FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION:

A. New Animal Drug Application No.: 141-061

B. Sponsor: Pfizer Inc 235 East 42nd Street, New York, N.Y. 10017

C. Established Name: Doramectin 1% injectable solution

D. Trade Name: DECTOMAX® Injectable Solution

E. Marketing status: Over-the-counter

II. INDICATIONS FOR USE:

For the treatment and control of the following nematode and arthropod parasites in cattle.

Gastrointestinal roundworms
- *Ostertagia ostertagi (*Adults and fourth-stage larvae[11])
- *Ostertagia ostertagi (*Inhibited fourth-stage larvae)
- *Ostertagia lyrata (*Adults and fourth-stage larvae)
- *Haemonchus placei (*Adults and fourth-stage larvae)
- *Trichostrongylus axei (*Adults and fourth-stage larvae)
- *Trichostrongylus colubriformis (*Adults and fourth-stage larvae)
- *Trichostrongylus longispicularis (*Adults)
- *Cooperia oncophora (*Adults and fourth-stage larvae)
- *Cooperia pectinate (*Adults)
- *Cooperia zurnabada (syn.mcmasteri) (*Adults)
- *Bunostomum phlebotomum (*Adults)
- *Strongyloides papillosus (*Adults)
- *Oesophagostomum radiatum (*Adults and fourth-stage larvae)
- *Trichuris spp. (*Adults)

Lungworms
- *Dictyocaulus viviparous (*Adults and fourth-stage larvae)

Eyeworms
- *Thelazia spp. (*Adults)

Grubs
- *Hypoderma bovis*
- *Hypoderma lineatum*

Lice
- *Haematopinus eurysternus*
- *Linognathus vituli*
- *Solenopotes capillatus*

Mange mites
- *Psorpotes bovis*
- *Sarcoptes scabiei*

[1] Doramectin injectable solution protects cattle against infestation or reinfestation with *Ostertagia ostertagi* for up to 21 days.

III. DOSAGE FORM:

DECTOMAX® Injectable Solution contains 10 mg doramectin/mL.

IV. ROUTE OF ADMINISTRATION AND RECOMMENDED DOSAGE:

A single subcutaneous or intramuscular dose of 1 mL per 110 lb body weight (200 mcg/kg).

V. EFFECTIVENESS:

A. Preclinical Investigation:

The *in vivo* activity of doramectin against nematodes and arthropods was initially established in laboratory animals and further investigated using a ruminant induced infection model. The results confirmed the doramectin activity previously observed in the primary screening models.

Based on these early findings a preliminary dose-response study was conducted in cattle to investigate the efficacy of various doses of doramectin given by subcutaneous (SC) injection against key species of gastrointestinal (GI) and pulmonary nematodes in the target species. Results indicated that *Cooperia oncophora* was the least sensitive nematode species and a dosage of 200 mcg/kg body weight (BW) was required to provide for effective control. Based on these findings, this species was targeted for definitive dose determination studies which also included representative arthropod parasites in order to confirm that 200 mcg/kg BW was the appropriate doramectin dose for effective broad spectrum activity.

B. Dose determination:

Four dose titration studies were conducted. In one, six dose levels of doramectin (50, 100, 150, 200, 300 or 400 mcg/kg BW) were evaluated against nematode species *Cooperia oncophora* and *Ostertagia ostertagi* and in each of the others, three dose levels (100, 200 or 300 mcg/kg BW) were evaluated against *C. oncophora,* a mite species, *Psoroptes bovis* and a louse species, *Linognathus vituli*, respectively. Doramectin was administered by SC injection.

Nematode percentage efficacies were calculated at each dose level using the following formula:

$$\text{Percentage Efficacy} = \frac{\text{Arithmetic Mean number of nematodes in non medicated cattle} - \text{Arithmetic mean number of nematodes in doramectin treated cattle}}{\text{Arithmetic mean number of nematodes in non medicated cattle}} \times 100$$

Table 1: Summary of Pivotal Dose Determination Studies

Study No.	Investigator	Parasite species	Optimal Dose
5232E-03-90-041	Coop	*Cooperia oncophora*	200 mcg/kg
5232E-03-90-041	Coop	*Ostertagia ostertagi*	150 mcg/kg
5232E-03-91-067	Coop	*Cooperia oncophora*	300 mcg/kg
1031C-60-91-005	Clymer	*Psoroptes bovis*	100 mcg/kg
5232E-03-90-042	Titchener	*Linognathus vituli*	100 mcg/kg

Overall Conclusion: Administered by SC injection, a doramectin dosage of 200 mcg/kg BW or greater was required for effective treatment of *C. oncophora*, the presumptive dose limiting nematode species. At this dosage (200 mcg/kg BW) doramectin was effective against the other nematode and arthropod species tested. It was concluded, therefore, from this series of studies that 200 mcg/kg BW was the most appropriate dose of doramectin for achieving efficacy against the nematode and arthropod parasite species within its projected spectrum of activity.

1. Individual Dose Determination Studies

    a. Dose Determination Study, #5232E-03-90-041, R.L. Coop, Moredun Animal Health Ltd., Edinburgh, Scotland

    Thirty-eight (38) calves confirmed free of nematode infection were allocated to either a negative control group (8 calves) or one of six doramectin groups (5 calves per group): 50, 100, 150, 200, 300 or 400 mcg/kg BW. On Day 0 calves were artificially infected with *O. ostertagi* and *C. oncophora.* Treatment with doramectin or saline was administered on Day 26, when all species were in the adult stage. Calves were slaughtered 14 or 15 days later for worm burden determinations.

    A summary of the results is presented in Table 2. *O. ostertagi* was controlled at all doramectin doses, but a dose of 200 mcg/kg BW or greater was required to adequately control *C. oncophora.*

Table 2: Therapeutic Efficacy of Doramectin Against Induced Infections of Cattle Nematodes

Species	Treatment/Dosage (mcg/kg)	Animals/Group	% Efficacy
C. oncophora	Non-medicated	8	-
C. oncophora	Doramectin (50)	5	22
C. oncophora	Doramectin (100)	5	49
C. oncophora	Doramectin (150)	5	80
C. oncophora	Doramectin (200)	5	93
C. oncophora	Doramectin (300)	5	95
C. oncophora	Doramectin (400)	5	97
O. ostertagi	Non-medicated	8	-
O. ostertagi	Doramectin (50)	5	96
O. ostertagi	Doramectin (100)	5	95
O. ostertagi	Doramectin (150)	5	100
O. ostertagi	Doramectin (200)	5	100
O. ostertagi	Doramectin (300)	5	100
O. ostertagi	Doramectin (400)	5	100

2. Dose Determination Study, #5232E-03-91-067, R.L. Coop, Moredun Animal Health Ltd., Edinburgh, Scotland

   Forty (40) calves were randomly allocated to one of four treatment groups (a negative control and three doramectin groups) and artificially infected with L3 larvae of *C. oncophora*. Following a period of time sufficient to allow for parasite development to the adult stage, animals were treated SC with either doramectin or saline and slaughtered 14 or 15 days later for determination of worm burdens.

   A summary of the results is presented in Table 3. A doramectin dosage of 200 mcg/kg BW or greater was required for effective control of *C. oncophora.*

   Table 3: Therapeutic Efficacy of Doramectin Against Induced Infections of *C. oncophora*

Treatment/Dosage (mcg/kg)	Animals/Group	% Efficacy
Non-medicated	10	---
Doramectin (100)	10	77
Doramectin (200)	10	88
Doramectin (300)	10	95

3. Dose Determination Study, #1031C-60-91-005, Dr. B.C. Clymer, Central Arizona Veterinary Laboratory, Amarillo, Texas

   Thirty-six (36) *P. bovis*-infected calves were randomly allocated to one of four treatment groups (negative control and three doramectin groups). Test animals were treated with either doramectin or saline, and mite counts conducted on skin scrapings taken at 0, 7, 14, 21 and 28 days post-treatment.

The percentage efficacy was calculated at each dose level by determining the percentage of animals in each treatment group with no mites detected on Day 28.

A summary of the results is presented in Table 4. Doramectin was 100% effective in eliminating artificially induced infestations of *P. bovis* at dosage levels of 100 mcg/kg or greater.

Table 4: Therapeutic Efficacy of Doramectin Against Induced Infestations of *P. bovis.*

Treatment/Dosage (mcg/kg)	Animals/Group	% Calves with No Mites Detected on Day 28
Non-medicated	6	0
Doramectin (100)	10	100
Doramectin (200)	10	100
Doramectin (300)	10	100

4. Dose Determination Study, #5232E-03-90-042, Dr. R.N. Titchener, West of Scotland School of Agriculture, Auchincruive, Scotland

Twenty-four (24) calves with confirmed *L. vituli* infestations were randomly allocated to a negative control and three doramectin-treated groups. On Day 0 test calves were treated SC, based on group assignment, with either saline or one of the three doramectin doses. Lice counts were subsequently conducted at weekly intervals for 28 days.

Efficacy was calculated as the percentage of calves in each group with no lice detected on Day 28.

A summary of the results is presented in Table 5. A doramectin dose of 100 mcg/kg BW or greater was 100% effective against *L. vituli* infestations in cattle.

Table 5: Therapeutic Efficacy of Doramectin Against Naturally Acquired Infestations of *L. vituli.*

Treatment/Dosage (mcg/kg)	**Animals/Group**	**% Calves with No Lice on Day 28**
Non-medicated	6	0
Doramectin (100)	6	100
Doramectin (200)	6	100
Doramectin (300)	6	100

C. Relative Bioavailability:

A relative bioavailability study was conducted to compare plasma kinetics of doramectin following intramuscular (IM) and subcutaneous (SC) administration to growing beef cattle. A clinical endpoint study was also conducted to compare efficacy of doramectin by the IM and SC routes.

The two studies demonstrated that doramectin efficacy was equivalent when administered by the SC or IM routes of administration. Therefore, the entire clinical efficacy database developed for doramectin using the SC route was used to establish claims for both the IM and SC routes of administration.

1. Study #1532N-60-91-007, Mr. D. E. Mouzin and Mr. M. J. Lynch, Animal Health Product Development Dept., Central Research Division, Pfizer Inc, Terre Haute, Indiana and Drug Metabolism Department, Central Research Division, Pfizer Inc, Groton, Connecticut. (* refers to confidence limits)

Variable	IM Route Mean	SC Route Mean	Lower*	Upper*
Area Under Curve	444.0938	412.716	-16.9119%	1.706555%
Maximum Concentration (ng/mL)	33.125	27.795	-35.7799%	-2.5723%
Time to Max. Concentration (days)	4.7	5.9	-9.34528%	50.0232%

Forty (40) calves were randomly allotted on the basis of sex and weight to two groups of 20 animals each and treated with doramectin at a dose of 200 mcg/kg BW either SC or IM. Blood samples were collected at regular intervals until 30 days post-dose, and analyzed for doramectin levels using a validated HPLC method.

The 90% confidence limits for the difference between mean $AUC_{0 \text{ day-30 days}}$ values for the SC and IM groups fell within +/-20% of the mean value for the SC group. Therefore, the extent of absorption of doramectin injectable solution was equivalent by the IM and SC routes of administration in cattle. The differences in the SC and IM routes with respect to C_{max} and T_{max} were judged to be of no clinical significance.

Based on the results of this study, doramectin provides comparable bioavailability when administered by the IM or SQ routes.

2. Study #5232E-03-89-025, Dr. C. Hong, M.A.F.F. Central Veterinary Laboratory, Weybridge, Surrey, Kent-UK

Thirty-two calves were randomly assigned to three treatment groups: non-medicated, 200 mcg/kg BW doramectin by SC injection or 200 mcg/kg BW doramectin by IM injection. On Day 0 calves were experimentally infected with *C. oncophora* and were subsequently treated on Day 26 according to group assignment. Fourteen or 15 days following treatment (Days 40 or 41), animals were sacrificed for determination of worm burdens.

Results are summarized in Table 6. A single injection of doramectin at a dosage of 200 mcg/kg by either the SC or IM route provided comparable efficacy in the therapy against an induced *C. oncophora* infection.

Table 6: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against an Induced Infection of *C. oncophora* in cattle.

Treatment/Route	Animals/Group	Percentage Efficacy
Non-medicated	10	--
Doramectin SC	11	87
Doramectin IM	11	90

D. Efficacy Confirmation - Gastrointestinal (GI) and lung nematodes:

Summary

A series of 17 efficacy studies, involving cattle with either naturally or artificially acquired infections were conducted in a wide range of geographical settings. These studies evaluated the efficacy of doramectin injectable, administered SC at a dose of 200 mcg/kg BW, against GI roundworms and lungworms. Studies were designed to evaluate doramectin efficacy against both adult stage and against normally developing and inhibited L4 larvae.

Efficacy evaluations against adult stage parasites were assessed in studies using either natural or artificially acquired infections, whereas efficacy against L4 larvae was assessed in studies using artificially acquired infections in which the development age of nematode species being evaluated was known at the time of treatment. Efficacy against inhibited larvae was assessed in naturally infected cattle.

Conclusion: A single SC injection of doramectin, administered to cattle at a dose of 200 mcg/kg BW was efficacious against adult stages of *Ostertagia ostertagi, O. lyrata*, *Haemonchus placei*, *Trichostrongylus axei*, *T. colubriformis*, *T. longispicularis*, *Cooperia oncophora*, *C. pectinata*, *C. punctata*, *C. surnabada*, *Bunostomum phlebotomum, Strongyloides, papillosus*, *Oesophagostomum radiatum*, *Trichuris ovis* and *Dictyocaulus viviparus*.

A single SC injection of doramectin at 200 mcg/kg BW was also efficacious against L4 larvae of *O. ostertagi*, *H. placei*, *T. axei*, *T. colubriformis*, *C. oncophora*, *C. punctata*, *C. surnabada*, *O. radiatum*, *D. viviparus* and inhibited L4 stages of *O. ostertagi*.

INDIVIDUAL DOSE CONFIRMATION STUDIES

1. Dose Confirmation Study #1231C-60-89-001, Dr. C.E. Couvillion, College of Veterinary Medicine, Mississippi State University

   Twenty-two calves were artificially infected with *Dictyocaulus viviparus* larvae and divided into two groups. One group of 11 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 7.

Table 7: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult Stage *D. viviparus* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Dictyocaulus viviparus (Adult)	99

2. Dose Confirmation Study #1231C-60-90-003, Dr. C.E. Couvillion, College of Veterinary Medicine, Mississippi State University

   Twenty calves were artificially infected with mixed doses of nematode parasites. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 8.

Table 8: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult and L4 Stage Cattle Nematodes - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Cooperia oncophora (Adult)	89
Cooperia punctata (Adult)	100
Cooperia surnabada (Adult)	96
Ostertagia ostertagi (L4 stage)	99
Oesophagostomum radiatum (L4 stage)	100

3. Dose Confirmation Study #1231C-02-90-005, Dr. A. Villineuve, University of Montreal, Faculty of Veterinary Medicine, Saint Hyancinthe, Quebec, Canada

   Thirty calves were artificially infected with mixed doses of *O. ostertagi* and *C. oncophora* nematode parasites and divided into three groups of 10 animals each. One group of 10 calves served as untreated controls. The other two groups were treated with a single injection of doramectin timed to evaluate efficacy against either L4 or adult stage nematodes. At necropsy, worm counts in the two doramectin-treated groups were compared to the negative control group to determine doramectin efficacy. The results are summarized in Table 9.

Table 9: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult and L4 Stage Cattle Nematodes - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Cooperia oncophora (Adult)	99
C. oncophora (L4 stage)	98
Ostertagia ostertagi (Adult)	98
O. ostertagi (L4 stage)	98

4. Dose Confirmation Study #1231C-60-90-006, Dr. C.E. Couvillion, College of Veterinary Medicine, Mississippi State University

   Twenty calves were artificially infected with mixed doses of nematode parasites. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 10.

Table 10: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against L4 Stage Cattle Nematodes

Parasite	% Efficacy
Ostertagia ostertagi (L4 stage)	100
Trichostrongylus axei (L4 stage)	100
Cooperia oncophora (L4 stage)	100
Cooperia punctata (L4 stage)	99
Cooperia surnabada (L4 stage)	99

5. Dose Confirmation Study #1231C-60-90-008, Dr. C.E. Couvillion, College of Veterinary Medicine, Mississippi State University

   Thirty calves were artificially infected with mixed doses of nematode parasites and divided into three groups of 10 animals each. One group of 10 calves served as untreated controls. The other two groups were treated with a single injection of doramectin timed to evaluate efficacy against either L4 or adult stage nematodes. At necropsy, worm counts in the two doramectin-treated groups were compared to the negative control group to determine doramectin efficacy. The results are summarized in Table 11.

Table 11: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult and L4 Stage Cattle Nematodes - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Haemonchus placei (Adult)	100
H. placei (L4 stage)	100
Ostertagia ostertagi (Adult)	99
O. ostertagi (L4 stage)	100
Trichostrongylus colubriformis (Adult)	100
T. colubriformis (L4 stage)	100
Oesophagostomum radiatum (Adult)	100
O. radiatum (L4 stage)	100

6. Dose Confirmation Study #1231C-60-90-010, Dr. T.A. Yazwinski, University of Arkansas, Fayetteville, Arkansas

   Twenty calves were artificially infected with mixed doses of nematode parasites and divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 12.

Table 12: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against L4 Stage Cattle Nematodes - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Haemonchus placei (L4 stage)	100
Ostertagia ostertagi (L4 stage)	99
Cooperia oncophora (L4 stage)	100
Cooperia punctata (L4 stage)	100
Trichostrongylus axei (L4 stage)	100

7. Dose Confirmation Study #1231C-60-90-011, Dr. T.A. Yazwinski, University of Arkansas, Fayetteville, Arkansas

   Twenty calves were artificially infected with *Bunostomum phlebotomum* larvae and divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were

compared to determine doramectin efficacy. The results are summarized in Table 13.

Table 13: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult Stage *Bunostomum phlebotomum* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Bunostomum phlebotomum (Adult stage)	100

8. Dose Confirmation Study #1231C-60-90-014, Dr. L. L. Smith, Larry Smith Farm, Viroqua, Wisconsin

   Twenty calves were artificially infected with *Dictyocaulus viviparus* larvae and divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 14.

Table 14: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against L4 Stage *Dictyocaulus viviparus* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Dictyocaulus viviparus (L4)	100

9. Dose Confirmation Study #5232C-03-89-037, Dr. R.L. Coop, Moredun Animal Health, Ltd., 408 Gilmerton Road, Edinburgh, Scotland

   Twenty calves were artificially infected with mixed doses of nematode parasites and divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 15.

Table 15: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult and L4 Stage Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Cooperia oncophora (L4 stage)	99
Trichostrongylus axei (L4 stage)	100
Trichostrongylus colubriformis (L4 stage)	100
Dictyocaulus viviparus (L4 stage)	100
Ostertagia ostertagi (Adult)	99

10. Dose Confirmation Study #1232C-60-89-001, Dr. C. E. Couvillion, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi

    Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 16.

Table 16: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult and Inhibited *O. ostertagi* Larvae Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Ostertagia ostertagi (Adult)	100
O. ostertagi (Inhibited L4)	99
Cooperia punctata (Adult)	100
Trichostrongylus axei (Adult)	100
Oesophagostomum radiatum (Adult)	100

11. Dose Confirmation Study #1232C-60-89-002, Dr. H. Ciordia, Georgia Agricultural Experiment Station, University of Georgia, Experiment, Georgia

    Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 17.

Table 17: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult and Inhibited *O. ostertagi* Larvae Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Cooperia oncophora (Adult)	100
Cooperia pectinata (Adult)	100
Cooperia punctata (Adult)	100
Haemonchus placei (Adult)	100
Ostertagia ostertagi (Adult)	100
O. ostertagi (Inhibited L4)	99
Trichostrongylus axei (Adult)	100
Trichostrongylus colubriformis (Adult)	100
Bunostomum phlebotomum (Adult)	100

12. Dose Confirmation Study #1232C-60-89-003, Dr. G.L. Zimmerman, Oregon State University, Corvallis, Oregon

    Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 18.

Table 18: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Cooperia oncophora (Adult)	99
Cooperia surnabada (Adult)	100
Ostertagia ostertagi (Adult)	99
Trichostrongylus axei (Adult)	99
Trichostrongylus longispicularis (Adult)	100
Oesophagostomum radiatum (Adult)	100

13. Dose Confirmation Study #1232C-60-89-004, Dr. T.A. Yazwinski, University of Arkansas, Fayetteville, Arkansas

    Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 19.

Table 19: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Haemonchus placei (Adult)	100
Cooperia oncophora (Adult)	99
Cooperia punctata (Adult)	100
Cooperia surnabada (Adult)	100
Ostertagia ostertagi (Adult)	100
Ostertagia lyrata (Adult)	100
Trichostrongylus axei (Adult)	100
Oesophagostomum radiatum (Adult)	100
Trichuris ovis (Adult)	99

14. Dose Confirmation Study #1232C-60-89-005, Dr. T.A. Yazwinski, University of Arkansas, Fayetteville, Arkansas

    Thirty naturally infected calves were divided into two groups. One group of 15 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in table 20.

Table 20: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult and L4 Stage Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Dictyocaulus viviparus (Adult)	100
Haemonchus placei (Adult)	100
H. placei (L4 stage)	100
Cooperia oncophora (Adult)	99
Cooperia punctata (Adult)	99
Cooperia surnabada (Adult)	99
Ostertagia ostertagi (Adult)	100
Ostertagia lyrata (Adult)	100
Trichostrongylus axei (Adult)	100
Oesophagostomum radiatum (Adult)	100
Trichuris ovis (Adult)	99

15. Dose Confirmation Study #1232C-60-89-006, Dr. H. Ciordia, Georgia Agricultural Experiment Station, University of Georgia, Experiment, Georgia

    Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in table 21.

Table 21: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Haemonchus placei (Adult)	100
Cooperia oncophora (Adult)	99
Cooperia. pectinata (Adult)	100
Cooperia punctata (Adult)	99
Ostertagia ostertagi (Adult)	100
Trichostrongylus axei (Adult)	100
Oesophagostomum radiatum (Adult)	100
Trichuris ovis (Adult)	100

16. Dose Confirmation Study #1232C-60-90-010, Dr. H. Ciordia, Georgia Agricultural Experiment Station, University of Georgia, Experiment, Georgia

    Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in table 22.

Table 22: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against *Strongyloides papillosus* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Strongyloides papillosus (Adult)	100

17. Dose Confirmation Study #1232C-60-90-011, Dr. T.A. Yazwinski, University of Arkansas, Fayetteville, Arkansas

    Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, worm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in table 23.

Table 23: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Adult Nematode Parasites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Cooperia oncophora (Adult)	99
Cooperia surnabada (Adult)	100
Cooperia punctata (Adult)	100
Bunostomum phlebotomum (Adult)	100
Strongyloides papillosus (Adult)	100
Trichostrongylus axei (Adult)	100
Oesophagostomum radiatum (Adult)	100
Trichuris ovis (Adult)	100

E. Field Efficacy Against Nematodes:

Summary

A series of ten studies was conducted to confirm, under field conditions, the effectiveness of doramectin injectable, administered by the subcutaneous route at 200 mcg/kg, against naturally acquired gastrointestinal nematode infections. Studies were conducted to a common protocol at different locations

throughout North America including northeast, southeast, northwest and southwest USA and Canada. These studies were conducted under a range of climatic conditions, with both feedlot and pasture management systems being represented. In each study, animals with confirmed infections of gastrointestinal nematodes were assigned to a doramectin-treated or a non-medicated group and treated accordingly. Efficacy was based on percentage reduction in mean fecal egg count in doramectin-treated animals assessed 21 days post-treatment, the non-medicated controls serving to confirm that no self-cure had occurred. Results of the studies are summarized in Table 24.

Table 24: Summary of Nematode Field Efficacy Studies

Study	Location	Management System	Number of Doramectin Treated Animals	% Reduction in EPG 21 Days Post Treatment
1232C-60-90-007	Texas, USA	Pasture	82	100
1232C-60-90-008	Texas, USA	Feedlot	90	100
1233C-60-90-007	Mississippi, USA	Pasture	83	100
1233C-60-90-001	N. Carolina, USA	Pasture	76	100
1233C-60-90-003	Wisconsin, USA	Pasture	74	100
1233C-60-90-008	Idaho, USA	Feedlot	96	100
1233C-60-90-009	Oregon, USA	Pasture	100	100
1233C-02-90-004	Saskatchewan, Canada	Pasture	90	100
1233C-02-90-005	Alberta, Canada	Feedlot	56	98
1233C-02-90-006	British Columbia, Canada	Pasture	51	100

F. Efficacy Confirmation - Eyeworms:

Summary

Two studies were conducted to evaluate the efficacy of doramectin injectable administered by the SC route at a dosage of 200 mcg/kg BW, against *Thelazia* spp. One study (1231C-02-90-004) was conducted with calves in which the infection had been induced by purposeful exposure to infected face flies and the other (1231C-02-90-012) with naturally infected calves.

Conclusion: A single SC injection of doramectin administered at a dosage of 200 mcg/kg was 100% efficacious against adult *Thelazia* spp.

INDIVIDUAL EYEWORM STUDIES

1. Dose Confirmation Study #1231C-02-90-004, Dr. M.J. Kennedy, Alberta Agricultural Health Division, O.S. Longman Bldg., 6909-116 St., Edmonton, Alberta, Canada

   Twenty-four naturally infected calves were divided into two groups. One group of 12 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, eyeworm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 25.

Table 25: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Eyeworms - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Thelazia skrjabini (Adult)	100

2. Dose Confirmation Study #1231C-02-90-012, Dr. M.J. Kennedy, Alberta Agricultural Health Division, O.S. Longman Bldg., 6909-116 St., Edmonton, Alberta, Canada

   Twenty naturally infected calves were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group served as untreated controls. At necropsy, eyeworm counts in the two groups were compared to determine doramectin efficacy. The results are summarized in Table 26.

Table 26: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Eyeworms - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Thelazia skrjabini (Adult)	100
Thelazia gulosa (Adult)	100

G. Efficacy Confirmation - Grubs:

Summary

Five studies were conducted to assess doramectin efficacy when administered by the subcutaneous route at a dose of 200 mcg/kg BW against natural infections of *Hypoderma bovis* and *H. lineatum* larvae.

Conclusions: A single SC doramectin dose of 200 mcg/kg BW was efficacious in the treatment of *Hypoderma lineatum* and *H. bovis*.

INDIVIDUAL HYPODERMA STUDIES

1. Dose Confirmation Study #1032C-60-89-004, Dr. B.C. Clymer, Cactus Feedyard, Dumas, Texas

   Ninety calves with natural *Hypoderma lineatum* infections were divided into two equal groups. One group of 45 calves was treated with a single injection of doramectin. The other group served as untreated controls. The results are summarized in Table 27.

Table 27: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle *Hypoderma lineatum* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Hypoderma lineatum	100

2. Dose Confirmation Study #1032C-60-89-009, Dr. J.E. Lloyd, High Plains Research Station, University of Wyoming, Cheyenne, Wyoming

   Sixty calves with natural *Hypoderma lineatum* and *H. bovis* infections were divided into two equal groups. One group of 30 calves was treated with a single injection of doramectin. The other group served as untreated controls. The results are summarized in Table 28.

Table 28: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle *Hypoderma lineatum* and *H. bovis* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Hypoderma lineatum	100
H. bovis	100

3. Dose Confirmation Study #1032-60-90-025, Dr. J.E. Lloyd, High Plains Research Station, University of Wyoming, Cheyenne, Wyoming

   Sixty calves with natural *Hypoderma* spp. infections were divided into two equal groups. One group of 30 calves was treated with a single injection of doramectin. The other group served as untreated controls. The results are summarized in Table 29.

Table 29: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle *Hypoderma lineatum* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Hypoderma lineatum	100

4. Dose Confirmation Study #5032C-12-89-004, Dr. A. J. Weatherly, Farm of Monsieur Guneau Thostes, 21460 Epoisses, France

   Fifty-nine calves with natural *Hypoderma bovis* infections were divided into two groups. One group of 29 calves was treated with a single injection of

doramectin. The other group of 30 calves served as untreated controls. The results are summarized in Table 30.

Table 30: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle *Hypoderma bovis* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Hypoderma bovis	100

5. Dose Confirmation Study #1032C-02-92-036, Dr. D. D. Colwell, Agriculture Canada Research Station, Lethbridge, Alberta, Canada

   Sixteen calves with natural *Hypoderma* spp. infections were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 6 calves served as untreated controls. The results are summarized in Table 31.

Table 31: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle *Hpyoderma lineatum* - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Hypoderma lineatum	100

H. Efficacy Confirmation - Lice:

Summary

A series of six controlled studies were conducted involving cattle harboring naturally acquired infestations of lice. These studies evaluated the efficacy of doramectin injectable, administered by the subcutaneous route at a dose of 200 mcg/kg bodyweight. Studies were conducted to a common protocol at a range of geographic locations throughout North America in order to obtain broad strain representation for each of the economically important species.

Conclusion: A single SC injection of doramectin at a dosage of 200 mcg/kg was 100% effective against infestations of *Hematopinus eurysternus*, *Linognathus vituli*, and *Solenoptes capillatus*.

INDIVIDUAL LICE STUDIES

1. Dose Confirmation Study # 1032C-60-90-013, Dr. J.E. Lloyd, Paradise Research Unit, University of Wyoming, Laramie, Wyoming

   Fifteen calves with natural infestations of lice were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 32.

Table 32: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Lice - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Linognathus vituli	100

2. Dose Confirmation Study # 1032C-60-90-018, Dr. T.A. Yazwinski, University of Arkansas, Fayetteville, Arkansas

   Fifteen calves with natural infestations of lice were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 33.

Table 33: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Lice - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Linognathus vituli	100

3. Dose Confirmation Study # 1032C-60-90-021, Dr. J.E. Lloyd, Paradise Research Unit, University of Wyoming, Laramie, Wyoming

   Fifteen calves with natural infestations of lice were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 34.

Table 34: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Lice - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Solenoptes capillatus	100
Linognathus vituli	100

4. Dose Confirmation Study # 1032C-60-90-029, Dr. L. L. Smith, Larry Smith Farm, Viroqua, Wisconsin

   Fifteen calves with natural infestations of lice were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 35.

Table 35: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Lice - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Haematopinus eurysternus	100

5. Dose Confirmation Study # 1032C-60-90-031, Dr. J.E. Lloyd, Paradise Research Unit, University of Wyoming, Laramie, Wyoming

   Fifteen calves with natural infestations of lice were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 36.

Table 36: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Lice - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Solenoptes capillatus	100
Linognathus vituli	100

6. Dose Confirmation Study #1032C-60-92-035, Dr. J.E. Lloyd, Beef Cattle Research Station, University of Wyoming, Laramie, Wyoming

   Fifteen calves with natural infestations of lice were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 37.

Table 37: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Lice - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Solenoptes capillatus	100
Haematopinus eurysternus	100
Linognathus vituli	100

I. Efficacy Confirmation - Mange Mites:

Summary

Two studies were conducted with artificially acquired *Psoroptes bovis* infestations and two with naturally acquired *Sarcoptes scabiei* infestations. These studies evaluated the efficacy of doramectin injectable administered by the subcutaneous route at a dosage of 200 mcg/kg BW.

Conclusion: A single SC injection of doramectin at a dosage of 200 mcg/kg BW was efficacious against infestations of *Psoroptes bovis* and *Sarcoptes scabiei*.

INDIVIDUAL MITE STUDIES

1. Dose Confirmation Study #1031C-60-89-001, Dr. B.C. Clymer, Clymer Research and Consulting Inc., P.O. Box 7313, Amarillo, Texas

Fifteen calves with experimental infestations of mites were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 38.

Table 38: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Mites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Psoroptes bovis	100

1. Dose Confirmation Study #1031C-60-89-002, Dr. H.G. Kinzer, New Mexico State University, Las Cruces, New Mexico

   Fifteen calves with experimental infestations of mites were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 5 calves served as untreated controls. The results are summarized in Table 39.

Table 39: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Mites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Psoroptes bovis	99

2. Dose Confirmation Study #5032C-81-92-058, Mr D.J. Shanks, S.C. Viticola S.A., Zimnicea, Judetul, Romania

   Twenty-eight calves with natural infestations of mites were divided into two groups. One group of 10 calves was treated with a single injection of doramectin. The other group of 18 calves served as untreated controls. The results are summarized in Table 40.

Table 40: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Mites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Sarcoptes scabiei	100

3. Dose Confirmation Study #5032C-81-92-059, Mr D.J. Shanks, AIECITT Bailesti, Oras Bailesti, Drumul Rastului, Judetul Dolj, Romania

   Thirty-one calves with natural infestations of mites were divided into two groups. One group of 16 calves was treated with a single injection of doramectin. The other group of 15 calves served as untreated controls. The results are summarized in Table 41.

Table 41: Therapeutic Efficacy of Doramectin at 200 mcg/kg BW Against Cattle Mites - Percentage Reduction Relative to Controls

Parasite	% Efficacy
Sarcoptes scabiei	100

J. Field Efficacy Against Arthropods:

Summary

Eight studies were conducted to evaluate the efficacy of doramectin injectable at a dosage of 200 mcg/kg given subcutaneously to cattle harboring natural infestations of lice. The studies were conducted to similar protocols at sites across the USA and Canada. In the USA, studies were conducted in the following states: North Carolina, Texas, Arkansas, Illinois, Wisconsin and Wyoming. One study was conducted in Alberta, Canada. In each study, animals with confirmed infestations of sucking lice were assigned to a doramectin-treated or a non-medicated group and treated accordingly. Efficacy was based on cure rate (i.e., the percentage of animals with no lice 28 days post-treatment) in doramectin-treated animals, the non-medicated controls serving to confirm that self cure did not occur.

Table 42: Summary of Lice Field Efficacy Studies

Study	**Location**	**Number of Doramectin Treated Animals**	**% Calves with No Lice on Day 28**
1033C-60-91-001	Arkansas, USA	30	100
1033C-60-91-002	Arkansas, USA	34	100
1033C-60-91-003	Texas, USA	30	100
1033C-60-91-004	Illinois, USA	30	100
1033C-02-91-006	Alberta, Canada	28	100
1032C-60-90-028	N. Carolina, USA	18	100
1032C-60-90-032	Wyoming, USA	52	100
1032C-60-90-033	Wisconsin, USA	30	100

K. Persistent Efficacy - Ostertagia ostertagi:

Summary

Two studies were conducted to evaluate persistent efficacy of doramectin injectable solution administered by the subcutaneous (SC) route at a dosage of 200 mcg/kg BW against artificial infections of *Ostertagia ostertagi*.

Conclusion: A single SC injection of doramectin at a dosage of 200 mcg/kg BW was effective in preventing challenge infections of *O. ostertagi* from establishing for up to 21 days post-treatment.

INDIVIDUAL *O. OSTERTAGI* STUDIES

1. Dose Confirmation Study #1231C-60-95-190, Dr. L.R. Cruthers, Professsional Laboratory & Research Services, Inc., Route 1, Box 3AA, Corapeake, North Carolina

   Forty calves were divided equally into four groups. One group of 10 calves served as untreated controls, all other groups were treated with a single injection of doramectin. Test animals were challenged with infective *O. ostertagi* larvae on days 14 to 28 post-treatment. At necropsy on Days 42, 43, or 46 post-treatment, worm counts in the three doramectin-treated groups were compared to the negative control group to determine doramectin efficacy. The results are summarized in Table 43.

Table 43: Persistent Efficacy of Doramectin at 200 mcg/kg BW Against *O. ostertagi* challenge - Percentage Efficacy at Persistence Intervals of 14, 21 and 28 Days Post-treatment

Persistence Interval	% Efficacy
28 days	98
21 days	90
14 days	99

2. Dose Confirmation Study #1231C-60-95-191, Dr. E.G. Johnson, Johnson Research, 24007 Highway 20/26, Parma, Idaho

   Forty calves were divided equally into four groups. One group of 10 calves served as untreated controls, all other groups were treated with a single injection of doramectin. Test animals were challenged with infective *O. ostertagi* larvae on days 14 to 28 post-treatment. At necropsy on Day 42 post-treatment, worm counts in the three doramectin-treated groups were compared to the negative control group to determine doramectin efficacy. The results are summarized in Table 44.

Table 44: Persistent Efficacy of Doramectin at 200 mcg/kg BW Against *O. ostertagi* challenge - Percentage Efficacy at Persistence Intervals of 14, 21 and 28 Days Post-treatment

Persistence Interval	% Efficacy
28 days	84
21 days	99
14 days	100

VI. ANIMAL SAFETY

The safety of doramectin injectable solution was evaluated in both breeding and non-breeding cattle. The objectives of the testing were to demonstrate an adequate margin-of-safety following overdose, to establish safety in breeding animals, to assess local tolerance at the site of injection, and to confirm safety in animals under representative field use conditions.

A. Drug Tolerance - Study 1:

1. *Experiment number*: 1431N-60-90-004

2. *Starting date:* November 5, 1990

3. *Termination date*: November 19, 1990

4. *Study director:* Dr. J.A. Jackson

5. *Study location:* Pfizer Animal Health Research Center, Terre Haute, Indiana

6. *Procedure and results*: Eight cattle (4 females, 4 male castrates) with a mean body weight of 136 kg were placed in one of two groups and treated subcutaneously with either doramectin at a dose of 2 mg/kg body weight (10X the recommended dose) or saline at 0.20 mL/kg body weight. Clinical observations were made during the period immediately following dosing and twice daily for 14 days post-treatment. Blood samples were collected for hematologic and clinical chemistry data prior to treatment and at 4, 7, and 14 days post-treatment. Calves were weighed on Days 4, 7 and 14.

   No adverse effects were attributable to doramectin treatment with respect to clinical condition or weight gain, and no pathologically significant changes occurred in any of the hematology and clinical chemistry variables. It was concluded that a single administration of doramectin injectable at 10 times the recommended dose was well tolerated by cattle.

B. Drug Tolerance - Study 2:

1. *Experiment number*: 1431N-60-90-005

2. *Starting date:* November 26, 1990

3. *Termination date*: December 10, 1990

4. *Study director:* Dr. J.A. Jackson

5. *Study location:* Pfizer Animal Health Research Center, Terre Haute, Indiana

6. *Procedure and results*: Eight cattle (4 females, 4 male castrates) with a mean body weight of 152 kg were placed in one of two groups and treated subcutaneously with either doramectin at a dose of 5 mg/kg body weight (25X the recommended dose) or saline at 0.5 mL/kg body weight. Clinical observations were made during the period immediately following dosing and twice daily for 14 days post-treatment. Blood samples were collected for hematologic and clinical chemistry data prior to treatment and at 4, 7, and 14 days post-treatment. Calves were weighed on Days 4, 7 and 14.

   No adverse effects were attributable to doramectin treatment with respect to clinical condition or weight gain, and no pathologically significant changes occurred in any of the hematology and clinical chemistry variables.

It was concluded that a single administration of doramectin injectable at 25 times the recommended dose was well tolerated by cattle**.**

C. Safety Margin Study:

1. *Experiment number*: 1433N-60-91-009

2. *Starting date:* June 24, 1991

3. *Termination date*: July 10, 1991

4. *Study director:* Dr. D.J. Fagerberg

5. *Study location:* Colorado Animal Research Enterprises, Inc., Fort Collins, Colorado

6. *Procedure and results*: Twenty-four (24) individually-housed cattle (12 of each sex) with a mean body weight of 180 kg were allotted to one of four equal sized groups and treated subcutaneously with either doramectin at doses of 200 (1X), 600 (3X) or 1000 (5X) mcg/kg BW or saline on each of three days, separated by 24-hour intervals. Clinical observations were made four times daily on dosing days 0, 1 and 2, twice daily on Days -2, -1, and 3 to 15, and once on Day 16, the last day of the study. Feed intake was measured daily from Days -3 to 16. Body weights were taken on Days -14, -3, 0 and 16. Clinical pathology tests (hematology, serum chemistry, and urinalyses) were conducted on specimens collected on Days -14, 0, 6 and 16. All test animals were euthanized on Day 16 and evaluated for gross pathology. Histopathologic examination was also conducted on all tissues from placebo and 5X groups, and on tissues with visible gross lesions in the 1X and 3X groups.

   No significant hematological, clinical chemistry, or pathological abnormalities were observed in cattle treated with doramectin. Transient post-dose excitability was observed in some treated animals, but these signs were also observed pre-treatment. The only clinical observation that may have been test article-related was transient post-dose salivation. The results of this study indicate that doramectin injectable administration at up to five times the recommended dose for three times the recommended duration of treatment was well tolerated by cattle.

D. Safety in Neonatal Calves:

1. *Experiment number*: 5432N-03-92-023

2. *Starting date*: April 16, 1992

3. *Termination date*: June 23, 1992

4. *Study director*: David M. Cameron, B.Sc.

5. *Study location*: Cambridgeshire, England

6. *Procedure and results*: 18 Friesian cows with known artificial breeding dates and their newborn calves were randomly allotted to one of three groups as the calves were born. The cows were allowed to calve naturally,

with minimal assistance if necessary. Calves were maintained in the pens with their dams throughout the 14-day test period, and were allowed to suckle freely. As each calf was born, it was examined within 1 to 12 hours after birth and, if considered physically and clinically normal, was allocated to experimental treatment using a randomized allocation list. There were 3 treatment groups with 6 animals per group. The treatment groups received saline, 200μg doramectin/kg (1X recommended dose), or 600 μg doramectin/kg (3X recommended dose) within approximately 1 to 12 hours of birth. The dams were treated at the same time with doramectin 200 μg/kg, irrespective of the treatment assigned to the calf. The calves were observed for 14 days following treatment. They were then euthanized, necropsied, and examined for gross pathological lesions.

All calves remained in good health throughout the study and no clinical abnormalities were observed which could be associated with experimental treatment. There were no clinical abnormalities observed in the cows which were considered likely to have an adverse effect on the calves. There were no macroscopic abnormalities found at necropsy which were considered to be related to experimental treatment.

E. Reproductive Safety in Female Cattle - Segment I study:

1. *Experiment number*: 1431N-60-91-008
2. *Starting date:* May 30, 1991
3. *Termination date*: September 17, 1991
4. *Study director:* C.M. Salamon
5. *Study location:* Hazelton Wisconsin, Inc., Madison, Wisconsin
6. *Procedure and results*: Ninety-six (96) heifers synchronized for, and confirmed in estrus were assigned at random to eight groups (12 heifers/group). Heifers in Groups T2, T4, and T6 received 600 mcg/kg BW (3X dose) of doramectin on post-estrus days 3, 11 or 18, respectively and heifers in Groups T1, T3, and T5 received saline on post-estrus days 3, 11, or 18, respectively. All heifers were inseminated at the next confirmed estrus. Heifers in groups T8 and T7 received 600 mcg/kg BW doramectin or saline, respectively 10 days post-insemination. All heifers were palpated 60 to 70 days after insemination to determine pregnancy status. Heifers were observed for approximately one hour post-dose and twice daily for abnormal clinical signs.

   There were no test material-related effects on reproductive performance, duration of the estrus cycle or pregnancy rates. Based on the results of this study, exposure of Holstein heifers to 600 mcg/kg BW doramectin (3X dose) during each of the stages of the estrus cycle or during early gestation resulted in no adverse effects on estrus, conception, or embryo implantation.

Table 45: Summary of Reproductive Variables

Group	Mean Estrus Cycle Length	Shortest Length	Longest Length	Number Inseminated	Number Pregnant	Pregnancy Rate %
T1	22	17	25	12	9	75%
T2	22	18	27	12	8	67%
T3	22	16	32	11	7	64%
T4	24	19	35	10	8	80%
T5	23	20	29	12	10	83%
T6	24	19	43	12	8	67%
T7	21	15	31	11	8	73%
T8	22	17	32	12	7	58%

T1 - Saline Day 3
T2 - Doramectin Day 3
T3 - Saline Day 11
T4 - Doramectin Day 11
T5 - Saline Day 18
T6 - Doramectin Day 18
T7 - Saline 10 days post-insemination
T8 - Doramectin 10 days post-insemination

F. Reproductive Safety in Female Cattle - Segment II/III Study:

1. *Experiment number*: 1436N-60-91-009

2. *Starting date:* March 31, 1992

3. *Termination date*: March 3, 1993

4. *Study Director:* Dr. D.J. Fagerberg

5. *Study location:* Colorado Animal Research Enterprises, Inc., Fort Collins, Colorado

6. *Procedure and results*: One hundred (100) heifers synchronized for, and confirmed in estrus were assigned at random to either doramectin or saline treatment groups (50 animals/group) and were artificially inseminated. After insemination, the 50 heifers in each treatment were randomly assigned to receive a single treatment between 12 and 55 days post-insemination, followed by a second treatment of animals diagnosed as pregnant at 220 days post-insemination. On its assigned treatment day, each heifer received a subcutaneous injection of either saline or doramectin (doramectin at 600 mcg/kg, 3X the commercial dose).

   All of the heifers were inseminated over a two-day. Of the heifers treated in the first trimester, 23 of the 29 saline-treated animals were diagnosed pregnant on Day 220 post-insemination, but only 20 of the 29 (69%) gave birth. Twenty-eight (28) of 32 doramectin-treated heifers were diagnosed pregnant on Day 220, all of which gave birth (88%).

Table 46

Group	# at start (inseminated)	# returned to heat and not treated	# not returned to heat and treated	# pregnant on Day 220	# calved	% live calf of those bred
T1	50	21	29	23	20	40%
T2	50	18	32	28	28	56%

All calves were born alive and no evidence of abortion was noted in either group at any time during the study. There was no significant difference between treatment groups in duration of gestation, length of parturition, incidence of dystocia, post-parturient health or agalactia.

Table 47

Group	Mean gestation period	% live calves of total born	mean duration of parturition	% dystocia	% agalactia	% post-calving ill health
T1	278.0	100%	87 min	25.0%	5%	0%
T2	279.8	100%	92 min	24.1%	0%	0%

There were no differences between the groups in neonatal calf viability (ability to stand, suckle, and walk) or in their survival rate to 7 days. There were no congenital abnormalities in calves from the doramectin-treated group.

Table 48

Group	Live calves at birth	Mean birth weight	Normal[1] calves at birth	% Viability	Healthy calves at 7 days	% Survivability
T1	21	30.8 kg	20	95.2%	19[2]	95%
T2	29	31.7 kg	29	100%	29	100%

[1]- Able to suckle, stand and walk
[2]- One calf removed from the study due to agalactia in the dam

There were no adverse effects on pregnancy, parturition, post-parturient health or lactation in heifers treated with doramectin. In addition, there were no adverse effects on neonatal viability among calves born to heifers treated with doramectin. Therefore, it was concluded that doramectin treatment of pregnant heifers at three times the recommended dose during the period of organogenesis and again during the third trimester of pregnancy had no adverse effects upon embryo development, maintenance of pregnancy, parturition, or neonatal calf viability and survivability.

G. Bull Reproductive Safety Study:

1. *Experiment number*: 1434N-60-92-012
2. *Starting date:* July 18, 1993
3. *Termination date*: October 6, 1993
4. *Study director:* Dr. D. J. Fagerberg
5. *Study location:* Colorado Animal Research Enterprises, Inc., Fort Collins, Colorado
6. *Procedure and results*: Twenty (20) healthy bulls were randomly assigned to two groups (10 animals/group). Each animal received single injections of doramectin and saline, administered at 3 mL/50 kg (600 mcg/kg in the case of doramectin) BW.

   Bulls were observed twice daily to assess general health status, and semen was collected for evaluation three times per week throughout the 70-day study period. Reproductive system evaluations included scrotal circumference measurements and examinations of the prepuce, penis, scrotum, testes, epididymides, spermatic cord, vesicular glands, prostate gland, ampullae of the vas deferens and pelvic urethral muscles. Semen specimens were evaluated for volume of ejaculate, color, spermatozoal mass activity, percent motility, concentration and morphology. Also at the time of semen collection, animals underwent physical examinations (including complete reproductive system evaluations), were weighed, and rectal temperatures were recorded. General physical condition was also assessed at these times.

Table 49: Semen Volume and Sperm Motility, Concentration, Output and Defects Summary

Treatment Group	Least Square Means (Adjusted for Pretreatment Differences) and [95% Confidence Bounds]					
	Seminal Fluid Vol. (mL)	Sperm Motility (%)	Sperm Conc./mL Semen	Total Sperm Output	% Sperm with Defects	
					Major	Minor
T01 (Placebo)	6.4 [5.5 - 7.4]	67.0 [62.4 - 71.6]	6.78×10^8 [5.78 - 7.79×10^8]	4.34×10^9 [3.5 - 5.19×10^9]	11.36 [8.32 - 14.4]	17.93 [16.19 - 19.67]
T02 (Dora)	5.8 [4.6 - 6.7]	63.5 [59.0 - 68.1]	6.96×10^8 [5.95 - 7.96×10^8]	4.11×10^9 [3.26 - 4.96×10^9]	13.35 [10.31 - 16.39]	17.32 [15.58 - 19.06]
p =	0.1352	0.2319	0.7960	0.6918	0.1123	0.5797

No significant differences found between treatment groups

Major defects were noted among the 11.36% of the placebo group sperm and 13.35% of the doramectin group sperm. Minor defects were noted among 17.93% and 17.32% of the placebo and doramectin group sperm, respectively. All types of major and minor defects were encountered among at least one specimen of every bull during the post-treatment

period. Among the major defects, proximal droplets were found in nearly every specimen (96% and 98% of placebo and doramectin, respectively). The next most common major defect was craters in or mal-developed spermatozoa (>80% of all specimens in both groups). Coiled tails or middle piece defects were noted in 55 - 60% of all specimens. The tailless defect was found among 69% of the placebo and 65% of the doramectin specimens. Abaxial implantation and abnormal acrosomes were encountered among 43 - 48% of tested specimens. The evaluations of sperm defects indicated that there were no treatment-related effects.

There were no significant differences between treatment and control groups with respect to reproductive organs/structures or semen quality. Therefore, it was concluded that doramectin treatment administered to breeding bulls at three times the recommended dose had no adverse effects on semen quality or any reproductive organs/structures.

H. Injection site toleration:

1. *Experiment number*: 1434N-60-92-003
2. *Starting date:* April 21, 1992
3. *Termination date*: May 23, 1992
4. *Study director:* D.E. Mouzin
5. *Study location:* Animal Health Research Center, Pfizer Inc., Terre Haute, Indiana
6. *Procedure and results*: Thirty (30) healthy calves of uniform weight were randomly assigned to three groups (10 animals/group). Each animal received single injections of doramectin and saline, administered at 1 mL/50 kg (200 mcg/kg in the case of doramectin) BW on opposing sides, both intramuscularly (IM) in the semimembranosus muscle and subcutaneously (SC) in the neck. An assessment of pain was made at the time of treatment. Injection sites were examined visually and by palpation at intervals following treatment. Groups of ten animals were slaughtered at 4, 15 and 30 days post-treatment. Injection sites were evaluated for gross abnormalities at necropsy and those exhibiting gross lesions were examined histopathologically.

   The majority of IM and SC doramectin injection sites showed pale discoloration at 4 days post-injection but by 30 days there was no significant difference in the frequency of discoloration between sites injected with saline and those injected with doramectin, regardless of the route by which the treatment was administered. It was concluded that doramectin 1% injectable solution is well tolerated at the injection sites by cattle.

VII. HUMAN FOOD SAFETY:

A. Toxicology:

Unless otherwise specified, all testing was conducted with doramectin (UK-67,994) in sesame oil. The doramectin activity was 92.5%. All pivotal testing

was conducted in full compliance with the Good Laboratory Practice (GLP) Regulations (21 CFR 58). All the genetic toxicology studies, conventional toxicology, and multigeneration reproduction safety studies were conducted at the Pfizer Central Research facilities located at Groton, Connecticut. The teratology/fetotoxicity studies were conducted at Pfizer's Research facility in Amboise, France.

GENOTOXICITY STUDIES

1. Microbial Reverse Mutation Assays (Ames Test)

    a. Protocol No: 87-657-02

    b. Starting date: July 1987

    c. Termination date: October 1987

    d. Investigator: H. E. Holden, Ph.D.

    e. Procedure and Findings:

    UK-67,994 (DMSO solvent) was tested for induction of reverse mutation in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100. Cells were exposed to compound, to compound and incomplete S9 mixture, or to compound and complete S9 mixture. Compound levels ranged from 0.005 to 10 mg/plate. Metabolic activation was provided by Aroclor 1254-induced liver S9 prepared from male rats [Crl: COBS CD(SD)BR] and mice [Crl: COBS CD-1(ICR)BR]. Incomplete S9 mixture contained S9 fraction and all cofactors present in the complete S9 mixture except NADP; this exception rendered the mixture metabolically inactive. The incomplete S9 mixture was used as a negative control condition for comparison to results of cultures treated with complete S9 mixture. No indication of mutagenic activity was observed.

    Cells from the same strains were exposed to urine collected from mice dosed intraperitoneally once with 0.2, 2 or 4 mg/kg of the same lot of compound. There was no evidence of mutagenic excretory product in these studies.

2. Mouse Lymphoma (L5178Y/TK Gene Mutation Assay)

    a. Protocol No: 87-657-02

    b. Starting date: July 1987

    c. Termination date: October 1987

    d. Investigator: H. E. Holden, Ph.D.

    e. Procedure and Findings:

    Three-hour exposure of L5178Y cells to UK-67,994 (DMSO solvent) produced no substantial dose-related increase in mutant frequency at test concentrations producing 20-80% total relative growth. The

presence of rat liver S9 greatly reduced the drug-induced cytotoxicity, but did not result in the production of mutagenic metabolites. Cells were exposed to compound, or to compound and S9 mixture prepared from naive (uninduced) male rat liver S9. In the direct test conducted without S9, concentrations of 8-35 mcg/mL were used. Concentrations ≥ 24 mcg/mL produced excessive cytotoxicity and these cultures were not clonable for mutant selection. In the test conducted with S9 activation, cultures were treated at concentrations of 13-62 mcg/mL. Concentrations ≥ 51 mcg/mL were excessively cytotoxic and not clonable for mutant selection.

3. Unscheduled DNA Synthesis

   a. Protocol No.: 87-657-02

   b. Starting date: July 1987

   c. Termination date: October 1987

   d. Investigator: H. E. Holden, Ph.D.

   e. Procedure and Findings:

      UK-67,994 (DMSO solvent) was tested for its ability to produce unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes. Concentrations of 1.7, 5.0, 7.5, 10, 15 and 20 mcg/mL were used in the UDS assay. UK-67,994 was tested up to cytotoxic concentrations (≥ 10 mcg/mL), as indicated by the marked reduction in cell viability. The two highest concentrations produced excessive cytotoxicity and could not be evaluated for induction of UDS. In cultures exposed to UK-67,994 at concentrations up to 10 mcg/mL for 18 hours, no significant increase in UDS was observed.

4. *In Vivo* Micronucleus Assay

   a. Protocol No.: 90-657-21

   b. Starting date: August 1990

   c. Termination date: August 1990

   d. Investigator: H. E. Holden, Ph.D.

   e. Procedure and Findings:

      UK-67,994 (in distilled water containing 0.5% methylcellulose) was tested for induction of micronuclei in male and female mouse bone marrow. Mice were dosed by oral gavage once per day for three days at dose levels of 500, 1000 and 2000 mg/kg/day.

      No evidence of micronucleus induction related to treatment with UK-67,994 was observed in any of these studies. There was evidence of target organ toxicity as indicated by a reduction in the ratio of polychromatic erythrocytes to normochromatic erythrocytes at mid and high dose levels.

TOXICITY STUDIES

1. Three-month oral (gavage) study in rats with *in utero* exposure.

   a. Experiment No: 89-657-17

   b. Starting date: May 15, 1989

   c. Termination date: August 24, 1989

   d. Investigator: D.O. Fisher

   e. Procedure and Findings:

      Twenty Long-Evans rats/sex/group were dosed by gavage with 0, 0.5, 2.0 and 8 mg/kg of doramectin for 90 days. The animals were selected from F1 pups produced in a two-generation study and were, therefore, exposed to drug *in utero* and during lactation. Dosing started approximately 3-4 weeks post weaning. The high dose females had statistically significant increases in both absolute and relative liver weights.

   f. NOEL: The No-Observed-Effect-Level (NOEL) for this study was 2.0 mg/kg/day.

2. Three month oral (gavage) study in Beagle dogs.

   a. Experiment No: 88-657-13

   b. Starting date: July 25, 1988

   c. Termination date: October 27, 1988

   d. Investigator: S. W. Stadnicki, Ph.D.

   e. Procedure and Findings:

      Four dogs/sex/group were dosed at 0, 0.5, 1.0 and 2.0 mg/kg/day for 91 consecutive days. Adverse effects were limited to dose-related, transient, reversible mydriasis in one low, two intermediate, and five high dose animals.

f. NOEL: None assigned

3. Three month oral (gavage) study in Beagle dogs

   a. Experiment No.: 89-657-19

   b. Starting date: June 26, 1989

   c. Termination date: September 27, 1989

   d. Investigator: S. W. Stadnicki, Ph.D.

   e. Procedure and Findings:

      Three dogs/sex/group were dosed at 0, 0.1 and 0.3 mg/kg/day for 92 consecutive days. Adverse effects were limited to transient, reversible mydriasis in 1/6 dogs in the high-dose group.

   f. NOEL: The No-Observed-Effect-Level (NOEL) for this study was 0.3 mg/kg/day.

4. Fetotoxicity in Rats by the Oral Route

   a. Experiment No's :

      a) 88079

      b) 88080

   b. Starting date:

      a) May 31, 1988

      b) May 31, 1988

   c. Termination date:

      a) June 23, 1988

      b) June 15, 1988

   d. Investigator: M. J. Kessedjian

   e. Procedure and Findings:

      In the main study (88079), twenty Crl: COBS-VAF-CD(SD)BR pregnant albino rats per group were dosed by gavage with 0, 1.5, 3.0 and 6.0 mg/kg/day of doramectin from Days 6 to 15 of gestation.

      A pharmacokinetic study (88080) was conducted in parallel, in which 5 inseminated rats were dosed with UK-67,994 at 6 mg/kg/day over the same period (Day 6 to 15). On Day 15, blood was withdrawn and the females were sacrificed. Amniotic fluid was collected and the fetuses were removed for UK-67,994 assay.

      Adverse signs were limited to a threshold increase in embryo mortality in the 6 mg/kg/day group.

f. NOEL: The No-Observed-Effect-Level (NOEL) of 3.0 mg/kg/day and a safety factor of 1000 were set for these studies.

5. Fetotoxicity Study in Rabbits by the Oral Route

   a. Experiment No's:

      a) 88106

      b) 88107

   b. Starting date:

      a) July 4, 1988

   c. Termination date:

      a) August 5, 1988

      b) July 22, 1990

   d. Investigator: M. J. Kessedjian

   e. Procedure and Findings:

      In the main study (88106) UK-67,994 was administered to groups of 20 New Zealand White artificially inseminated female rabbits for 12 days (Day 7 to 18 post insemination) at daily dose levels of 0.75, 1.5 and 3.0 mg/kg.

      In the pharmacokinetic study (88107), 4 inseminated rabbits received UK-67,994 at 3 mg/kg/day over the same period (Days 7 to 18 post injection). On Day 18, blood was withdrawn and the females were sacrificed. Amniotic fluid was collected and fetuses were removed for UK-67,994 assay.

      Direct teratogenic effects were limited to some delay in fetal pubic bone ossification at the 1.5 and 3.0 mg/kg/day dose levels.

      Teratogenic effects, i.e., cleft palate were not seen at dose levels that did not also produce maternal toxicity (3.0 mg/kg/day), and therefore were considered indirect toxic effects on the fetuses.

   f. NOEL: The No-Observed-Effect-Level (NOEL) of 0.75 mg/kg/day and a safety factor of 1000 were set for these studies.

6. Fetoxicity in Mice by the Oral Route

   a. Experiment No's:

      a) 88091

      b) 88092

b. Starting date: a & b) June 16, 1988

c. Termination date:

a) July 12, 1988

b) June 29, 1988

d. Investigator: M. J. Kessedjian

e. Procedure and Findings:

In the main study (88091), UK-67,994 was administered by gavage to groups of 20 Crl:COBS-VAF-CD1(ICR)BR albino inseminated female mice from day 6 to 13 post-insemination at daily doses of 1.5, 3 and 6 mg/kg.

In the pharmacokinetic study (88092), 10 Crl:COBS-VAF-CD1(ICR)BR albino inseminated mice received UK-67,994 at 6 mg/kg/day over the same period (days 6 to 13 post-insemination). On day 13, blood was withdrawn and all females were sacrificed. Amniotic fluid was collected and fetuses were removed for UK-67,994 assay.

There were no deaths and no drug-related clinical signs. There were no effects of the treatment on maternal body weights and reproductive parameters. The embryo mortality rate was higher at the top dose level but the difference was not statistically significant. There was not evidence of teratogenicity.

f. NOEL: The No-Observed-Effect-Level (NOEL) for embryotoxicity of 3 mg/kg/day and a safety factor of 1000 were set for this study.

7. Two-Generation Oral (gavage) Study in Long-Evans Rats

a. Experiment No: 89-657-20

b. Starting date: October 1989

c. Termination date: August 1990

d. Investigator: D. O. Fisher

e. Procedure and Findings:

Long-Evans rats were dosed for two generations (45/sex/group in F_0 and 30/sex/group in F_1 generation) with 0, 0.1, 0.3 and 1.0 mg/kg/day of doramectin. Body weight gain during lactation was adversely affected by treatment in F_1 females of 1.0 mg/kg/day group. The mean weights of F_2a and F_2b pups of the high dose group were lower than controls on Day 21 of lactation.

This finding was determined to be a direct effect of the drug, since in a previous study, doramectin was shown to be excreted in the milk at high concentration.

f. NOEL: The No-Observed-Effect-Level (NOEL) for reproductive effects was set at 0.3 mg/kg/day and a safety factor of 100 was set for this study.

CALCULATION OF A SAFE CONCENTRATION (S.C.)

The lowest NOELs in the toxicity studies were 0.3 mg/kg/day for maternotoxic effects in the two-generation study in rats, and 0.3 mg/kg/day for the mydriasis observed in the 90-day dog study. However, the NOEL of 0.3 mg/kg/day was not used to calculate the S.C. because (a) a safety factor of 100 is applicable to the two-generation rat study, (b) the mydriasis observed in the dogs was a transient effect, and (c) mydriasis is not induced in the humans given therapeutic avermectin treatment. Therefore, the NOEL used to calculate the S.C. of doramectin is 0.75 mg/kg/day which corresponds to that set for the fetotoxicity study in rabbits. The safety factor applied to this study was 1000 because cleft palate was observed in the 3.0 mg/kg/day group and delayed ossification of the fetal pubic bones occurred in the 1.5 and 3.0 mg/kg/day groups. In addition, a safety factor of 1000 is applied to this study because doramectin is structurally related to ivermectin which has been shown to cause cleft palate in rats, mice, and rabbits. Using the appropriate NOEL and safety factor, an acceptable daily intake (ADI) of up to 0.75 micrograms/kg/day of doramectin residue in food was determined using the formula:

$$\text{ADI} = \frac{\text{NOEL}}{\text{Safety factor}}$$

$$\text{ADI} = \frac{0.75 \text{ mg/kg/day}}{1000 \text{ Safety factor}} = 0.75 \text{ µg/kg/day}$$

A safe concentration in muscle tissue of cattle is calculated from the acceptable daily intake, assuming the average weight of a man to be 60 kg and the daily human intake of muscle to be 300 g, as follows:

$$\text{Safe concentration in muscle} = \frac{(60 \text{ kg})(0.75 \text{ µg/kg/day})}{300 \text{ g/day}} = 150 \text{ ppb}$$

The safe concentration of residues in liver, kidney and fat are determined from this number using appropriate food consumption values (food factor) for these tissues. Therefore, the safe concentrations are:

Liver: 150 ppb x 3 (food factor) = 450 ppb

Kidney: 150 ppb x 6 (food factor) = 900 ppb

Fat: 150 ppb x 6 (food factor) = 900 ppb

B. Total Residues and Metabolism:

1. Total Residues

The levels of total drug-related residues of doramectin in the tissues of cattle treated with [^{3}H]-doramectin were determined in a tissue residue study conducted by Pfizer.

a. TITLE: Radiotracer Residue Depletion Study in Edible Tissues and Injection Site of Cattle Treated Intramuscularly with [^{3}H]-doramectin.

b. PROTOCOL NO.: 1535N-60-93-016

c. STUDY DESIGN:

a) Dose: 200 mcg/kg BW as an intramuscular injection in the neck

b) Radiotracer: doramectin radiolabeled with tritium at the C-5 position

c) Test animals: 26 crossbred beef cattle (13 male castrate, 13 female), average wt.: 328 kg

d) Withdrawal schedule: 7, 14, 21, 28, 35, and 42 days post-dosing

Following treatment with radiolabeled doramectin, four animals (two of each sex) were sacrificed at each collection time, and tissue samples of liver, muscle, kidney, perirenal fat, and injection site were collected and radioassayed for total drug-related residues.

d. Results and Conclusions:

The results from the study are shown in Table 50.

Table 50: Mean doramectin residue concentrations (±1 SD) in cattle tissue (Study No. 1535N-60-93-016).

Withdrawal Period (days)	Liver Levels (ppb)	Injection Site Levels (ppb)	Kidney Levels (ppb)	Muscle Levels (ppb)	Fat Levels (ppb)
7	470 ± 110	2540 ± 1800	108 ± 15	40 ± 5	551 ± 42
14	415 ± 180	672 ± 790	60 ± 4	20 ± 6	265 ± 27
21	257 ± 69	421 ± 400	35 ± 8	13 ± 3	180 ± 30
28	120 ± 24	571 ± 630	22 ± 5	10 ± 4	115 ± 26
35	42 ± 28	<24 ± 38	7 ± 3	<3 ± 1	<36 ± 17
42	24 ± 1	18 ± 10	4 ± 1	<3	23 ± 6

2. METABOLISM IN CATTLE

The profiling of doramectin metabolites in cattle was conducted with tissues from animals in study 1535N-60-93-016 and also with tissues from a preliminary total residue study. The calf livers were from animals treated with 200 mcg/kg tritium-labeled doramectin and then sacrificed 3 days and 21 days later. Samples of the livers were extracted with acetonitrile-methanol, and the extracts were eluted through a solid phase extraction

column with methanol. Fat samples were extracted with acetonitrile. Reverse phase HPLC was used to generate the profiles of extractable metabolites in both tissues.

The metabolite workup of the liver from a animal sacrificed three days after dosing revealed that 95% of the radioactivity was extractable, indicating that bound residues constitute, at most, a small fraction of the total residue. Unchanged doramectin was the major metabolite of doramectin in liver, and three minor metabolites also were observed. The compounds identified in liver over the range of 3 to 21 days of withdrawal are listed below, along with the relative amounts present.

Liver Metabolites	Percent of Extracted Radioactivity
doramectin	58 - 70%
3''-O-desmethyldoramectin	7 - 9%
24-hydroxymethyldoramectin	0 - 4%
24-hydroxymethyl-3''-O-desmethyldoramectin	7 - 8%

Unchanged doramectin was the major metabolite in cattle fat (91%) along with a small amount (7.4%) of a minor metabolite identified by mass spectrometry techniques as an epimer of doramectin.

3. METABOLISM IN RATS AND DOGS

The profiling of doramectin metabolites in rat liver and feces was conducted with samples from Sprague-Dawley rats administered a single 5 mg/kg oral dose of tritium-labeled doramectin. The rats were sacrificed 48 hrs after dosing. The dog liver and feces were obtained from a female Beagle dog that was dosed with a single 3.5 mg/kg oral dose of tritium-labeled doramectin. The liver was collected at the time of sacrifice of the dog at 48 hrs post dosing.

Approximately 37% and 51% of the liver total radioactivity was extracted from the rat and dog liver samples, respectively. HPLC analysis of those extracts and of extracts of dog and rat feces showed the presence of unchanged doramectin and the same three metabolites as observed in calf liver. The levels of those compounds are listed below as percentages of the sample activity recovered.

Metabolite	**Rat Liver**	**Rat Feces**	**Dog Liver**	**Dog Feces**
doramectin	18%	22%	28%	6%
3''-O-desmethyldoramectin	12%	19%	12%	8%
24-hydroxymethyldoramectin	3%	14%	ND*	5%
24-hydroxymethyl-3''-O-des-methyldoramectin	2%	16%	ND	4%

* ND = not detected

4. COMPARATIVE METABOLISM

The profiles of doramectin metabolites present in extracts of cattle liver were compared with profiles from extracts of urine and feces from rats and a dog, and a good match was observed.

Epi-doramection was present as a minor metabolite in cattle fat, but it was not observed in any of the samples from the test species. Because of the close structural relationship of *epi*-doramectin with doramectin and the good match of the cattle liver metabolites, further toxicological evaluation of *epi*-doramectin was judge not to be warranted.

Thus, it was concluded that the metabolism of doramectin is similar in cattle, rats, and the dog and that the test species were exposed to all of the major metabolites observed in cattle tissues.

C. Selection of Target Tissue and Marker Residue for Doramectin in Cattle:

The data in the table of total residue values shown above in Table 50 establish that liver contains the highest levels of total drug-related doramectin residues and that it is the tissue in cattle from which residues are the last to deplete to the safe concentration. Similarly high concentrations occur in fat; however, these did not exceed the safe concentration at any time point tested. These observations suggested that liver was the most likely target tissue for doramectin in cattle.

Liver was confirmed as the target tissue and parent doramectin was assigned as the marker residue following further assay of the tissue samples from the radiolabeled study (1535N-60-93-016) using the proposed determinative HPLC procedure for unchanged doramectin. The results of the assays of the liver samples are shown in Table 51. The ratio of unchanged drug to total residues ranged from 68% on day 7 to 53% on day 35 in liver tissue, with an overall mean of 60%. The ratio of unchanged drug to total residues was found to be 60% on day 21, the time point closest to the time at which mean total residue concentrations fall below the safe concentration.

Table 51:Mean doramectin residue concentrations in cattle liver in study 1535N-60-93-016.

Withdrawal Period (days)	**Liver Total Residue Level (ppb)**	**Unchanged Doramectin (ppb)**	**Percent Unchanged Doramectin**
7	470 $\pm$ 110	319 $\pm$ 78	68 $\pm$ 4
14	415 $\pm$ 180	253 $\pm$ 110	61 $\pm$ 3
21	257 $\pm$ 69	154 $\pm$ 38	60 $\pm$ 1
28	120 $\pm$ 24	72 $\pm$ 10	61 $\pm$ 9
35	42 $\pm$ 28	22 $\pm$ 16	53 $\pm$ 2
42	24 $\pm$ 1	13 $\pm$ 1	56 $\pm$ 4

The residue data listed above confirmed liver as the target tissue. Those data also demonstrated that parent doramectin was present in sufficiently high

concentration and had the proper depletion characteristics in liver to serve as the marker residue in that tissue.

D. Tolerance for the Marker Residue:

The Rm (tolerance) for doramectin was set from the data obtained in the analysis of the liver samples from cattle in the total residue study (1535N-60-93-016). The liver samples from that study were assayed for unchanged doramectin by the determinative assay of the regulatory method as shown earlier in Table 51.

A value of 100 ppb unchanged doramectin is assigned as the tolerance for the drug in cattle liver, and that value compensates for the occasional high injection site residue that was observed in the pivotal tissue residue depletion studies. If the high injection site residues did not occur and the tolerance could be set solely on the basis of residues in the liver, a tolerance of 300 ppb would have been assigned. The 100 ppb tolerance in liver corresponds to a withdrawal time of 35 days that is needed because of the injection site residues (see Part E. Studies Establishing the Withdrawal Period).

E. Studies Establishing the Withdrawal Period:

Depletion of the marker residue was determined in two studies - one following subcutaneous doramectin administration and one following intramuscular doramectin administration.

1. TITLE: Doramectin Residue Depletion Study in Edible Tissues of Cattle

   a. PROTOCOL NO.: 1535N-60-90-049

   b. Study design:

      a) Dose: 200 mcg/kg BW as a subcutaneous injection in the neck

      b) Test animals: 26 crossbred beef cattle (13 male, 13 female), 245 kg avg wt

      c) Withdrawal schedule: 14, 21, 28, and 35 days post-dosing

      Following treatment with doramectin, six animals were sacrificed at each collection time, and tissue samples of liver, muscle, kidney, perirenal fat, and injection site were collected and assayed for unchanged doramectin, the marker residue.

   c. Results:

      The results from the study are shown in Table 52:

Table 52: Mean doramectin (± 1 SD) concentration in cattle tissue (Study No. 1535N-60-90-049)

Withdrawal Period (days)	Liver Levels (ppb)	Injection Site (ppb)	Kidney Levels (ppb)	Muscle Levels (ppb)	Fat Levels (ppb)
14	88 ± 14	7300 ± 6200	23 ± 2	13 ± 1.9	288 ± 36
21	44 ± 16	1900 ± 1300	11 ± 5	< 7.1 ± 4.2	182 ± 104
28	25 ± 11	380 ± 300	8.8 ± 4	< 4.1 ± 1.7	94 ± 34
35	14 ± 6	930 ± 910	< 4.5 ± 2	< 3.1 ± 1.5	57 ± 22

2. TITLE: Depletion of Drug Residues From Tissues of Cattle Treated Parenterally With Doramectin - Single IM Injection of 200 mcg/kg

   a. PROTOCOL NO.: 1535N-60-91-050

   b. Study design:

      a) Dose: 200 mcg/kg BW as an intramuscular injection in the neck

      b) Test animals: 26 crossbred beef cattle (13 male, 13 female), 322 kg avg wt

      c) Withdrawal schedule: 14, 21, 28, and 35 days post-dosing

      Following treatment with doramectin, six animals were sacrificed at each collection time, and samples of liver, muscle, kidney, perirenal fat, and injection site were collected and assayed for unchanged doramectin, the marker residue.

   c. Results:

      The results from the study are shown in Table 53:

Table 53. Mean doramectin concentration in cattle tissue (Study No. 1535N-60-91-050).

Withdrawal Period (days)	Liver Levels (ppb)	Injection Site (ppb)	Kidney Levels (ppb)	Muscle Levels (ppb)	Fat Levels (ppb)
14	89 ± 26	838 ± 1653	24 ± 4	12 ± 3	182 ± 36
21	39 ± 9	1033 ± 1198	12 ± 2	7 ± 2	97 ± 25
28	13 ± 7	162 ± 317	4 ± 2	3 ± 1	48 ± 28
35	10 ± 5	177 ± 309	3 ± 1	< 2 ± 1	37 ± 19

3. Withdrawal time

   Statistical analyses of the marker residue depletion studies (1531N-60-90-049 and 1531N-60-91-050) using a liver-based tolerance of 300 ppb gave withdrawal times of 20 days (intramuscular dosing) and 22 days (subcutaneous dosing). However, the withdrawal time was extended to 35 days in order to compensate for the occasional high injection site residue

that occurs in cattle. The 35-day withdrawal time corresponds to a tolerance of 100 ppb, which is the value formally assigned as the tolerance for doramectin in cattle liver.

F. Study to evaluate possible doramectin residues in milk from heifers treated prior to calving:

1. TITLE: Doramectin Residues in Milk from Heifers Treated Approximately 75 Days Prior to Calving

   a. PROTOCOL NO.: 1533N-60-94-002

   b. STUDY DESIGN:

      a) Dose: 200 mcg/kg BW as a subcutaneous injection

      b) Test animals: 32 primiparous Holstein heifers (30 treated with doramectin, 2 controls)

      c) Milk sampling times: 72, 84 and 96 hours post-calving

      Thirty (30) primiparous heifers with known artificial insemination dates and confirmed to be normally pregnant were treated with doramectin 75 days prior to their expected calving date.

      Heifers were monitored through the remainder of their pregnancy and calves were removed from their dams shortly after birth. Milk samples were collected at approximately 72, 84 and 96 hours post-partum and were subsequently assayed for doramectin residues using an HPLC method with a limit of quantitation of 0.5 ng/mL.

   c. Results and Conclusions:

      Pregnant heifers treated with 200 mcg/kg doramectin injectable solution between 59 and 90 days prior to calving had no quantifiable levels of doramectin in milk collected 72, 84, or 96 hours after parturition. These data support the label statement that doramectin injectable solution may be administered to heifers up to the age of 20 months, without concern for possible residues in milk being consumed by humans.

G. Regulatory Method:

1. Doramectin determinative assay procedure

   The determinative analytical method is an HPLC procedure that is capable of measuring the marker residue, doramectin, in cattle liver at concentrations ranging from 20 ppb to 400 ppb. The procedure involves the extraction of unchanged doramectin from liver homogenate with acetonitrile. The extracted drug is derivatized using trifluoroacetic anhydride and triethylamine followed by treatment with methanolic ammonia. That yields a chemically stable, fluorescent derivative which is then measured by HPLC with fluorescence detection.

2. Doramectin Confirmatory assay procedure

   The doramectin confirmatory assay is an HPLC-MS/MS method capable of confirming the presence of doramectin in cattle liver. The structural confirmation of doramectin is based on its extraction from liver homogenate and LC/MS/MS analysis.

3. Method validation

   A method trial of the determinative and confirmatory assay procedures has been completed by FDA, USDA, and CFSAN laboratories, and the procedures have been accepted as the regulatory method for detection and confirmation of doramectin residues in cattle liver.

4. DISPLAY OF METHOD

   The regulatory method is on file in Dockets Management Branch (HFA-305), Park Building, 12420 Parklawn Drive, Rockville, Maryland 20855. The method is attached to the FOI Summary.

VIII. 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 of the implementing regulations. The data demonstrate that DECTOMAX® Injectable Solution is effective for the treatment and control of gastrointestinal roundworms, lungworms, eyeworms, grubs, lice, and mange mites in cattle when administered subcutaneously or intramuscularly as a single injection of 200 μg doramectin per kilogram body weight. DECTOMAX® Injectable Solution has been further proven to protect cattle against infection or reinfection with *Ostertagia ostertagi* for up to 21 days when administered subcutaneously or intramuscularly as a single injection of 200 μg doramectin per kilogram body weight.

Based on a battery of toxicology tests, the safe concentrations of doramectin residues are 150 ppb in muscle, 450 ppb in liver, 900 ppb in kidney, and 900 ppb in fat. Based on metabolism studies in cattle, a tolerance (Rm) of 100 ppb for the marker residue, parent doramectin, has been established in liver. The tolerance (Rm) refers to the residue measured by the regulatory method described herein.

A pre-slaughter withdrawal period of 35 days was calculated from two residue depletion studies of doramectin residues in cattle, following the administration of DECTOMAX® Injectable Solution by either subcutaneous or intramuscular administration. Statistical analyses of the marker residue depletion studies using a liver-based tolerance of 300 ppb would give withdrawal times of 20 days (intramuscular dosing) and 22 days (subcutaneous dosing). However a withdrawal time of 35 days is appropriate in cattle to compensate for the occasional high injection site residues.

The data submitted for DECTOMAX® Injectable Solution for cattle support the marketing of the product as an over-the-counter new animal drug. Adequate directions for use have been written for the layman, and the conditions for use prescribed on the labeling are likely to be followed in practice. Therefore, the Center for Veterinary Medicine (CVM) has concluded that this product shall have over-the-counter marketing status.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding contained in an environmental assessment may be seen in the Dockets Management Branch (HFV-305), Park Building (Room 1-23), 12420 Parklawn Dr., Rockville, Maryland 20855.

Under Section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of approval because no active ingredient (including any ester or salt of the drug) has been approved in any other application. DECTOMAX® Injectable Solution is under U.S. patent number 5,089,480, which expires on February 18, 2009.

IX. ATTACHMENTS:

The following labeling is attached.

Approved bottle label for DECTOMAX® Injectable Solution for 100 mL, 250 mL, and 500 mL bottles

Approved insert for DECTOMAX® Injectable Solution

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.