the diet for 90 consecutive days. The 800, 2000 and 5000 ppm dosages produced a significant decrease ($p<0.05$) on female body weights, an increase in male thyroid weight, and changes in the chemical properties of urine (pH in males and females, albumin, and specific gravity in females, and serum glutamic pyruvic transaminase in males at termination). It was concluded, however, that the 800 ppm dose level (which equated to 40 mg/kg bw/day test substance intake level) was the NOEL for the compound in this study.

iii. 90-Day Feeding Study in Beagle Dogs with N-methyl-2-pyrrolidone

Report Number: 6414
Report Date: September 3, 1980 -
Study Location: Food and Drug Research Laboratories, Inc.
Waverly, NJ

Experimental Design and Conclusion: This study was conducted to evaluate the potential systemic toxicity of dietary NMP when administered as a liquid to beagle dogs for 90 days at dose levels of 0, 25, 79, and 250 mg/kg bw/day. Forty-eight (24 male and 24 female) beagle dogs ranging from 5 to 6 months of age at the beginning of the study were included. Body weights and food consumption were measured weekly, animals were observed daily, and blood samples for hematology and biochemical determinations were collected and analyzed pretest, 60 days after initiation, and at termination. At the termination of the study (90 days), gross and microscopic examinations were performed.

No signs of toxicity or behavioral abnormalities were observed during the study. Intermittent significant variations in total cholesterol, serum albumin, and serum total protein were noted, but toxicological significance was not supported by any histopathological finding. No test article-related histopathological findings were noted in any organs and analysis of organ weight failed to reveal any significant treatment differences.

The only unusual finding was a very minimal growth rate of all dogs in the study. Although food consumption was similar among all groups, the high dose males gained almost no weight compared to the controls after the 90-day period. The NOEL for the study was 79 mg/kg bw/day based on the lack of body weight gain of the highest dose group.

d. Teratogenic, Developmental and Reproductive Studies

i. Teratologic Evaluation of N-methyl-2-pyrrolidone After Dermal Application in Sprague-Dawley Rats

Report Number: 6161
Report Date: November 18, 1979
Study Location: Food and Drug Research Laboratories, Inc.
Waverly, NJ

Experimental Design and Conclusion: This study was conducted to evaluate the teratogenic potential of NMP administered dermally to pregnant Sprague-Dawley rats at 0 (negative, positive, and aspirin control groups), 75, 237, or 750 mg/kg bw/day on days 6 through 15 of gestation. Six groups were utilized providing 25 female rats per group. On Day 20 of gestation, all animals were euthanized for uterine examination and fetal examination for skeletal and soft tissue abnormalities. The frequency and severity of dry skin and bright

yellow urine increased with dosage. Maternal toxicity was evidenced by significantly lower body weights on Days 15 and 20 of gestation, and reduced weight gains during gestation in the 750 mg/kg bw/day females. The high dose also resulted in fewer live fetuses per dam, an increase in the percentage of resorption sites (although the percentage of dams showing resorptions was unaffected), and a significant reduction in fetal weights. Fetal examination revealed a variety of skeletal variations at the 750 mg/kg bw/day dose which indicates possible retardation in fetal development and possibly a teratogenic potential of the compound at high doses. Treatment with 750 mg/kg bw/day of the compound could be considered maternally toxic since it resulted in observations similar to those of the positive controls administered hexafluoroacetone solution. No maternal or fetal effects were seen in the animals treated with 75 or 237 mg/kg bw/day of the compound. No teratogenic effects were seen at the dose levels tested. The NOEL for maternal and fetal toxicity was 237 mg/kg bw/day.

ii. Developmental Toxicity Study of N-methyl-2-pyrrolidone in New Zealand White Rabbits

Report Number: 637-002
Report Date: December 17, 1991
Study Location: International Research and Development Corporation
Mattawan, MI

Experimental Design and Conclusion: Inseminated New Zealand White SPF female rabbits were used to determine the developmental toxicity including the teratogenic potential of NMP. The rabbits were randomly assigned to one placebo control and three treatment groups (55, 175, and 540 mg/kg bw/day) consisting of twenty animals each. The treatments were administered orally by gavage as a single daily dose on Gestation Days 6 through 18 at a volume of 3.0 mL/kg. Cesarean section examinations were performed on all females on Gestation Day 29, followed by teratologic examination of the fetuses. Maternal toxicity was observed at 540 mg/kg bw/day. Statistically significant inhibition of maternal body weight gain and food consumption was observed at this level and an abortion at 540 mg/kg bw/day was confirmed. At 175 mg/kg bw/day, there appeared to be a dose-related trend toward inhibited body weight gain; however, this inhibition was only statistically significant during the first treatment subinterval (gestation Days 6-12). Additionally, reduced feed consumption was not statistically significant when compared to the control group. Developmental toxicity was observed at 540 mg/kg bw/day manifested by increased post-implantation loss, increased incidences of cardiovascular and skull malformations and

developmental variations. The NOEL based on this study was considered to be 55 mg/kg bw/day with respect to maternal toxicity, and 175 mg/kg bw/day with respect to developmental toxicity.

iii. Multigeneration Rat Reproduction Study with N-methyl-2-pyrrolidone

Report Number: 236535
Report Date: November 26, 1991
Study Location: Exxon Biomedical Sciences, Inc., East Millstone, NJ

Experimental Design and Conclusion: A two generation reproduction study with two litters per generation was conducted with NMP at dose levels of 50, 160, and 500 mg/kg bw/day in the diet. No reproductive effects were seen in the P1 generation. However, high dose F1b (P2) male mating indices and fertility indices were lower than controls for both litters. High dose female (P2) fertility and fecundity indices were lower than controls for both litters. Additionally, there were differences in survival indices and growth rate of all litters with 500 mg/kg bw/day of the compound. The NOEL for reproductive and developmental effects was established as 160 mg/kg bw/day of the compound. The 160 mg/kg bw/day dose was established as the parental, reproductive and developmental NOEL in this study.

e. Summary of the Studies to Assess the Carcinogenic Mode of Action

Pursuant to 21 CFR § 500.84(c)(2), FDA considers that "no residue" of a compound remains in edible tissues when the residue of carcinogenic concern in the total diet of people does not exceed the concentration of the test compound in the total diet of test animals that corresponds to a maximum lifetime risk of cancer in the test animals of 1 in 1 million. The sponsor has petitioned a waiver of requirements according to 21 CFR § 500.90 and has clearly explained the reasons why alternative procedures will provide the basis for concluding that approval of the compound satisfies the requirements of the anticancer provisions of the act. Based on the non-genotoxic mode of action summarized below, the alternative approach to the regulation for carcinogenic residues of NMP in the human diet is scientifically acceptable.

In mechanistic studies, NMP activated the hepatic Peroxisome Proliferator-Activated Receptor alpha (PPARα) *in vitro*. In *in vivo* studies of different durations for up to four weeks in duration, NMP exposure was associated with the induction of hepatic peroxisomal palmitoyl CoA oxidase and nonperoxisomal Cyp4a enzymes. NMP administration was also associated with increases in hepatic peroxisome number and volume,

indicators of cell replication, hepatocellular hypertrophy, and liver weight. These mechanistic data are consistent with key events for the non-genotoxic PPARα hepatocellular tumor promoting mode of action. This mode of action is characterized by key causal events specific to non-PPARα non-genotoxic hepatocellular tumor promoters, such as hepatic mixed function oxidase inducers. This mode of action is supported by NMP-induced non-peroxisomal hepatic enzyme Cyp2b. The progression of dose- and exposure-dependent observations and lesions typology reported in repeated dose safety through cancer studies provide additional data that are highly correlated with the mechanistic data, enhancing the weight-of-evidence analysis for the proposed mode of action.

The pleiotropic effects of increased liver weight and hepatocellular hypertrophy are known hallmarks of PPARα and Constitutive Androstane Receptor activation. PPARα activation is known to induce key causal events of enhanced cell proliferation and/or inhibited apoptosis in background level DNA-damaged hepatocytes, leading to preneoplastic foci of basophilic, eosinophilic, and clear cell lesions. In repeated dose studies, NMP-induced dose- and exposure-dependent increases in liver mass (weight and hepatocellular hypertrophy) in mice, but not rats. Upon lifetime administration in the mouse bioassay, dose-related hepatocellular hypertrophy and altered hepatic foci (preneoplastic foci) were associated with hepatocellular adenomas and carcinomas at the same dose in mice. The observation of basophilic, eosinophilic, and clear cell preneoplastic foci in the mouse bioassay is consistent with increased hepatocellular proliferation observed in mechanistic studies. The concomitant association of these foci with adenomas and carcinomas supports clonal expansions of these foci as a key causal event in liver tumors in NMP-treated mice. The progression of lesion type and associations across studies and time is consistent with that published for PPARα and mixed function oxidase-inducers.

2. Determination of No Observed Effect Level (NOEL)

The most appropriate toxicity study for determining the Human Health Protective Value of NMP residues in edible tissues is the 90-day oral toxicity study in mice. The no-observed-effect level (NOEL) of NMP for this study is 167 mg/kg bw/day.

3. Human Health Protective Value for NMP

The Human Health Protective Value is defined as the concentration of total residue of carcinogenic concern of the test compound in the total human diet that represents no significant increase in risk of cancer to the human

consumer. The Human Health Protective Value for NMP is 66.8 parts per million (ppm), and was calculated as follows:

Human Health Protective Value

$$= \frac{\left(\text{Point of Departure}/\text{Safety Factor}\right) \text{ x } \text{Average Human Body Weight}}{\text{Total Human Diet}}$$

$$= \frac{\left(167 mg/kg\ bw/day / 100\right) \text{ x } 60 kg\ bw}{1.5 kg/day}$$

= 66.8 mg/kg food in total diet (66.8 ppm)

4. S_m (Concentrations for Total Residues of NMP in Edible Tissues)

The S_m is defined in 21 CFR § 500.82(b) and § 500.84 (c)(2) as the permitted concentration of residues of carcinogen concern in a specific edible product. Because the total human diet (1500 g per day) is not derived from food producing animals, a correction for food intake is made in determining the S_m. The S_m is calculated based on the Human Health Protective Value, assuming that up to 500 g of the total diet is due to the consumption of meat. Of this 500 g total daily meat consumption, 300 g is assumed to be comprised of muscle, 100 g comprised of liver, and 50 g comprised of kidney and 50 g comprised of fat. Thus, one fifth of the total diet, or $\left(\frac{300 g\ muscle}{1500 g\ total\ diet}\right)$, would be comprised of muscle. For a Human Health Protective Value of 66.8 parts per million (ppm), the concentration of total residues of carcinogenic concern, S_m muscle, would then be calculated as 66.8 ppm ÷ 1/5 or 334 ppm. The S_m for each edible tissue is calculated as follows:

Consumption values, Fraction of Total Diet, and calculated S_m values for NMP (excipient) residues of carcinogenic concern in edible cattle tissues.			
Tissue	**Consumption Factor (g)**	**Fraction of Total Diet**	**S_m (mg/kg or ppm)**
Muscle	300	1/5	334
Liver	100	1/15	1002
Kidney	50	1/30	2004
Fat	50	1/30	2004

B. Residue Chemistry:

1. Summary of Residue Chemistry Studies

Eprinomectin

a. Total Residue and Metabolism Study

Total residue and metabolism data for eprinomectin in cattle were described in the FOI Summary for the NADA 141-079 approval (IVOMEC EPRINEX Pour-On for beef and dairy cattle, FOI Summary dated April 16, 1997). Additional data generated on the extended-release injectable formulation are described below.

Study PR&D 0049701: "A Study to Evaluate the Depletion of Radio-Residues in Cattle Treated with ^{3}H Eprinomectin at 1 mg/kg Body Weight in a Long-Acting Injectable Formulation Containing ^{14}C Labeled N-Methyl Pyrrolidone"

1) Purpose: This pivotal residue study was conducted to determine total radioactive residues of ^{3}H Eprinomectin and ^{14}C N-Methyl Pyrrolidone (NMP) in the edible tissues, plasma, and excreta following subcutaneous administration of eprinomectin extended-release injectable (ERI) to cattle, and to confirm liver as the target tissue, eprinomectin B_{1a} as the marker residue and the ratio of marker residue to total residues.

2) Study Director: L. Wilkes
Analytical Development Corporation
Colorado Springs, CO

In-Life Phase Principal Investigator: C. Heird
Southwest Bio-labs, Inc.
Las Cruces, NM

3) General Design of the Study

a. - Test Substance: A solution containing ^{3}H-labeled eprinomectin and ^{14}C-labeled NMP. The radiochemical purities of these two compounds in the dosing solution were 97.8% and 99.8%, respectively, as determined by radio-high performance liquid chromatography (HPLC) analyses.

b. Test Animals: Mixed-breed beef cattle approximately eight months of age and weighing between 214 and 264 kg.

c. Number of Animals: Sixteen (eight males and eight females)

d. Treatment Groups: One heifer and one steer were randomly assigned to each of six treatment groups, one untreated control group, and a seventh treated group to be used as replacement cattle.

e. Dose: Animals in treatment groups received a single subcutaneous injection in the neck region with the ERI formulation at the intended rate of 1 mg eprinomectin/kg body weight.

f. Sample Collection: Animals comprising a treatment group were sacrificed at either 5, 10, 50, 120, 150, or 180 days post-treatment. The two control animals were sacrificed 140 days post-treatment. Plasma and excreta samples were collected throughout the study. Tissues were collected after euthanasia.

g. Assay: Liquid scintillation counting, radio-HPLC and HPLC with fluorescence detection.

4) Results -- Total Residue (based on total radioactivity):

Table IVB.1: Concentration of ^{3}H-labeled Total Radioactive Residues in Tissues of Cattle Following Injection of ^{3}H-Eprinomectin as the Eprinomectin ERI at 1 mg/kg Body Weight

Day Post-Dose	Sex	^{3}H-Eprinomectin Total Radioactive Residue (ppm)					
		Liver	Kidney	Hindquarter Muscle	Diaphragm Muscle	Fat	Injection Site
5	M	1.11	0.178	NQ	0.014	0.036	85.0
	F	0.730	0.122	NQ	0.007	0.019	46.0
10	M	0.690	0.192	NQ	0.011	0.033	213
	F	1.15	0.126	NQ	NQ	0.017	53.6
50	M	0.238	0.033	NQ	NQ	NQ	102
	F	0.382	0.042	NQ	NQ	NQ	0.681
120	M	0.866	0.136	NQ	0.007	0.019	47.7
	F	0.613	0.089	NQ	NQ	NQ	5.15
150	M	0.376	0.054	NQ	NQ	NQ	0.019
	F	0.269	0.038	NQ	NQ	NQ	0.387
180	M	0.264	0.038	NQ	NQ	NQ	10.6
	F	0.191	0.023	NQ	NQ	NQ	0.023
	M	0.042	NQ	NQ	NQ	NQ	9.12

NQ = Less than 2x background (liver, kidney, H. muscle, D. muscle NQ < 0.007 ppm; fat NQ < 0.014 ppm, injection site NQ < 0.013 ppm)

5) Conclusions for Eprinomectin Total Residues in Edible Tissue

a. The injection site total residues depleted slowest to below the injection site safe concentration of 160 ppm.

b. Except for the injection site, liver had the highest total radioactive residue (TRR) levels for ^{3}H- Eprinomectin, and kidney had the second highest TRR. Highest concentrations in liver (~1 ppm) and kidney (0.1-0.2 ppm) were at 5 and 10 days post dose. For liver and kidney, residue concentrations increased at 120 days post dose (to ~0.7 ppm for liver and ~0.1 ppm for kidney), followed by further decline out to 180 days. All liver samples contained total residues that were below the safe concentration of 5.8 ppm for eprinomectin residues in liver.

c. The radiolabeled study confirmed the target tissue and marker residue assignments previously determined based on the data generated on the pour-on product [FOI Summary for NADA 141-079, dated April 16, 1997, 21 CFR 556.227]. The target tissue is liver and the marker residue is eprinomectin B_{1a}.

6) Results -- Metabolism in Cattle:

Eprinomectin underwent very little metabolism; Eprinomectin B_{1a} was the only major ^{3}H-labeled residues. The finding was consistent with the metabolism profiles obtained in cattle treated with Ivomec Eprinex Pour-On (NADA 141-079, FOI dated 16 April 1997).

b. Comparative Metabolism

Comparative metabolism for eprinomectin has been described in the FOI Summary for the approval of the pour-on product (NADA 141-079, FOI Summary dated April 16, 1997).

c. Eprinomectin Tissue Residue Depletion Study

Study PR&D 0050001: "A Study to Evaluate the Depletion of Residues in Cattle Treated with Eprinomectin at 1 mg/kg Body Weight in a Long-Acting Injectable Formulation"

1) Purpose: This study was designed to measure concentrations of eprinomectin B_{1a} in edible tissues of cattle after subcutaneous injection of Eprinomectin ERI to cattle.

2) Study Director: T. Wehner
Merial Limited
North Brunswick, NJ.

In-life Phase Principal Investigators: D. Wallace and B. Kunkle
Merial Limited
Missouri Research Center
Fulton, MO

Analytical Phase Principal Chemist: J. Kruplak
Analytical Development Corp.
Colorado Springs, CO

3) General Design:

a. Test Substance: Eprinomectin extended-release injectable - formulation containing NMP.

b. Test Animals: Ruminating Angus or Angus cross cattle approximately eight to nine months of age and weighing between 147 and 272 kg.

c. Number of Animals: Eighty-two (41 male castrates and 41 females)

d. Treatment Groups: Thirteen treatment groups of three males and three females in each group; two untreated control groups with one male and one female in each group.

e. Dose: One dose with final formulation *via* a single subcutaneous injection in front of the shoulder at 1 mg eprinomectin/kg body weight

f. - Sample Collection: All cattle comprising a treated group were sacrificed at either 21, 42, 63, 84, 105, 126, 147, 168, 189, 210, 231, 252, or 273 days post-treatment. Liver, kidney, perirenal fat, hindquarter muscle, diaphragm muscle, and injection site muscle were collected. The animals in one control group were sacrificed 20 days post-treatment, and the animals in another control group were sacrificed 266 days post-treatment.

g. Assay: Eprinomectin marker residue, eprinomectin B_{1a}, was quantitated using a validated HPLC fluorescence method. The method has a validated limit of quantification of 10 ppb for liver and muscle and 2 ppb for fat and kidney.

4) Results: Total Residues

Table IV.B.2: Concentration Range of Eprinomectin B_{1a} in Liver and Injection Site Tissues of Treated Animals		
Withdrawal Period (Days)	**Liver (ppm)**	**Injection Site (ppm)**
21	0.075 – 0.243	1.63 – 321
42	0.077 – 0.140	0.199 – 140
63	0.040 – 0.238	6.41 – 75.0
84	0.160 – 0.460	NQ[a] – 64.8
105	0.297 – 0.478	0.026 – 19.4
126	0.066 – 0.280	ND[b] – 25.2
147	0.052 – 0.119	ND[b] – 19.3
168	0.016 – 0.114	NQ[a] – 25.0
189	0.013 – 0.087	ND[b] – 9.46
210	NQ[a] – 0.028	ND[b] – 6.66
231	0.012 – 0.024	ND[b] – 1.38
252	ND[b] – 0.018	ND[b] – 1.22

[a] NQ is defined as ≥0.002 ppm but <0.010 ppm. -
[b] ND is defined as <0.002 ppm. -

5) Conclusions: The injection site residue is rate limiting for withdrawal period determination. At 48-day withdrawal and after, all edible tissues derived from the Eprinomectin ERI treated cattle are safe for human consumption.

N-Methyl-2-Pyrrolidone (Excipient)

a. Total Residue and Metabolism Study

Study PR&D 0049701: "A Study to Evaluate the Depletion of Radio-Residues in Cattle Treated with ^{3}H Eprinomectin at 1 mg/kg Body Weight in a Long-Acting Injectable Formulation Containing ^{14}C Labeled N-Methyl Pyrrolidone"

Study PR&D 0049701, described in an earlier section of this FOI Summary, provides sufficient data on the depletion of NMP total residues following treatment of cattle with the product formulation containing ^{14}C-labeled NMP. The reader is referred to that section for complete information regarding the study design. The NMP-related study results are described hereunder.

1) Results -- Total Residues (based on total radioactivity):

Table IV.B.3. Total Radioactive Residue of ^{14}C-NMP in Edible Tissues of Cattle Treated With 1 mg/kg BW Eprinomectin ERI Containing ^{14}C Labeled NMP							
Day Post-Dose	**Sex**	**^{14}C-NMP Total Radioactive Residue (ppm)**					
		Liver	**Kidney**	**HQ[a] Muscle**	**Diaphragm Muscle**	**Fat**	**IS[b]**
5	M	2.39	1.11	0.607	0.468	NQ	0.435
	F	1.69	0.929	0.312	0.390	NQ	NQ
10	M	1.29	0.516	0.392	0.415	1.79	NQ
	F	1.03	0.626	0.331	0.401	1.14	NQ
50	M	NQ	NQ	NQ	NQ	NQ	NQ
	F	NQ	NQ	NQ	NQ	0.718	NQ
120	M	NQ	NQ	NQ	NQ	NQ	NQ
	F	NQ	NQ	NQ	NQ	NQ	NQ
150	M	NQ	NQ	NQ	NQ	NQ	NQ
	F	NQ	NQ	NQ	NQ	NQ	NQ
180	M	NQ	NQ	NQ	NQ	NQ	NQ
	F	NQ	NQ	NQ	NQ	NQ	NQ
	M	NQ	NQ	NQ	NQ	NQ	NQ

[a] Hindquarter
[b] Injection site
NQ = less than 2 x background LOQ (liver, H. muscle, NQ < 0.30 ppm; kidney, D. muscle NQ < 0.29 ppm; fat NQ <0.59 ppm; injection site NQ < 0.31 ppm).

2) Conclusions for Total Residue in Edible Tissues:

a. The NMP total radioactive residue concentrations in all edible tissues were at least hundreds fold below their respective S_m at Day 5 post-treatment and thereafter.

b. The data support assigning liver as the target tissue.

3) Results -- Metabolism Profiles in Cattle

Table IV.B.4: Metabolic Profile of ^{14}C-NMP in Plasma, Urine and Feces at 0.5 to 1 Days Post Treatment							
Matrix	Animal	**%Total Radioactive Residue**					
		Succinimide	**2HNMS**	**5HNMP**	**2-Pyr**	**NMS**	**^{14}C-NMP**
Plasma	Male	1.7	1.1	16.0	-	39.6	-
	Female	1.8	1.0	14.0	-	33.0	-
Urine	Male	10.9	1.5	33.1	1.2	22.6	0.7
.	Female	5.5	1.3	41.2	1.7	26.7	1.5
Feces	Male	8.0	2.1	14.8		23.3	1.8
	Female	10.1	1.8	19.6	-	20.1	1.7

4) Conclusions for NMP Metabolism in Cattle

a. In plasma, urine, and feces obtained 0.5 to 1 days post treatment, the major metabolites of ^{14}C-NMP in cattle dosed subcutaneously with Eprinomectin ERI were N-methylsuccinimide (NMS) and 5-hydroxy-NMP (5HNMP). Succinimide, 2-hydroxy-N-methylsuccinimide (2HNMS), and 2-pyrrolidone (2-Pyr) were present as minor metabolites. NMP parent was below the quantification limit in plasma and was present at less than 2% of the total residues in the excreta.

b. A conservative R_m of 8 ppm for parent NMP in liver, the target tissue, is assigned. R_m is defined in 21 CFR § 500.82(b) as the concentration of the marker residue in the target tissue when the total residue of carcinogenic concern is equal to S_m (see the toxicology section of this FOI Summary for the calculation of the S_m values).

b. Comparative Metabolism

Metabolite profile in the rat treated with NMP was provided based on the literature information[1,2,3].

1) Results: The following table summarizes the metabolic profiles of NMP in rats and cattle as reported in the literature.

Table IV.B.5: Comparison of NMP Metabolic Profiles in Rats and Cattle

Metabolite	Rats	Cattle
5HNMP	+	+
NMS	+	+
2-HNMS	+	+
2-Pyr	+	+
Succinimide	-	+

[1]Wells DA, AA Hawi, and GA Digenis. 1992. Isolation and identification of the major urinary metabolite of N-methylpyrrolidinone in the rat. Short Communication. Drug Metabolism and Disposition 20(1):124-126.

[2] Payan J-P, D Beydon, J-P Fabry, I Boudry, B Cossec, and E Ferrari. 2002. Toxicokinetics and metabolism of N-[^{14}C]methylpyrrolidinone in male Sprague-Dawley rats. A saturable NMP elimination process. Drug Metabolism and Disposition 30(12):1418-1424.

[3]Carnerup MA, AM Saillenfait, and BAG Jönsson. 2005. Concentrations of N-methyl-2pyrrolidone (NMP) and its metabolites in plasma and urine following oral administration of NMP to rats. Food and Chemical Toxicology 43:1441-1447.

2) Conclusions:

Toxicological studies for assessing toxicity of NMP were conducted in toxicological model species, including the rat. The literature information supports the notion that rats have been autoexposed to the same metabolites found in cattle. The NMP residues in all edible tissues were at least hundreds fold below the respective S_m values at Day 5 post treatment and thereafter. At the withdrawal period of 48 days assigned for the product, the amount of NMP metabolites in the edible tissues would not cause human food safety concerns.

c. NMP Residue Depletion Study

The eprinomectin residue withdrawal drives the withdrawal period determination for Eprinomectin ERI. A residue depletion study to determine withdrawal period for NMP residues is not needed.

2. Target Tissue and Market Residue Assignments

Eprinomectin -- The target tissue for residue monitoring is liver and the marker residue is eprinomectin B_{1a}.

N-methyl pyrrolidone (NMP) -- The target tissue for residue monitoring is liver and the marker residue is parent NMP.

3. Tolerance and R_m Assignments

The tolerance for eprinomectin B_{1a} in liver is 1.5 ppm. This tolerance, a revision of the previously assigned tolerance (4.8 ppm, NADA 141-079, FOI Summary dated April 16, 1997), is needed to ensure that the target tissue (liver) and the marker residue (eprinomectin B_{1a}) continue to monitor the safety of all the edible tissues, including the depletion of eprinomectin residues at the injection site.

The R_m for parent NMP in liver is 180 ppm.

4. Withdrawal Period

The withdrawal period for Eprinomectin ERI is 48 days. The withdrawal period for the product was determined based on the eprinomectin residue data at the injection site.

A withdrawal period of 48 days is consistent with the depletion of eprinomectin and NMP residues in all edible tissues following treatment with Eprinomectin ERI.

C. Microbial Food Safety:

Information or data on microbial food safety is not necessary at this time because eprinomectin is a parasiticide rather than a traditional antimicrobial drug. Eprinomectin is not normally known to, nor has it been reported to, affect antimicrobial resistance among bacteria of public health importance. Therefore, a review of the microbial food safety of eprinomectin was not conducted by the Agency.

D. Analytical Methods for Residues:

1. Eprinomectin Determinative Procedure

The determinative assay procedure, which is based on the US Department of Agriculture Food Safety and Inspection Service (USDA/FSIS) method IVR/DOR1, dated October 1998, is a reversed-phase HPLC procedure with fluorescence detection that is capable of quantitating eprinomectin in cattle liver and muscle to concentrations as low as 10 ppb. The method involves vortex extraction of the sample using acetonitrile, extract cleanup on solid phase extraction columns, reconstitution in acetonitrile, and precolumn derivatization before quantitation by HPLC with fluorescence detection using an external standard curve.

2. Eprinomectin Confirmatory Procedure

The eprinomectin confirmatory assay utilizes the same extraction and purification steps as the determinative procedure. The structural confirmation of eprinomectin is based on high pressure liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis monitoring five precursor/product ion transitions in Multiple Reaction Monitoring (MRM) mode and comparing the results to those obtained with standards.

3. NMP Regulatory Method

NMP is extracted with methanol and acetonitrile and injected onto a hydrophilic interaction HPLC analytical column. The NMP is eluted from the column and detected with tandem mass spectrometry (LC-MS/MS). NMP is determined by response ratio to deuterated NMP, as the internal standard, from an external standard calibration curve. The quantitatively determined peak is confirmed by monitoring four precursor/product ion transitions in Multiple Reaction Monitoring (MRM) mode and comparing the results to those obtained with standards.

4. Display of the Methods

The methods for eprinomectin B_{1a} and NMP are on file with the Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to LONGRANGE:

User Safety Warnings
Not for use in Humans. Keep this and all drugs out of the reach of children.
The material safety data sheet (MSDS) contains more detailed occupational safety information. To report adverse effects, to obtain an MSDS, or for assistance, contact Merial at 1-888-637-4251.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that LONGRANGE Extended-Release Injectable Parasiticide, when used according to the label, is safe and effective for treatment and control of internal and external parasites of cattle on pasture with persistent effectiveness. Additionally, data demonstrate that residues in food products derived from cattle treated with LONGRANGE Extended-Release Injectable Parasiticide will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because a proper diagnosis of the parasites present in a herd of animals and the follow-up required to ensure the drug maintains effectiveness is important for the safe and effective use of this product. A veterinarian is trained in the parasitological procedures necessary for safe and effective use of this product.

B. Exclusivity:

Under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the *date of the approval*.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.

VII. APPENDIX:

Original text:

The R_m for parent NMP in liver is 8 ppm.

Revised text:

The R_m for parent NMP in liver is 180 ppm.