intervet

an Akzo Nobel company

March 22, 2007

Mr. Mark Bradley
Program Manager, USDA/AMS/TMP/NOP
1400 Independence Ave., S.W.
Room 4008-So.
Ag Stop 0268
Washington, D.C. 20250

Dear Mr. Bradley,

Intervet Inc. formally submits this petition to the U.S. Department of Agriculture's National Organic Program to request the amendment of §205.603(a)(12) of the National Organic Standards to include fenbendazole as a synthetic substance allowed for use in organic livestock production.

In accordance with the instructions on the National Organic Program website, we have provided answers to all of the questions below, and in a manner that satisfies the criteria in 7 USC 6517 and 6518, commonly known as the Organic Foods Production Act.

We, of course, are ready to provide any additional information that you, the National Organic Standards Board, or the Technical Advisory Panels may require to complete your review process including copies of any of the literature cited within this submission.

Please contact me at 302.933.4040 if you have any questions.

Sincerely,

Celia B. Shelton, PhD
Manager, Regulatory Affairs – Pharmaceuticals
Intervet Inc.

Enclosure

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PETITION

To the
U.S. Department of Agriculture
National Organic Program

To Amend 7 CFR §205.603(a)(12)
To Include Fenbendazole
As A Synthetic Substance Allowed
For Use in Organic Livestock Production

Submitted March 22, 2007

By
Intervet Inc.
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Millsboro, DE 19966
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1. **The substance's common name.**

   Common Name: Fenbendazole (Safeguard®)
   Chemical Name: methyl N-(5-phenylsulfanyl-3H-benzoimidazol-2-yl)carbamate
   Chemical Formula: $C_{15}H_{13}N_3O_2S$

2. **The official name, address, and telephone number for Intervet:**

   Intervet Inc.
   29160 Intervet Lane
   P. O. Box 318
   Millsboro, DE 19966
   USA
   Telephone: 302.933.4040
   Email: celia.shelton@intervet.com
   Website: www.intervet.com

3. **The intended or current use of the substance.**

   Fenbendazole (Safeguard®) is an anthelmintic, i.e.: a medication capable of causing the evacuation of parasitic intestinal worms. It is being petitioned for inclusion on §205.603(a)(12) of the National List of Synthetic Livestock Materials Allowed.

4. **A list of livestock activities for which the substance will be used. This needs to include the rate and method of application for the material, including the different forms (drench, paste, etc.). Also, describe the mode of action for the material.**

   Fenbendazole is approved for use in cattle, including beef animals and dairy cows, as a treatment and control of several types of gastronomical worms, including: lungworms (ductyocaulus viviparous), stomach worms (brown stomach worm, barberpole worm and small stomach worm), and intestinal worms (hookworm, threadnecked intestinal worm, small intestinal worm, bankrupt worm, and nodular worm).

   The substance is approved for use in the following manners:
   a.) Safeguard/Panacur 10% suspension: 2.3ml of drench per 100 lb. body weight
   b.) Safeguard .5% (top dress pellets): feed one pound of material per 1,000 pounds of body weight
   c.) Safeguard 1.96%: (flaked meal or soft mini pellet) feed .25 pound of material per 1,000 pounds of body weight
   d.) Safeguard paste: 5 g of material per 220 pounds of body weight

   Mode of action: Fenbendazole binds to β-tubulin, inhibiting assembly of microtubules, resulting in cell and parasite death. According to the Merck Veterinary

Manual, "The wide safety margin of benzimidazoles is due to their greater selective affinity for parasitic β-tubulin than for mammalian tissues." (Merck, 2006)

5. **The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product. Petitioners with concerns for confidential business information can follow the guidelines in the Instructions for Submitting Confidential Business Information (CBI) listed in #13.**

   The Manufacturing Process Information and the basic components in the final product are included in **Attachment A**. This is considered Confidential Business Information.

6. **A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.**

   Fenbendazole has not been reviewed by any state, private or international certification program.

7. **Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.**

   Products containing fenbendazole are regulated by the Food and Drug Administration's Center for Veterinary Medicine (CVM). The following products are approved for use in the United States, and their New Animal Drug Application designations (NADA numbers) are provided:
   a.) Safeguard / Panacur 10% suspension: NADA # 128-620
   b.) Safeguard .5% (top dress pellets): NAC No.: 11061772
   d.) Safeguard 1.96%: (flaked meal) NAC No.: 11061781
   e.) Safeguard 1.96%: (soft mini pellet) NAC No.: 11061791
   f.) Safeguard 290 gm paste: NADA # 132-872
   Freedom of Information Summaries are provided in **Attachment B.**

8. **The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance.**

   CAS number: 43210-67-9

   Copies of the approved product labels are included as **Attachment C.**

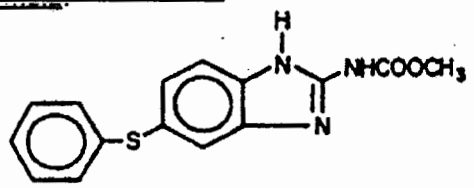
9. **The substance's physical properties and chemical mode of action including (a) chemical interactions with other substances, especially substances used in organic production; (b) toxicity and environmental persistence; (c) environmental impacts from its use or manufacture; (d) effects on human health; and, (e) effects on soil organisms, crops, or livestock.**

Fenbendazole is a white to light brownish-gray, odorless, tasteless crystalline powder which is insoluble in water, but highly soluble in DMSO. Fenbendazole is not a macrolide antibiotic.

Fenbendazole is a member of a well-known and widely used chemical class of compounds, the benzimidazoles, and is related in chemical structure and pharmacological properties to other drugs commercially available in the United States, such as thiabendazole, oxfendazole, oxibendazole, mebendazole and albendazole. Another related compound available on the international market includes febantel. Both thiabendazole and mebendazole are currently approved for use in humans in the United States.

Substance:	Fenbendazole (United States Adopted Name)
CAS registry No.:	43210-67-9
CAS Nomenclature: carbamic acid methyl ester.	[5-(phenylthio)-1H-benzimidazol-2-y1}-
Also:	methyl 5-(phenylthio)-2-benzimidazol-carbamate.

Structural Formula:



Molecular Formula:	$c_{15}H_{13}N_3O_2S$
Description:	White to light brownish or grayish powder, essentially odorless.
Melting Point	Approximately 233° (with decomposition)
Solubility:	Insoluble in water (approx. 10-40 ppb.) Insoluble or only slightly soluble in the usual solvents. Freely soluble in DMSO.
Octanol/Water Partition Coefficient:	Log K_{ow}3.9
U.V. Absorption Spectrum:	Representative spectrum with maximum absorptivity at 296 nm
Mode of Administration:	Oral

Insoluble in Water

Fenbendazole is very insoluble in water. The solubility was determined by passing saturated dilutions through filters with .45 micron pore size. The water solubility was determined to be between 10 and 40 ppb. It is clear from these data that fenbendazole is water-insoluble.

Not Hydrolyzed in Tested Range of Conditions

One study was done to determine if fenbendazole is decomposed depending on various pH values.

Three aqueous reaction mixtures of fenbendazole were stored at 25°C in the dark at pH's of 5, 7 and 9. At specified time intervals, through 28 days, aliquots of the reaction mixtures were extracted with dichloromethane and analyzed by high performance liquid chromatography (HPLC). The levels of fenbendazole found by HPLC were unchanged throughout the time period. At selected intervals, the dichloromethane extract from the sample aliquots were also assayed by thin layer chromatography (TLC) which show one spot attributable to parent fenbendazole upon visualization by ultraviolet light (UV). After 28 days, no significant hydrolysis of fenbendazole was indicated by HPLC or TLC.

Slow Biodegradation

The biodegradation of fenbendazole was determined in an experimental setting. Fenbendazole was incubated with a secondary effluent for 30 days. During the experiment, aliquots were removed for dissolved organic carbon (DOC) analyses at intervals of 1, *2,* 3, 4, 7, 10, 15, 21 and 30 days. In addition, aliquots were removed at *1,* 2 and 30 days of incubation for high performance liquid chromatography (HPLC) analyses of fenbendazole.

The biodegradation of fenbendazole was extremely difficult to follow using DOC determinations because of the insolubility of fenbendazole in aqueous media. During the incubation period, fenbendazole apparently precipitated in the incubation flasks resulting in non-homogeneous mixtures. The DOC determinations from the aliquots fluctuated considerably but suggested a general trend toward biodegradation. Extraction of the total remaining mixtures in the incubation flask after 30 days followed by HPLC analyses indicated that there was no degradation of fenbendazole.

These tests provide a reliable indication of long-term biodegradation, Therefore, it can be concluded from this study that fenbendazole may biodegrade very slowly under the test conditions.

(a) Chemical interactions with other substances, especially substances used in organic production

As stated above, fenbendazole is insoluble in water, and only slightly soluble with the usual solvents. The substance binds with soil, and thus does not impact other substances used in organic production.

Specific studies have been conducted on fenbendazole concerning impact on earthworms (both Eisenia foetida and Lumbricus terrestris). The studies (detailed in section 9(e)(iv)) demonstrated the absence of an acute lethal effect of fenbendazole on Eisenia foetida at concentrations below 100 ppm. On a separate study on Lumbricus terrestris, the LC_{50} for earthworms exposed to fenbendazole for 28 days was calculated by moving average angle analysis to be 180 ppm fenbendazole. The concentration of fenbendazole in soil with waste from treated animals would be significantly lower (390 ppb).

Dung beetles *(Onthophagus gazelle)* are considered an important tool in organic livestock production and pasture management. A toxicity investigation on exposure of dung beetles to fenbendazole was conducted by Springborn Laboratories, Inc. That investigation (explained in greater detail in Section 9(e)(iv)) determined no detectible impact on dung beetles.

(b) Toxicity and environmental persistence

1) Toxicity

The toxicology data on fenbendazole submitted with the original application to NADA 128-620 (48 FR 42809; Sept. 20, 1983) allowed the establishment of a safe concentration of 1.67 ppm for total residues of fenbendazole in milk. From the residue and metabolite data submitted with a later supplemental application, a tolerance of 0.6 ppm was established as the tolerance for residues in milk of the fenbendazole metabolite fenbendazole sulfoxide (the marker residue). Because the maximum levels of residues found in milk of fenbendazole-treated cattle are well below the safe concentration and tolerance noted above, no discard of milk (zero milk withdrawal) is required. The preslaughter withdrawal time of 13 days established for treated dairy cattle is the same as that established for cattle under the original NADA 137-600.

This product is not considered a carcinogen and is not listed by OSHA, IRAC or NTP.

Acute toxicity studies were conducted for evaluation by the Joint FAO/WHO Expert Committee on Food Additives. Doses of fenbendazole were administered to mice, rats, rabbits, dogs, swine and sheep. (Scholz & Schultes, 1973)

Toxicity studies were reviewed also by the European Medicines Agency. Fenbendazole was shown to be of low acute toxicity. Oral LD50 values in laboratory rats and mice were greater than 10000 mg/kg.

2) Environmental Persistence

Rapid Photolytic Decomposition

A study designed to conform to Method 3.10 of the FDA Environmental Assessment Technical Assistance Document was conducted by Springborn Laboratories, Inc. to measure the photo-degradation of fenbendazole in aqueous solution.

Photolytic decomposition is a known degradative pathway for benzimidazoles. The effect of simulated sunlight on the photolytic degradation of aqueous solutions of fenbendazole was tested at pH 5, 7 and 9. Actinometer (reference material) solutions of paranitroacetophenone (PNAP) were analyzed concurrently with the pH 5, 7 and 9 test solutions.

Sampling and analysis for [14 C] fenbendazole consisted of an extraction method where 4- 5 separate tubes for the light-exposed and dark control solutions were separately combined, each containing approximately 12-mL. to provide triplicate replicates for solid phase extraction (SPE). Eluent from the solid phase columns were analyzed utilizing high performance liquid chromatography (HPLC) with fraction collection and subsequent radioassay. Radiochromatograms (histograms) were conducted to quantify the concentration of fenbendazole present and to determine its degradation rate. Samples for PNAP were analyzed by high performance liquid chromatographic analysis with UV detection.

Since degradation was so rapid, and insufficient quantities of photolyzed samples existed for identification of degradates. Additional exposures at pH 5, 7 and 9 were conducted upon completion of the definitive portion of the study, with a large number of replicates, to provide enough volume for photodegradate identification. The combined volume of these replicates was extracted using a solid phase system and a photodegradate profile determined based on chromatographic comparison of retention times with supplied standards. None of the degradation products comprised more than 10% of the original concentration of fenbendazole, indicating that photolysis was severely destructive to the molecule.

The half-life ($T^1/_2$, days) of fenbendazole at pH 5, 7 and 9 are presented below.

pH	$T^1/_2$ days
5	0.713
7	0.527
9	0.471

This study conclusively demonstrates a rapid degradation process for fenbendazole exists (less than one day) with photolysis proceeding to many insignificant degradate compounds in which none comprise more than 10% of the original concentration.

(c) Environmental impacts from its manufacture or use:

1) Manufacture:

Fenbendazole is manufactured in bulk at the plant of Hoechst AG in Frankfurt, Germany. Fenbendazole bulk drug substance is shipped to the United States to the Somerville, NJ plant of Hoechst-Roussel Pharmaceuticals, Inc. for manufacturing and packaging of a 10% suspension in the New Jersey facilities.

Fenbendazole bulk drug is formulated into an aqueous suspension using common inert pharmaceutical grade excipients which are recognized in the U.S.P. or N.F. Energy requirements for manufacturing are similar to those which would be used in any conventional pharmaceutical operation involved in the production and packaging of liquid products. No irreversible or irretrievable commitment of resources is involved.

The manufacturing process of fenbendazole suspension consists of carefully controlled weighting, mixing, and filling operations conducted in a pharmaceutical manufacturing plant. These processes are controlled to arrive at a full material balance, and no effluents or pollutants are formed. This action does not require any significant use of the environment. There has been no experience of short-term or long-term effects. Therefore, there is no effect upon the depletion of natural resources due to manufacture of the drug.

2) Use

For practical purposes, fenbendazole is only introduced into the environment when it is excreted by treated animals. Because the primary route of introduction of fenbendazole into the environment is through excretion by the target animal, the firm conducted several studies of the fate of this drug in the environment (All studies are part of the original application NADA 128-620 (48 FR 42809, September 20, 1983)

Studies in which radiolabeled fenbendazole was given to cattle at a dose 5 mg fenbendazole/kg body weight found that 48% of the parent compound was excreted in feces and 0.5% was excreted in urine [See table on Page 14]

Orally administered fenbendazole is excreted as intact parent compounds and several metabolites. The principal metabolites are p-hydroxy-FBZ and an amine metabolite.
Studies have been conducted to measure the impact of fenbendazole on soil and water resources.

Target animals excrete quantities of the drug as parent compound and metabolites. The excretion of fenbendazole plus metabolites was measured in studies with cattle treated with radiolabeled fenbendazole. The studies showed that practically the entire dose as measured by radioactivity, is excreted within a few days.

In one study, researchers assumed that 100% of the administered dose is excreted within seven days. They also assumed that a 1,500 lb. diary cow would be treated at a dose level of 5 mg. fenbendazole/kg body weight resulting in a total dose of 3,400 mg (3.4 g.) per animal given three times each year. This is the maximum introduction scenario based on labeled recommendations.

A 1,500 lb. dairy cows voids as manure 8% of her body weight each day (Principles of Dairy Science, G.H. Schmidt, L.D. Ban Vieck, M.F. Hutjans, page 430 (1988)). This equals 120 lbs., or 54.4 kg. manure per day. Because the total fenbendazole dose is voided over seven days, each 380/.8 kg. (54.4 kg x 7 days) of waste will contain 3.4 g fenbendazole which will equal 8.9 ppm. Assume a maximum of 40 metric tons of cattle excreta is present on one acre of agricultural land.

Impact on Water Resources

Studies demonstrate that fenbendazole has no impact on runoff or leachate water.

No Migration to Runoff or Leachate Water

In one study researchers assumed there will be two inches of rainfall over an acre of land during the year. Two inches of rainfall on an acre of land weighs approximately 205,500 kilograms. The study assumed 10 animals per acre per year. Therefore, the amount of fenbendazole on one acre would equal:

10 dairy cows x 3.4 g/cow x 3 treatments/year = 102 g fenbendazole per acre per year.

Fenbendazole is not soluble in water. If it is possible to have the entire residue in the run-off, the maximum concentration of fenbendazole in the run-off, assuming no degradation, equals:

$$\frac{102 \text{ grams}}{205{,}500 \text{ kg of water}} = .496 \text{ mg/kg (496 ppb) FBZ in runoff}$$

It would be expected that the amount of fenbendazole released into water runoff would be very much lower than 496 ppb because fenbendazole is very insoluble in water and absorbs tightly to soil particles. Therefore, fenbendazole is not expected to migrate from application sites into runoff or leachate water, and hence, is not expected to be available to aquatic species. Exposure would be limited by adsorption and available pathways for rapid degradation (e.g. photolysis).

No Runoff from Fecal Matter

Separate studies have shown that the same metabolites are found in the feces of swine and cattle treated with fenbendazole. Feces from pigs treated with 14C fenbendazole were mixed with soil to a final concentration equivalent to 11 .07 micrograms of 14C fenbendazole/g of soil. The soil feces mixture was incubated with a 10 fold excess of distilled water for 72 hours with constant shaking to achieve an equilibrium distribution of fenbendazole ÷ metabolites between the soil and the aqueous phase. The final concentration of 14C fenbendazole in the aqueous phase was .045 micrograms/mL which represented 3.19% of the initial 14C activity.

The result of this study shows that fenbendazole metabolites just as fenbendazole parent substance is bound tightly to particulate matter and do not migrate into surface waters. (Bio/dynamics, Bound Brook, NJ.)

No significant Impact on Aquatic Environment

Under "worst case" conditions (assuming that all fenbendazole administered to dairy cattle is excreted via their manure, is extracted from the manure by two inch rainfall and enters into water run-off), the estimated water run-off concentration of fenbendazole is 496 ppb. This would be the highest concentration of fenbendazole in any aquatic environment since it assumes three treatment periods per year which are not consecutive, does not account for dilution as it enters bodies of water such as streams, rivers, ponds and lakes (secondary aquatic environments), does not account for the fact that fenbendazole and fenbendazole metabolites are bound tightly to the soil and do not migrate into surface waters, and that upon entry into these secondary aquatic environments, fenbendazole and fenbendazole metabolites rapidly decompose through the process of photo-degradation. The half-life in water is less than one day. Dilution and photochemical decomposition in the secondary aquatic environments reduces the environmental concentrations of fenbendazole and its metabolites such that the

effects from fenbendazole on vertebrate and invertebrate populations are expected to be transient and would not be considered to be significant.

Impact on Soil Resources

Lack of Release into Soil

In one study, researchers assumed that:

a. No degradation in the manure before applying to the soil.
b. Manure is added to the soil at the rate of 40.0 metric tons per acre. Amount of fenbendazole in 40 metric tons equals 0.356 kg.
c. (3.4 g fenbendazole/380.8 kg manure per week) X 40,000 kg per acre = 0.356 kg
d. Fenbendazole in 40 metric tons manure or 8.9 mg/kg (ppm) manure.
e. The manure will be incorporated into the top 6" of soil (weight of the top 6- of soil in one acre equals 909,000 kg).

The amount of fenbendazole in the top six (6) inches of soil would equal:

$$\text{Drug Conc. In soil (mg/kg)} = \text{Drug conc. in manure (mg/kg)} \times \frac{\text{Kg manure applied to soil}}{\text{acre of soil}} \times \text{acre of soil kgs in top 6" of soil}$$

$$\text{Drug Conc. In soil} = 8.9 \text{ mg/kg} \times \frac{40{,}000 \text{ kg manure}}{1 \text{ acre}} \times \text{acre} = 0.39 \text{ mg/kg (390 ppb) FBZ in soil}$$

9.09×10^5 kg

As demonstrated above, the amount of fenbendazole (assuming no degradation) released into the soil would be extremely minimal.

Adsorption of Fenbendazole to Particulate Matter

An absorption study was done to determine how tightly fenbendazole is bound to particulate matter in the soil. Radiolabeled fenbendazole was used and 3 soil and 1 sediment were fortified with the radiolabeled drug at 5 different concentration levels. After continuously shaking the soil/water mixture for 48 hours, the level of radioactivity was determined in water, dichloromethane, soil extracts and extracted soil.

The adsorption isotherms of fenbendazole were determined to be log 3 for a sample of New Jersey soil, New Jersey sediment and Texas soil. The adsorption isotherms for a Louisiana soil was determined to be log 2.8. A clear correlation was found between the adsorption isotherm values and the soil variables or organic matter, sand and silt content.

Overall, fenbendazole was adsorbed very tightly to the soil samples. The study demonstrated again that fenbendazole was bound tightly to all soils examined.

(d) Effects on human health

Human Health Studies

The acute oral toxicity of fenbendazole was evaluated in laboratory and target animals. Standard protocols were used for studies in mice and rats. Large animals (horses, cattle, sheep) were also treated with relatively high doses of fenbendazole. Fewer large animals were exposed to the various dose levels since the individual animals were studied more thoroughly. In those studies no toxicity was found after the highest administered dose, with the exception of the study in rabbits, which was conducted as a pilot study. One out of 3 animals died after 3,200 mg/kg and 2 out of 3 after 5,000 mg/kg.

The results of single dose, oral acute toxicity studies are summarized in the following table:

ACUTE ORAL TOXICITY OF FENBENDAZOLE
SINGLE DOSE MG/KG B. W.

		Toxic Doses Greater Than
Mice		10,000 mg/kg*
Rats		10,000 mg/kg*
Dogs		500 mg/kg
Sheep		5,000 mg/kg
Horses		1 ,000 mg/kg
Cattle		2,000 mg/kg
Rabbits	LD_{50}	3,200 mg/kg

**These doses were the highest that could be administered technically because of the large volume.*

Fenbendazole was also studied for its effect on reproducing animals. Studies were done in rats, rabbits, horses, cattle and swine. No adverse effects were found. Details are described in the Freedom of Information summary which is part of the NADA (48 FR 42809, September 20, 1983). Chronic toxicity studies (up to 90 days) have been performed with dogs and rats. The levels fed in the studies were much higher than levels expected to occur in the environment. The data are summarized below:

Chronic (90 day) Studies with Laboratory Animals

The 90-day studies in rats (up to 2,500 mg/kg) and dogs (up to 125 mg/kg) did not reveal any clinical signs of toxicity in any of the animals. No drug related postmortem lesions were found.

In addition, 6 month oral toxicity studies in dogs, a 3 generation reproduction study in rats, a lifetime oral toxicity study in rats in which offspring from the 3 generation study were used, and a lifetime mouse study were conducted to determine if fenbendazole is a carcinogen. No oncogenic properties of the drug were found. Based on these studies, a finite tolerance of 12 ppm fenbendazole residues in cattle liver was established.

In a separate study, there was no evidence of carcinogenicity in a study in which groups of 60 per sex per dose Charles River CD-1 mice were given fenbendazole in the diet at concentrations designed to produce 0, 45, 135 or 405 mg/kg bw per day for up to 2 years. Survival was reduced in treated groups compared to controls. In Charles River CD rats, the animals were exposed to dietary doses of fenbendazole of 0, 5, 15, 45 or 135 mg/kg, including an initial *in utero* phase where the dams received the same dosages. Effects on survival were seen at the high dose and bodyweight gain was affected at 45 and 135 mg/kg. Alkaline phosphatase was consistently elevated at 15 to 135 mg/kg and serum glutamic-oxalacetic transaminase (SGOT) at 135 mg/kg only. Histological changes were seen primarily in the liver including hepatocellular hypertrophy, hyperplasia and vacuolation, bile duct proliferation and biliary cyst formation. The overall NOEL was 5 mg/kg.

Does Not Contribute to Antibiotic Resistance

Because fenbendazole is not a macrolide antibiotic, there is no risk of passing on resistant food borne pathogens to humans.

Antibiotic residue test screening was conducted on milk samples from three treated cows chosen randomly. The samples were collected at 12-hour intervals for 72 hours post-dose. Tests performed included the Charm II assay, Delvotest P, and BSDA. Zero-time samples were included in all antibiotic screening tests; Delvotest P and BSDA also included milk collected from the control animal at 12-hour intervals for 72 hours post-dose. Examinations indicated that the incurred residues from treated cows had no discernible or consistent effect on the assays. No sample from any cow examined gave a positive response to the Delvotest P and BSDA. Assay results of ten antibiotic classes indicated that fenbendazole and its metabolites do not interfere or cross-react with any consistency in the Charm II assay.

It was concluded that the fenbendazole sulfoxide marker residue level was below the tolerance level; therefore, total residues were below the established safe

concentration for milk. A zero-day withdrawal period was approved for use of fenbendazole oral suspension at 5 mg/kg bodyweight in dairy cattle of breeding age. It was further concluded that use of fenbendazole does not interfere with routine antibiotic drug screening.

(e) Effects on soil organisms, crops or livestock

1) Effects on Livestock

The effect of fenbendazole has been extensively reviewed by the U.S. Food and Drug Administration (FDA), and by the European Medicines Agency (EMA)

Cattle

An orally administered single dose of fenbendazole is excreted as intact parent compound and several metabolites:

TABLE

	Feces	Urine
Parent Compound	48%	0.5%
SO'-Metabolite	8%	--
2-amino-5-Metabolite	--	3%
p-OH-Metabolite	--	6.5%
Not identified	3 metabolites= 17%	2 metabolites = 3%
Total	73%	13%

This is a result of studies in which radiolabeled fenbendazole was given to cattle at a dose 5 mg fenbendazole/kg body weight.

A finite tolerance of 10 ppm in cattle liver was established based on extensive safety studies. Residue levels in the liver fall below the tolerance level before the 7th day after treatment.

Sheep

In studies provided for sheep, 5 days after oral treatment with fenbendazole (10 mg/kg bw), tissue concentrations of oxidised fenbendazole residues were: 33.5, 79.0, 29.3 and 3658 5 ug/kg respectively for fat, kidney, muscle and liver. Nine days after treatment, these concentrations had depleted to, less than 5, less than 5.7, 6.2 and 744.5 ug/kg respectively for kidney, fat, muscle and liver. These residue concentrations detected in sheep tissues were consistent with a similar radiometric study.

Swine

In a study in pigs, 5 days after oral treatment with fenbendazole (5 mg/kg bw), fenbendazole residue concentrations were below the analytical limit of quantification (less than 5 µg/kg) in all edible tissues. Tissue concentrations of fenbendazole residues at earlier time points were not reported. In an old radiometric (5 mg 14C-fenbendazole/kg bw) study in the pig previously reviewed by the CVMP, residue concentrations in the liver were: 260, 70 and less than 20 µg/kg respectively and in kidney 50, 30 and 10 µg/kg respectively on days 5, 14 and 21 after treatment.

Muscle tissues contained residue concentrations below the analytical limit of quantification (less than 10 µg/kg) at all time points (concentrations in fat were not reported). Based on the data in these two studies it can be estimated that the routine analytical method was only able to measure a small fraction of the tissue residue content of pig tissues 5 days after treatment (liver: less than 5µg/kg by HPLC or 260 µg/kg radiometric).

2) Effects on Crops

Seed Germination and Root Elongation

A study was undertaken to define the effect of fenbendazole on corn (*Zea mays),* cucumber *(Cucumis sativus),* perennial ryegrass *(Latium perenne),* soybean *(Glycine max),* tomato *(Lycopersicon esculentum)* and wheat *(Triticum aestivum)* germination and root elongation. This study was conducted by Springborn Laboratories, Inc. in accordance with FDA Environmental Assessment Technical Assistance Document 4.06.

Seeds of corn, cucumber and perennial ryegrass were exposed to fenbendazole suspensions of 970, 480, 240, 110, 61 and 0 ppm while wheat seeds were exposed to fenbendazole suspensions of 1000, 530, 310, 150, 61 and 0 ppm. Soybean and tomato seeds were exposed to fenbendazole suspensions of 1000, 530, 310, 150, 61, 36, 3.6. 0.36 and 0 ppm. Each treatment group consisted of six replicates of 50 seeds each. All tests were conducted in the absence of light. The test was initiated by adding 50 seeds to each appropriately labeled petri dishes containing treated or control filter paper and 15 ml ASTM Type 2 water.

At test termination, percent germination and root length data for the treatments were statistically compared on a per replicate basis to the solvent control data. No morphological abnormalities were observed in any seeds at test termination. A No-Observed-Effect Concentration (NOEC) was defined as the highest treatment level where there was no statistically toxicant-related reduction in percent germination and root length when compared to the solvent control. The Lowest-Observed-Effect Concentration (LOEC), defined

as the lowest concentrations demonstrating a statistically significant effect, was determined for each species. Results are as follows:

	Germination		Root Elongation	
Species	NOEC (mg/L)	LOEC (mg/L)	NOEC (mg/L)	LOEC (mg/L)
Corn	970	>970	970	>970
Cucumber	970	>970	970	>970
Ryegrass	970	>970	970	>970
Soybean	1000	>1000	1000	>1000
Tomato	1000	>1000	1000	>1000
Wheat	1000	>1000	1000	>1000

Seedling Growth

The effect of fenbendazole on seedling growth was determined in a study in which six species of angiosperms were selected. They included three monocotyledons. Corn *(Zea mays),* wheat *(Triticum aestivum),* and perennial ryegrass *(Lolium perenne),* and three dicotyledons, soybean *(Glycine max),* tomato *(Lycopersicon esculentum)* and cucumber *(Cucumis sativus).* This study was conducted by Springborn Laboratories, Inc. in accordance with FDA Environmental Assessment Technical Assistance Document 4.07.

A range of six concentrations were chosen for the definitive tests which were expected to yield NOEC and LOEC values for each species. The measured treatment levels were 1600, 810, 360, 150, 64, 36 and 0 (control) mg fenbendazole/kg support medium. At test initiation, appropriately labeled replicate pots, each containing 1.5 kg of treated or control silica sand, were surface watered with 250 ml of nutrient solution. Germinated seedlings of uniform root and shoot development were selected by random assignment for planting in the treated or control support medium (silica sand). For each species, five seedlings were planted in each of five replicate pots per concentration and controls. Artificial lighting of 1000 to 1200 foot-candles was provided on a day/night schedules (16 hours light/8 hours dark) to allow for proper shoot orientation and the initiation of photosynthesis. During the test, all pots were subirrigated daily, and in addition the 360, 810 and 1600 mg/kg pots were watered on the surface on days 0, 1, 2 and 4 for corn, cucumber and perennial ryegrass and on days 0.1 and 3 for soybean, tomato and wheat due to the hydrophobic nature of the test article on the sand.

Seedling shoot lengths were measured on days 1, 3, 5, 7, 14 and 21 to establish growth rate curves. Plant survival, dry shoot weight and dry root weight were measured at the conclusion of the 21-day test period. The results are as follows:

Species	NOEC[a]. (mg/kg)	LOEC[a] (mg/kg)
Corn[b]	1600	>1600
Cucumber[b]	1600	>1600
Ryegrass[b]	1600	>1600
Soybean[b]	1600	>1600
Tomato[c]	36	64
Wheat[b]	1600	>1600

[a] NOEC and LOEC based on the most sensitive parameter measured (percent survival, shoot length, shoot and root weight).
[b] No effect was observed for percent survival, shoot length, shoot dry weight and root dry weight at the highest measured concentration tested.
[c] NOEC and LOEC based on root weight, the most sensitive parameter for tomato.

Studies in Plants

Another study was conducted to determine if fenbendazole is accumulated in plants. Feces from a cow which had been treated with 14C fenbendazole at a dose level of 5 mg fenbendazole/kg body weight were used to determine if fenbendazole or its metabolites are taken up by plants.

Barley and bean plants were raised under laboratory conditions on sandy loam soil to which 3.5% of a mixture of urine and feces had been added. The plants and new crop, tested for their radioactive content at various times after sowing 6 days, 14 days. 11 weeks - showed concentrations varying between the level of detection and twice the level of detection of ppb. The comparative value for the soil was 490 ppb

3) Effects on Micro-Organisms

No Impact on Micro-Organisms (Including Soil Organisms)

A number of micro-organisms were exposed to fenbendazole and no activity of fenbendazole was found. The micro-organisms included:

Gram positive aerobic bacteria:
Staphylococcus aureus S.G. 511
Streptococcus pyogenes A (308)
Streptococcus faecium D

Gram negative bacteria:
Escherichia coli 055
Proteus mirabilis
Pseudomonas aeruginosa

Mycoplasma:
Mycoplasma gallisepticum 15302

The test method was a bacteriostatic (growth inhibition) test. Serial dilutions in Mueller-Hinton-Broth were used. The inoculum per ml medium was .05 ml of a 24 hour stationary fluid culture of the respective organism diluted 1:100. The minimum inhibitory concentration (MIC) was determined after an incubation of 18 hours at 37°C. MIC was the concentration of the last test tube in which no macroscopically visual bacterial growth was observed. The highest tested concentration of fenbendazole was 100 micrograms/mL. No antibacterial effect could be found against any of the tested aerobic bacteria.

In addition to these aerobic bacteria, anaerobic bacteria were also tested as follows:

Several strains of *Bacteroides fragilis*
Bacteroides ovatus
Bacteroides thetajotaomicron
Sphaerophorus varius
Sphaerophorus freundii
Peptococcus anaeroblus and *variabilis*
Peptostreptococcus anaerobius and *variabilis*
Propionibacterium acnes as well as several clostridia strains including
Clostridium erfringens
Clostridium septicum.

The highest tested concentration of fenbendazole was 100 micrograms/mL agar. No antibacterial effect could be found against any of the tested anaerobic bacteria.

Fenbendazole was further evaluated for in-vitro activity against *Trichomonas vaginalis* and *Entamoeba histolytica.* The study was done as an In-vitro model for activity against *Histomonas meleagridis.* No in-vitro effect was seen at concentrations of up to 200 micrograms/mL in-vitro.

Fenbendazole was tested against these protozoa in in-vivo experiments:
Eimeria tenella
Entamoeba histolytica
Trichomonas foetus
Aegyptianella pullorum
Trypanosoma brucei
Plasmodium vinckei
Babesia rodhaini

No activity was found in any of the experiments.
An antifungal test was also performed against:

Trichophyton mentagrophytes
Trichophyton rubrum
Microsporum canis
Candida albicans
Aspergillus niger

Two test media were used: malt extract peptone glucose agar and serum glucose agar. The concentration of fenbendazole was up to 100 micrograms/ml. No inhibition of fungi was observed in this study.

We conclude from the available information that fenbendazole would not have any effect on soil microbes because no growth inhibition could be demonstrated at the 100 and 200 ppm concentrations which are greater than the maximum solubility of the compound (10-40 ppb).

Dung Beetle Toxicity (*Onthophagus gazelle)*

An investigation was conducted by Springborn Laboratories, Inc. to determine the NOEC and LD_{50} of fenbendazole to dung beetles. The 7-day toxicity test with dung beetles *(Onthophagus gazelle)* included a single measured fenbendazole concentration of 770 mg/kg and a control. Five replicate vessels were maintained for the treatment and control. Treated cattle manure (1000 mg/kg, nominal) was divided into five 300 g aliquots formed into oval shaped patties and placed in the plastic pail vessels, each containing 2.4 kg of moistened artificial soil. Five replicates of 300 g aliquots of untreated cattle manure (control) were also maintained. Test vessels were randomly positioned in a temperature controlled water bath designed to maintain temperature at 28 ± 2° C. Relative humidity was maintained at 58 to 66%. Light intensity was 60 foot candles with a photoperiod of 18 hours light and 8 hours darkness. Each vessel was misted with deionized water once daily. Two male-female pair of dung beetles were placed in each replicate vessel. Survival rate, physical or behavioral abnormalities (e.g. lethargy) and presence of dung balls were recorded at test termination (day 7).

At test initiation (day 0) and test termination manure samples for the treatment level and the control were analyzed for fenbendazole concentration. The mean of the day 0 and the normalized day 7 concentrations defined the measured treatment level to be 770 mg/kg.

Mean survival among dung beetles exposed to the treatment level of fenbendazole tested (770 mg/kg, measured) was 100%. Based on the absence of mortality and sublethal-effects during the study, the 7-day LO_{50} was empirically estimated to be greater than 770 mg/kg. The No-Observed-Effect Level was determined to be 770 mg/kg. The concentration of fenbendazole in waste manure from treated animals would be significantly lower (8.9 ppm) than the NOEC of 770 ppm.

Earthworm Toxicity *(Eisenia foetida & Lumbricus terrestris)*

Eisenia foetida

A preliminary range-finding test using earthworms *(Eisenia foetida)* tested the toxicity of fenbendazole doses of 1,000, 500 and 100 mg drug/kg soil. Worm mortality was not observed until 14 days and then only in the 1,000 and 500 mg/kg groups. The 14 day LC_{50} was calculated to be 1,068 mg/kg with the 95% confidence interval being from about 900-1600 mg/kg. The worms at 100 mg/kg suffered no mortalities.

The study demonstrated the absence of an acute lethal effect of fenbendazole on earthworms at concentrations below 100 ppm. It did not determine the minimum effect level for sublethal effects since doses lower than 100 mg/kg were not tested.

Lumbricus terrestris

The subacute toxicity of fenbendazole on earthworms *(Lumbricus terrestris)* was evaluated in a study conducted by Springborn Laboratories, Inc. in accordance with "FDA Environmental Assessment Technical Document 4.12.

A preliminary range-finding test, consisting of two replicate test vessels per concentration and control, using earthworms *(Lumbricus terrestris)* tested the toxicity of fenbendazole doses of 1,000, 100. 10, 1.0. 0.10 and 0 (control) mg drug/kg artificial soil (dry weight basis). Percent survival was 95% or greater at all levels tested except 1000 mg/kg where 5% survival rate was observed. Definitive test concentrations were then established to be 960,500, 240, 120, 56 and 0 (control) mg fenbendazole/kg artificial soil (dry weight basis). For each exposure concentration and control, four replicate test vessels were utilized during the definitive test. When compared with burrowing time and percent weight change, statistical analysis of the data determined that earthworm survival was the most sensitive parameter to the toxicity of fenbendazole. At test termination survival in 960, 500, 240, 120, 56 and 0 (control) mg fenbendazole/kg artificial soil was 0, 25, 35, 53, 93, and 100%, respectively. Therefore, earthworm survival was used to establish the LC_{50}, LOEC and NOEC.

The LC_{50} for earthworms exposed to fenbendazole for 28 days was calculated by moving average angle analysis to be 180 ppm fenbendazole. The Lowest-Observed-Effect Concentration (LOEC) was determined to be 120 ppm fenbendazole, and the No-Observed-Effect Concentration (NOEC) was determined to be 56 ppm fenbendazole in artificial soil containing 50 g cattle manure per kg dry artificial soil. The concentration of fenbendazole in soil

with waste from treated animals would be significantly lower (390. ppb) than the NOEC of 56,000 ppb.

4) Summary of Terrestrial Effects of Fenbendazole

Terrestrial Environment

Under "worst case" conditions (assuming that all fenbendazole administered to dairy cattle is excreted via their manure, accumulates over a year and is mixed into the top six inches of soil at the rate of 40 metric tons per acre of land) the total initial concentration of fenbendazole is calculated to be 390 ppb. The comparison of the calculated environmental concentrations of fenbendazole in the terrestrial environment in conjunction with the effects levels below is not expected to have a significant impact on the environment.

Terrestrial Effect Levels

Microorganisms> >	NOEC	>	100,000 ppb
Seedling Growth (tomato most sensitive) > >	NOEC	=	36,000 ppb
	LOEC	=	64,000 ppb
Seed Germination/Root Elongation> >	NOEC	≥	970,000 ppb
Earthworm Toxicity> >	NOEC (28 d.)	=	56,000 ppb
	LOEC (28 d.)	=	120,000 ppb
	LC_{50} (28 d.)	=	180,000 ppb
Dung Beetle Toxicity> >	NOEC (7 d.)	=	770,000 ppb
	LD_{50} (7 d.)	>	770,000 ppb

Environmental risks can be estimated from the relationship between concentrations expected in the environment and the highest concentrations of fenbendazole at or below which no toxicological effects have been observed in laboratory studies. Quotients (Q) representing the relationship between the CEC or calculated environmental concentration and the NOEC or no-observed-effect concentration are presented-below where Q = CEC/NOEC. The Q values below illustrate a considerable margin of safety across a range of microbial, insect, invertebrate and plant species of importance to the terrestrial compartment of the environment. Typically, where Q <0.10, a 10 fold margin of safety, minimal risk to the environment is expected (USEPA 1994)3. Based on margins of safety ranging between about 100 and 2500 fold, the introduction of fenbendazole is not expected to impact the terrestrial environment.

	NOEC (ppb)	CEC (ppb)	Q
Microorganisms	100,000	390	0.004
Earthworm	56,000	390	0.007
Seed Germination	970,000	390	0.0004
Seedling Growth[1]	36,000	390	0.011
Dung Beetle	770,000	390	0.0005

[1] Based on most sensitive species - tomato.

Fenbendazole used at the proposed levels will not significantly adversely affect micro-organisms, soil biota, plants, fish or mammals exposed to environmental concentrations of the drug that can reasonably be expected to occur. Studies are included as part of original application NADA 128-620 (48 FR 42809, September 20, 1983).

10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies.

The Material Safety Data Sheet for fenbendazole is included as **Attachment D** with this petition. The Food and Drug Administration (FDA) does not require a National Institute of Environmental Health Studies report for fenbendazole. Therefore a NIEHS report has not been developed. The information contained in this petition under Section 9 covers the safety of human health and environment.

11. Research information about the substance which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List.

A listing of relevant research information and literature concerning fenbendazole is included as **Attachment E** with this petition.

12. A "Petition Justification Statement" which provides justification for inclusion of the substance on the National List. The petition should state why the synthetic substance is necessary for the production of an organic product. The petition should also describe the nonsynthetic substances or alternative cultural methods that could be used in place of the petitioned synthetic substance. Additionally, the petition should summarize the beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support the use of it instead of the use of a nonsynthetic substance or alternative cultural methods.

Parasite control today stands as perhaps the major factor limiting the development of certified organic livestock production.

The Organic Foods Production Act (OFPA) and the National Organic Standards are very clear in regard to limiting the use of synthetic parasiticides in organic livestock. §2110(d)(1)(B) of OFPA specifically prohibits the use of synthetic internal parasiticides on a routine basis.

§205.238 of NOS specifies parasiticides allowed under § 205.603 may be used on
(1) Breeder stock, when used prior to the last third of gestation but not during lactation for progeny that are to be sold, labeled, or represented as organically produced; and
(2) Dairy stock, when used a minimum of 90 days prior to the production of milk or milk products that are to be sold, labeled, or represented as organic.

§205.603 of the National List adopted as a part of the National Organic Program Final Rule on October 22, 2002 includes Ivermectin as the only parasiticide allowed for use in organic livestock production (with restrictions). However, many organic livestock producers have actively sought a viable alternative to Ivermectin for at least three reasons:

1. Ivermectin is a macrolide antibiotic.

   The use of macrolide antibiotics are inconsistent with organic management practices. In fact, the Secretary of Agriculture last year refused to accept the National Organic Standards Board's recommendation to add moxidectin to the National List of Substances Allowed in Organic Production and Handling. In the July 17, 2006 Federal Register, the USDA states, "Although moxidectin is approved for use in beef and dairy cattle by the FDA, the Secretary cannot accept the NOSB's recommendation to add moxidectin to the National List because it is a macrolide antibiotic." (Federal Register, Vol. 71, No. 136, Page 40630).

2. Ivermectin is toxic to dung beetles.

   Dung beetles play a crucial role in the recycling of nutrients in pastures, and in controlling horn flies and face flies. A single manure pat can generate 60-80 horn fly adults if protected from insect predators and competitors such as dung beetles. As dung beetles feed, they compete with the fly larvae for food and physically damage the flies' eggs. Fly populations have been shown to decrease significantly in areas with dung beetle activity. Dr. George Bornemissza found that 95% fewer horn flies emerged from cowpats attacked by *Onthophagus gazella*, than from pats where beetles were excluded. (Knutson, Allen. 2000) On an Oklahoma ranch it was estimated dung beetles following the herd buried a ton of wet manure per acre per day and removed 90% of the surface material.

   Dung beetles also play a biological role as a control agent for gastronomical parasites. The eggs of most gastrointestinal parasites pass out in the feces of the host. The eggs then hatch into free-living larvae and develop into the infective stage. They then migrate onto grass, where they can be ingested by grazing

animals, and complete their life cycle within the animal. If the manure/egg incubator is removed by beetles, the eggs perish and the life cycle of the parasite is broken.

On a pasture-management level, dung pat removal is beneficial for forage availability. Most ruminants will not graze closely to their own species' manure pats. Research has shown that the forage is palatable, but avoided because of the dung pile. Consequently, cattle manure deposits can make from 5% to 10% per acre per year unavailable. By completely and quickly removing the manure, dung beetles can significantly enhance grazing efficiency.

Dung beetles also enrich the soil by burying large quantities of nutrient-rich dung, and effectively mix and aerate soil through tunneling. One study documented that five adult pairs of dung beetles were capable of burying 37% of a dung pat. This calculated to a return of about 134 kg of N per hectare. In areas where larger and more fecund or vigorous beetles are present, beetles may bury 80-95% of the nitrogen in dung (Gillard 1967). Large amounts of nitrogen returned to soil could prove to be an important factor in the growth of plants (Bertone, 2004). The tunneling of the dung beetles also increases the ability of soil to absorb and retain water (Shelton, 2001).

Fenbendazole does not impact dung beetles because benzamidazoles bind to tubulin, which is specific for nematodes. Avermectins, however, have a broad range of activity in nematodes and arthropods. Ivermectin injectable, used at the recommended dose, reduced survival for 1 to 2 weeks. Ivermectin pour-on reduced survival of the larvae for 1 to 3 weeks. Most detrimental was Ivermectin administered as a bolus, with effects lasting up to 20 weeks.

3. Parasite resistance is growing to Ivermectin.

   The repeated use of the same drug class of anthelmintic is determined to be a considerable risk factor for development of resistance by parasites. According to Dr. Don Bliss, parasitologist at the MidAmerican Agricultural Research Center in Madison, WI, "Based on what I've found and what the World Association for the Advancement of Veterinary Parasitology (WAAVP) standards are for a dewormer, I believe we are seeing true parasite resistance with the endectocide pour-ons." (Anthelmintic Resistance Roundtable, 2002) Dr. Lou Gasbarre, manager of the USDA Agricultural Research Service farm in Beltsville, Md. notes, "What's happening in the U.S., unfortunately, is resistance to Ivermectin products." (Lefever, 2006).

Certainly, management practices are the foundation for parasite control in organic livestock production. §205.238 requires producers to establish and maintain preventative livestock health practices through selection of species, appropriate housing, pasture conditions, and sanitation practices. That same section, however,

mandates that organic producers use all appropriate medication to restore an animal to health when methods acceptable to organic production fail.

To date, biological and other natural parasite controls have not proven effective to control emergency outbreaks of parasites.

While diatomaceous earth (DE) is utilized widely and effectively as a control for external parasites, its effectiveness as an internal control has not been reputably documented. Diatomaceous earth has no effect on lungworm and is not very appetizing. It may also be a lung irritant. Given that the level of dust is already quite high in barns, diatomaceous earth does not seem appropriate when the animals are fed indoors. The main motivation for adding diatomaceous earth to rations should not be to control internal parasites.

Fenbendazole can represent a viable tool that allows producers to have access to an "appropriate medication" that will not violate the principles of organic production:

1. Fenbendazole is not a macrolide antibiotic, and therefore violates neither the spirit or the letter of §205.238(c)(1) of the National Organic Standards.

2. Fenbendazole is also non-injurious to dung beetles (*Onthophagus gazelle),* which, in turn promotes ecologically sound nutrient management, and pest control measures vital to organic production; and

3. Fenbendazole has not demonstrated signs of parasite resistance. According to Dr. Bliss, "Fenbendazole has been on the market for over 20 years with one or two reports of anthelmintic resistance."

Unlike current approved parasiticides, Fenbendazole is administered orally. Thus, the elimination of the substance into the environment is limited to the excreta of the target animal. This is a true benefit because producers can release treated animals onto pasture without a risk of contaminating the new pasture.

Fenbendazole has been demonstrated to be benign in regard to its impact on soil, water, and biological sources critical to organic agriculture. It does not impact water quality or injure aquatic life. It binds tightly to soil particles and is insoluble in water. It does not damage microorganisms and has only negligible effects on earthworms. And, it has been demonstrated to have no negative impacts on human health.

Conclusion

Fenbendazole clearly meets the three major criteria specified in this section:

1. Why the synthetic substance is necessary.

   Internal parasites cannot always be controlled through species selection and management practices. The ineffectiveness of non-synthetic parasite control measures is a major inhibitor to the growth of the organic sector. In addition, the inability to effectively control parasites through non-synthetic means results in suffering--and even mortality--among livestock populations.

   Fenbendazole provides a solution which will effectively address the target nematodes without causing harm to the environment.

2. Alternative methods currently available are not effective, and the only allowed synthetic materials are incompatible with organic livestock production.

   Current non-synthetic substances, synthetic substances on the national list, and alternative cultural practices are not adequate. For example, diatomaceous earth has not been demonstrated to be effective on internal parasites.

   The only allowed synthetic material currently on the National List, Ivermectin, presents serious limitations, primarily because:
   - It is a macrolide antibiotic;
   - It is toxic to dung beetles; and
   - Internal parasites are developing a resistance to anthelmintics.

3. Fenbendazole is benign in terms of impact on environment, human health, or farm ecosystems.

   Studies referenced above have demonstrated that fenbendazole will not have negative impact on dung beetles, earthworms or plant life. The National Organic Standards clearly specify that synthetic parasiticides are not to be used as a substitute for cultural methods. Fenbendazole, however, will provide certified organic livestock producers with a viable material that can be utilized when cultural methods fail to prevent parasitic infestations.

Fenbendazole was approved for use in 1983, and therefore has a proven track record of more than 20 years. During this time period, a significant body of evidence has been developed to demonstrate the efficacy of fenbendazole, as well as its lack of negative affects on the environment.

References for Justification Statement:

BERTONE, M (2004) "Dung Beetles (Coleoptera: Scarabaeidae And Geotrupidae) Of North Carolina Cattle Pastures And Their Implications For Pasture Improvement," A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

BLISS, D; BOWMAN, D.; CRAIG, T; KVASNICKA, B; GASBARRE, L; MILLER, J; MONAHAN, C; MYERS, G (2005) "Anthelmintic Resistance: An Examination of its Growing Prevalence in the U.S. Cattle Herd," Anthelmintic Resistance Roundtable, Minneapolis, MN

DUVAL J (1994) The control of Internal Parasites in Ruminants, Ecological Agriculture Projects, McGill University (Macdonald Campus) , Ste-Anne-de-Bellevue, QCCanada

FEDERAL REGISTER (2006) "7 CFR Part 205, National Organic Program (NOP); Proposed Amendments to the National List of Allowed and Prohibited Substances (livestock); Proposed Rule" Vol. 71, No. 136 (July)

GILLARD, P. (1967) Coprophagous beetles in pasture ecosystems. J. Aust. Inst. Agric. Sci. 33: 30-34.

JOHNSON, P (2005) Dung Beetle, Ag Connection, University of Missouri Cooperative Extension, Columbia, MO

KNUTSON, A (2000). "Dung beetles–Biological control agents of horn flies," Texas Biological Control News, Texas Agricultural Extension Service, The Texas A&M University System. (Winter)

SHELTON, T, DVM (2001) Fenbendazole and its ecological implications," Pasture Management presentation.

13. A Commercial Confidential Information Statement

The applicant considers the information contained in Section No. 5 of this petition (Description of Manufacturing Process and Formulation Components) to be proprietary knowledge, and therefore considers this information confidential business information. Intervet Inc. has expended significant financial resources in the development and refinement of this manufacturing process, and maintains these records as trade secrets. Accordingly, this information is 1) commercially valuable, 2) used in the applicant's business, 3) maintained in secrecy. The release of this information outside of the company would be injurious to the company.

Attachment A **CBI Deleted**
Source of Substance and Manufacturing Process

CONFIDENTIAL
BUSINESS INFORMATION

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Source of Substance and Manufacturing Process

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Source of Substance and Manufacturing Process

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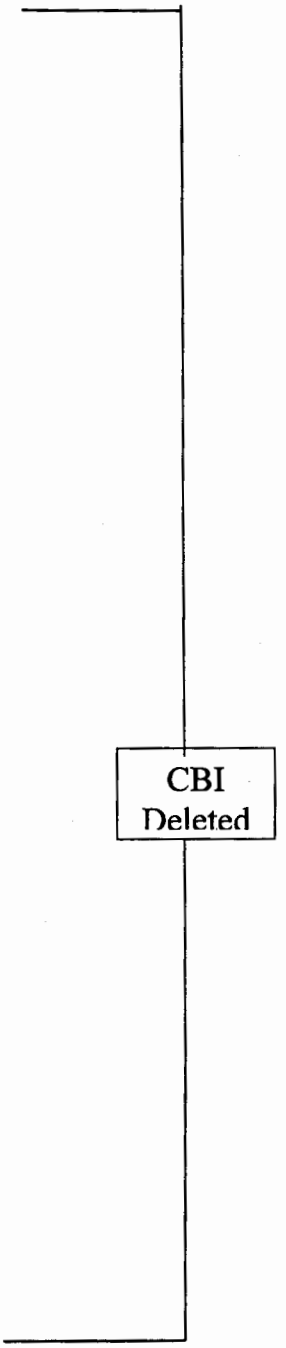
BUSINESS INFORMATION



Attachment A (Cont.) **CBI Deleted**
Source of Substance and Manufacturing Process

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Attachment A (Cont.) **CBI Deleted**
Source of Substance and Manufacturing Process

CONFIDENTIAL
BUSINESS INFORMATION

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Attachment A (Cont.)
Basic Components

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Attachment A (Cont.)
Basic Components

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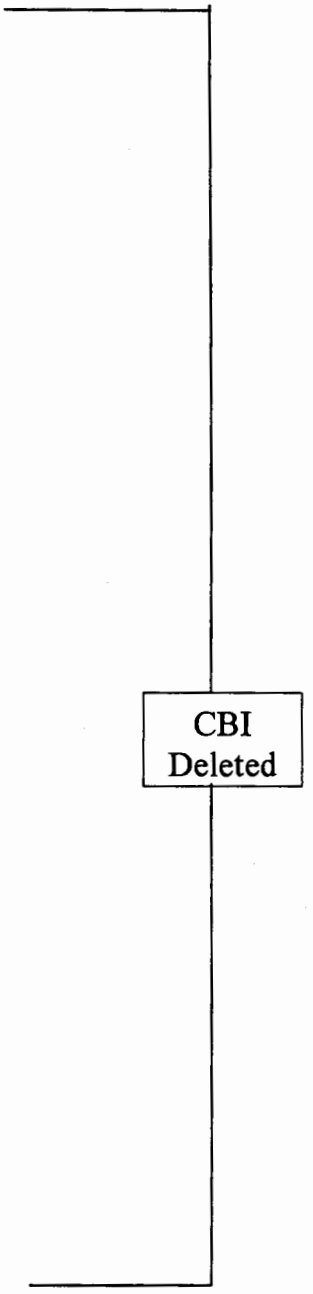
BUSINESS INFORMATION



F.O.I SUMMARY

FENBENDAZOLE SUSPENSION 10% FOR USE IN CATTLE

1. General Information

NADA	NADA 128-620
Name and Address of the Sponsor:	American Hoechst Corporation Animal Health Division Route 202-206 North Somerville, New Jersey 08876
Generic Name:	Fenbendazole
Trade Name:	PANACUR^{R} / SAFE-GUARD^{TM}
	This NADA provides for over-the-counter (OTC) distribution.

2. Indications for use: Cattle dewormer for the removal and control of: Lungworm: (Dictyocaulus viviparus), Stomach worms: Barberpole worm (Haemonchus contortus), Brown stomach worm (Ostertagia ostertagi), Small stomach worm (Trichostrongylus axei).

   Intestinal worms: Hookworm (Bunostomum phlebotomum), Threadnecked intestinal worm (Nematodirus helvetianus), Small intestinal worms (Cooperia oncophora and C. punctata), Bankrupt worm (Trichostrongylus colubriformis), Nodular worm (Oesophagostomum radiatum).

3. Dosage form: Suspension 10% (100 mg/ml).

   Dosage: Five (5) mg fenbendazole/kg body weight (2.3 mg/lb) administered orally with a suitable syringe.

Rev. 8/22/83

4. Effectiveness:

Screening studies in the research laboratories of Hoechst AG-Frankfurt, Germany, had shown that fenbendazole is a broad spectrum anthelmintic with high efficacy against gastrointestinal and other nematodes in various animal species. Consequently, the drug was evaluated regarding its efficacy on parasitic nematodes in cattle. A total of 15 controlled critical efficacy studies have been conducted by 6 investigators in 6 different geographical locations in the United States. Doses of 5 to 10 mg fenbendazole/kg body weight were used in these studies.

Fenbendazole was supplied to the investigators initially as a granular formula which facilitated accurate dosing. Later studies demonstrated that the 10% suspension is equally as effective. The drug was administered orally. It was evaluated for efficacy in "controlled critical trials". The term controlled critical trial means that groups of 10 cattle each were treated and postmortem worm counts at 7 days after treatment compared to those of 10 untreated controls. The animals were either experimentally or naturally infected with one or more species of nematodes. Each claim for a nematode species is supported by at least 2 adequate and well controlled studies.

Efficacy was expressed in % removal of worms as compared to controls. The % removal was calculated as follows:

$$\frac{\text{Number of parasites in control animals} - \text{Number of parasites in treated animals} \times 100}{\text{Number of parasites in control animals.}} = \% \text{ Removal}$$

The efficacy of fenbendazole was in the high 90% and therefore statistical analysis does not seem necessary. However, the data from all major controlled efficacy studies conducted in the U.S. have been analyzed statistically using both a parametric and nonparametric analysis. The results of the two statistical analyses are essentially the same and support the claim that in cattle at 5 mg/kg body weight, fenbendazole is a highly effective anthelmintic with a wide spectrum of activity ($P < 0.05$).

SUMMARY OF U.S. STUDIES

EFFICACY OF FENBENDAZOLE AGAINST ADULT PARASITES - 5 mg/kg

Parasite	(% Efficacy) Range	Investigator
Haemonchus contortus	76.6 - 99.2	Todd, Benz, Williams
Ostertagia ostertagi	96.9 - 100	Todd, Benz, Williams
Trichostrongylus axei	98.1 - 100	Todd, Benz, Williams, Craig
T. colubriformis	100	Todd, Benz
Cooperia oncophora	99.4 - 100	Todd, Benz, Craig
C. punctata	99.7 - 100	Benz, Williams, Craig
Nematodirus helvetianus	97.4 - 100	Todd, Yazwinski
Bunostomum phlebotomum	99.9	Williams, Yazwinski
Oesophagostomum radiatum	99.8 - 100	Todd, Benz
Dictyocaulus viviparus	98.0 - 100	Cheney, Todd

Investigators

Dr. G. Benz, Auburn, AL
Dr. T. Craig, College Station, TX
Dr. J. Cheney, Ft. Collins, CO
Dr. A. C. Todd, Madison, WI
Dr. J. C. Williams, Baton Rouge, LA
Dr. T. A. Yazwinski, Fayetteville, AR

Dose Titration Study, A. C. Todd, University of Wisconsin, Madison, Wisconsin - Study #4-C

This first study was conducted with a granular form of the drug instead of the 10% suspension because accurate dosing was easier with this formula.

Forty calves were experimentally infected with G.I. nematodes, and then divided into four groups (10 calves per group). They were orally treated with a single dose of granular fenbendazole at 0, 3.5, 5.0 and 7.5 mg/kg, respectively. At necropsy, the adult stage of the following parasites were recovered in adequate numbers to evaluate efficacy.

Parasite	5 mg/kg Granular % Removal
H. contortus	99.6%
O. ostertagia	100%
Cooperia spp.	99.9%

Statistical analysis indicated that 5 mg fenbendazole/kg body weight were more effective on Haemonchus contortus than 3.5 mg/kg. Since H. contortus is a parasite of major importance, the dose of 5 mg/kg is recommended.

Dose Titration Study, J. M. Cheney, Colorado State University, Fort Collins, Colorado - Study #5-C

This study was also conducted with a granular form of the drug instead of the 10% suspension because accurate dosing was easier with this formula.

Forty calves were experimentally infected with lungworms (Dictyocaulus viviparus), and divided into four groups (10 calves per group). They were orally treated with a single dose of fenbendazole at 0, 3.5, 5.0 and 7.5 mg/kg, respectively. At necropsy, all of the control calves had adequate infections of lungworms, while the worms in the treated group receiving 5.0 mg/kg were reduced by 98%.

Dose Titration Study, T. M. Craig, Texas A&M, College Station, Texas - Study #31-A

This study was designed as a dose titration study concerning efficacy on arrested larvae of Ostertagia ostertagi; which is not the subject of this NADA, however, the efficacy of the dose level of 5 mg fenbendazole/kg body weight is pertinent to this NADA.

Ten cattle with natural infections of G.I. nematodes were treated with 5 mg fenbendazole/kg body weight and the postmorten worm counts were compared to those of untreated controls with comparable infections. The 10% suspension was used in this study.

Compared to the controls, the worms in the treated group were reduced as follows:

Parasite	5 mg/kg % Removal
H. placei	100%
Trich. axei	99%
Cooperia spp.	100%
O. ostertagi	99%

Comparison Study, A. C. Todd, et al, University of Wisconsin, Madison, Wisconsin, Study No. 4-E

The efficacy of fenbendazole granules and suspension 10% was compared in 30 calves with experimental infections of lungworms (D. viviparus). Groups of 10 calves each were treated with 0 mg/kg, a single dose of fenbendazole granules at 5 mg/kg and a single dose of fenbendazole suspension 10% at 5 mg/kg. At postmortem, all of the controls harbored lungworms (average of 203 worms per animal), while they were reduced by 99.9% in both of the treated groups.

Comparison Study, A. C. Todd, et al, University of Wisconsin, Madison, Wisconsin, Study No. 4G-1

A total of 25 cattle with experimental infections was used to compare the efficacy of a single dose of fenbendazole granules and suspension 10%, and also low dose levels administered daily. Only the control group (5 cattle) and the two groups (5 cattle/group) receiving a single dose of either fenbendazole granules or suspension 10% are pertinent to this NADA. At postmortem, adequate numbers of the following parasites were recovered from the control group and compared to parasites left in the treated animals:

Parasite	5 mg/kg Granules % Removal	5 mg/kg Susp. % Removal
Haemonchus	100%	99%
Ostertagia	99%	100%
Trichostrongylus axei & colubriformis	100%	100%
Cooperia	99%	100%

Comparison Study A. C. Todd, et al. University of Wisconsin, Madison, Wisconsin, Study No. 4G-2

This trial has the same design and used the same number of cattle as preceeding Study No. 4G-1. Only the two groups receiving a single dose of the drug are pertinent to this NADA. Adequate numbers of the following parasites were recovered from the control group and compared to parasites left in the treated animals:

Parasite	5 mg/kg Granules % Removal	5 mg/kg Susp. % Removal
Haemonchus	99%	99%
Ostertagia	100%	100%
Trichostrongylus axei & colubriformis	100%	100%
Oesophagostomum	100%	100%

Dose Confirmation Study, A. C. Todd, University of Wisconsin, Madison, Wisconsin, Study No. 4-I

Twenty calves were experimentally infected with Nematodirus helvetianus, and divided into two groups of 10 calves each. One group received no fenbendazole, the other, Group 2, received a single dose of fenbendazole suspension at a dose of 5 mg/kg. At postmortem, the worm count in the controls was an average of 713 worms per animal, and in the treated group an average of 19 worms per animal. This represents a 97.4% removal of Nematodirus helvetianus.

Comparison Study, J. M. Cheney, et al, Colorado State University, Fort Collins, Colorado, Study No. 5-I

Thirty calves were experimentally infected with lungworms (Dictyocaulus viviparus) and divided into three groups (10 calves per group). One group remained unmedicated, the other 2 groups were administered a single oral dose of 5 mg fenbendazole/kg as the suspension 10% or the granules, respectively. At necropsy, the lungworms in the two treated groups were reduced as follows: suspension 100%, and granules 99%.

Comparison Study, G. W. Benz, Auburn University, Auburn, Alabama, Study No. 17-A

A total of 42 calves was divided into 4 groups (group 1 - unmedicated control, group 2 - suspension 5 mg/kg, group 3 - granules 5 mg/kg per individual animal, group 4 - granules 5 mg/kg per group). All of the calves were experimentally infected and received the drug as a single oral dose. The following parasites were recovered from the controls in adequate numbers and compared to those remaining in the treated animals:

Parasite	5 mg/kg Susp.10%, % Removal	5 mg/kg Granules % Removal
H. contortus	65.8%	6.8%
O. ostertagia	99.4%	98.9%
Trich. axei	100%	100%
Trich.colubriformis	100%	99.5%
C. oncophora	99.5%	87.9%
C. punctata	99.9%	92.3%
O. radiatum	99.8%	98.6%

Some treated animals in this study were found to carry unusually high numbers of Haemonchus contortus at postmortem. Additional animals were, therefore, infected with the same parasites and treated according to the same protocol as in Study #17-A. Two animals were replaced in the group treated with suspension 10%. Efficacy was calculated to be 76.6% after this adjustment. This was not in line with results in other studies where efficacy was consistently higher than 90%. The investigator, therefore, did a study with cattle infected with Haemonchus contortus alone (see Study #17-C).

Comparison Study, G. W. Benz, Auburn University, Auburn, Alabama, Study No. 17-C

This study was a follow-up to Study #17-A in which insufficient efficacy was observed against H. contortus. A total of 43 calves was experimentally infected with two different strains of H. contortus (Merck isolate, and USDA isolate). The calves were divided into four groups, one group with 10 animals and the remaining three groups had 11 animals each. One group infected with the Merck isolate and one group infected with the USDA isolate remained unmedicated. The calves in the remaining two groups were given a single oral dose of fenbendazole suspension 10% at 5 mg/kg. At postmortem, the control groups had adequate numbers of worms and the worms in the treated animals were reduced as follows: Merck isolate - 95.9% and USDA isolate - 91.1%.

Comparison Study, J. C. Williams, et al, Louisiana State University, Baton Rouge, Louisiana, Study No. 44-B

A total of 30 cattle with natural infections of G.I. nematodes was divided into 3 groups of 10 animals each. One group remained untreated, the other groups received a single oral dose of fenbendazole suspension 10% at 5 mg/kg or fenbendazole granules on a daily basis. Only the group treated with the suspension is pertinent to this study. The following parasites were recovered in sufficient numbers from the control group and compared to those found in the treated group:

Parasite		5 mg/kg Susp. 10%, % Removal
Haemonchus	adult	98.6%
Trich. axei	adult	100%
O. ostertagi	adult	96.9%
O. ostertagi	developing	82.9%
O. ostertagi	early L_4	74.7%
Cooperia	adult	97.7%
Bunostomum	adult	100%
Trich. colubriformis	adult	100%

Dose Titration, J. C. Williams, Louisiana State University, Baton Rouge, Louisiana Study No. 44-C

An experimental infection of hookworms (Bunostomum) was super imposed on 30 cattle with natural infections of G.I. nematodes including Bunostomum. The cattle were divided into three groups of 10 cattle each. One group remained untreated, the other groups received a single oral dose of fenbendazole suspension 10% at 5 and 10 mg/kg, respectively. Only results in the animals treated with 5 mg/kg are pertinent to this NADA. Sufficient numbers of the following parasites were recovered from the control animals and compared to those found in the treated animals:

Parasite		5 mg/kg % Removal
O. ostertagi	adult	99.6%
Trich. axei	adult	98.1%
Haemonchus	adult	97.8%
Cooperia spp.	adult	100%
Bunostomum	adult	99.9%

Dose Confirmation Study, T. A. Yazwinski, University of Arkansas, Fayetteville, Arkansas, Study #101-A

A total of 20 calves was experimentally infected with N. helvetianus and B. phlebotomum and divided into 2 groups of 10 animals each. One group was treated with a single dose of fenbendazole suspension 10% at a dose of 5 mg/kg. The other group served as untreated control. The control calves had adequate parasite burdens of both species to evaluate efficacy. As compared to the untreated controls, worm counts in the treated animals were reduced by 99% for N. helvetianus and B. phlebotomum.

Numerous efficacy studies with many different parasites were conducted in other countries. One controlled critical study done in South Africa is especially pertinent to this NADA and was an adequate and well controlled study. Calves were experimentally infected with nematode larvae and treated with 5 mg fenbendazole/kg body weight when the worms had matured to the adult stage. Seven days after treatment 11 treated animals and 7 untreated controls were subjected to a postmortem worm count. Compared to the untreated controls, high efficacy was observed against Haemonchus placei, Ostertagia ostertagi, Cooperia spp., Bunostomum phlebotomum and Oesophagostomum radiatum. (D. Malan, Hoechst Research Farm, Malelane, South Africa).

Well documented clinical studies were conducted in the United States according to a uniform protocol which was only slightly modified to accomodate local management conditions. Groups of cattle with at least a moderate worm infection, as determined by egg counts in their feces, were selected. Approximately the same number of animals were treated with 5 mg fenbendazole/kg body weight or left untreated as controls. Worm eggs in fecal samples were counted before and after treatment, the animals were visually observed for side effects.

Seven investigators studied fenbendazole suspension 10% in 6 different states. They treated a total of 916 cattle of various breeds and compared them to 963 untreated controls. Investigators, location and numbers of animals in the trials are tabulated on the following page.

CLINICAL FIELD TRIALS
WITH FENBENDAZOLE SUSPENSION 10% IN CATTLE
DOSAGE 5 MG/KG
United States

Study #	Investigator/ Location	Total Number of Animals					
		Treated	+* Before Treatment	+* After Treatment	Controls	+* Before Treatment	+* After Treatment
7-1	Bradley Gainesville, FL.	100	99	0	100	92	97
1-4	Todd Madison, WI.	63	23	11	61	20	22
6-4	Todd Madison, WI.	138	80	26	129	69	70
8-4	Todd Madison, WI.	27	8	0	27	9	2
13-27	Sharp Vernon, TX.	60	59	1	60	58	48
2-28	Bechtol Canyon, TX.	100	15	0	100	59	5
5-28	Bechtol Canyon, TX.	93	28	0	85	27	13
3-29	Schafer/Elars Ft. Collins, CO.	86 27	4	0	83 84	42	15
4-29	Schafer/Elars	100	69	0	96	65	49
12-79	Morter W. Lafayette, IN.	108	81	2	108	81	82
22-80	Worley Bozeman, MT.	14	13	0	30	29	11

*) + = cattle with nematode eggs in their feces. Study #22-80 lungworm larvae in their feces.

The clinical studies confirm the results of the critical studies by eliminating or reducing fecal egg counts in virtually all treated cattle.

The recommended treatment was found to be both safe and practical under field conditions.

Foreign controlled critical and clinical studies were also submitted. They confirm further the efficacy of fenbendazole against a wide spectrum of nematode parasites in cattle.

5. Target Animal Safety

Studies to evaluate the safety of fenbendazole in cattle were done in Hoechst Research Laboratories in Frankfurt, Federal Republic of Germany and in Malelane, South Africa as well as in the laboratories of independent investigators in the United States and other countries. If not indicated otherwise, studies summarized here were done in the research laboratories of Hoechst AG in Frankfurt, Federal Republic of Germany.

This application contains reports about acute toxicity studies in which high doses of fenbendazole were administered orally to cattle, mice (10,000 mg/kg), rats (10,000 mg/kg), rabbits (5,000 mg/kg dogs (500 mg/kg), sheep (5,000 mg/kg) and horses (1,000 mg/kg) (Dr. Cheney, Ft. Collins, Colorado).

Acute Oral Safety Evaluation of Fenbendazole in Mice

Investigator: Hoechst AG, Frankfurt, Federal Republic of Germany, HAG #30

Methods: Fenbendazole was administered as a single oral dose to groups of 10 male and 10 female juvenile NMRI mice. The compound was given in the highest dose that could be administered: 10,000 mg/kg body weight by means of a stomach tube.

Results: Acute symptoms were not observed. The body weight development was normal during the follow-up period.

Conclusions: According to this experiment the minimum lethal dose is higher than 10,000 mg of fenbendazole/kg body weight.

Acute Oral Safety Study with Fenbendazole in Rats

Investigator: Hoechst AG, Frankfurt, Federal Republic of Germany, HAG #42

Methods: Fenbendazole was administered as a single oral dose to one group of 10 male and 10 female juvenile Wistar rats. The compound was given with a stomach tube in the highest dose that could be administered: 10,000 mg/kg.

Results: The development of the rats was retarded during the 3 week follow-up period. Otherwise, no toxic symptoms were observed. Deaths did not occur.

Conclusions: According to this test, the maximum tolerated dose of fenbendazole in rats is greater than 10,000 mg fenbendazole/kg body weight.

Acute Oral Safety Study of Fenbendazole in Dogs

Investigator: Hoechst AG, Frankfurt, Federal Republic of Germany, HAG #38

Methods: Fenbendazole was administered as a single oral dose. One female dog received 300 mg, and another female and a male dog 500 mg fenbendazole/kg body weight.

Results: The animals were observed for 3 weeks. During this period, no changes in the behavior were observed which could be attributed to an effect of the substance.

Conclusions: According to this test, the maximum tolerated dose is above 500 mg fenbendazole/kg body weight.

Acute Oral Toxicity Study of Fenbendazole in Cattle

Investigator: Bio/dynamics Inc., East Millstone, NJ., U.S.A., AHC #075-A

Methods: A single dose of fenbendazole was administered orally, via drenching, to Aberdeen Angus cattle at levels of 630 (1 male), 1,000 (2 male and 2 female) and 2,000 (2 male and 2 female) mg/kg. After the administration of the compound, all animals were retained for a 14-day observation period followed by sacrifice of all survivors.

Results: One animal receiving 2,000 mg/kg died spontaneously prior to termination. Cause of death was considered to be pneumonia. Evaluation of all in-life and postmortem observations for cattle receiving 630, 1,000 and 2,000 mg/kg of fenbendazole did not reveal any changes considered related to the administration of the test compound.

Conclusions: It was therefore concluded that the maximum tolerated dose is greater than 2,000 mg/kg as there were no apparent effects at this level.

Acute Oral Toxicity Study in Cattle

Investigator: Hoechst AG, Frankfurt, Federal Republic of Germany, HAG #201/34

Methods: Three animals in each group were treated with 0, 500, 750, 1,000 and 2,000 mg fenbendazole/kg body weight orally.

Results: Only a short term increase of GDH, SDH occurred and the leukocyte count dropped after a single dose of 500 mg/kg. After a single dose of 2,000 mg/kg orally, 2 out of 3 test animals died from coagulation disorders with thrombosis of major vessels and hemorrhagic infarcts in various organs. After 1 x 1,000 mg fenbendazole/kg orally and also after 1 x 750 mg fenbendazole/kg orally one out of every three animals died with the same symptoms.

Laboratory results showed increased enzyme activity (particularly SGOT with the exception of cholinesterase) after 2,000 mg fenbendazole/kg. Alkaline phosphatase levels were low. The blood clotting time was accelerated, thrombocytes were increased and the blood sedimentation rate was dropped. All three animals developed leukopenia, one animal also showed a drop in the red cell count with a correspondingly reduced hematocrit and a slight drop in hemoglobin amount. Bilirubin was increased in the serum analysis. A slight and short term loss of serum electrolytes, potassium, calcium and inorganic phosphorous with a mild increase in chloride was seen. One animal showed an increase of protein in the urine.

Various parameters deviated from the physiological range after a single dose of 1,000 mg and 750 mg/kg. The deviation was not as marked as after 2,000 mg and the values normalized in the surviving animals after a few days. Bilirubin increased pathologically and alkaline phosphatase and glutamic dehydrogenase also increased markedly after 1,000 mg/kg.

At postmortem, 2 animals after 2,000 mg fenbendazole/kg showed microscopic evidence of thrombi with hemorrhagic infarcts in the lungs, kidneys and in the gastrointestinal tract. In addition, numerous focal necroses were found in the liver and activated coagulation products detected in hepatic cells. One animal after 1,000 mg and 750 mg fenbendazole/kg each showed hepatic parenchymal cells with activated coagulation products and the animal after 1,000 mg/kg showed isolated centrilobular necrosis.

Conclusions: The animals which died in this study, died with the signs of disseminated intravascular coagulopathy. This syndrome could not be reproduced in two other studies, one in the United States (Study AHC #075-A) and one in South Africa (Study HAG #330). Disseminated intravascular coagulopathy can be caused by a number of nonspecific factors. It was, therefore, concluded that the deaths observed in this study were probably not caused by the drug.

Toxicity Trials/Fenbendazole/Cattle

Investigator: Dr. C. A. Wilkins, Hoechst Research Institute, Malelane, Republic of South Africa, HAG #330

Methods: Four groups of two animals each were dosed with fenbendazole in a commercially available form of a 10% suspension at dosage rates of 0, 10, 100, 1,000 and 2,000 mg/kg body weight.

Results: These cattle were kept on the experimental floor under observation for a period of 21 days post-dosage.

Conclusions: None developed any symptoms and no signs of toxicity were found throughout the trial period.

Toxicity Tests with 10% Fenbendazole Suspension in Cattle

Investigator: Dr. L. J. Loots & Berg, Hoechst South Africa, HAG #310

Methods: 150 beef and 150 dairy animals were treated orally with 20 mg fenbendazole/kg in form of the 10% suspension. The procedure was repeated approximately every 30 days for 12 months.

Results: All animals were observed for approximately 48 hours after dosing. No signs of toxicity developed in any of the animals in the trial. A few did develop anaplasmosis during the trial period, one ox in the beef herd died as a result of bloat but no direct connection could be made to the dosing. 139 cows calved during the dosing. None of them had calves showing any signs of teratological defects.

Conclusions: No toxicity or adverse effects on the offspring were observed.

Toxicity Study with 10% Fenbendazole Suspension in Cattle

Investigator: Hoechst Research Institute, South Africa, HAG #328

Methods: 30 Simmentaler cattle were treated under intensive grazing conditions and at hot and dry climatic conditions with a single oral dose of 20 mg fenbendazole/kg in form of the 10% suspension. The animals were observed daily for 7 days and thereafter weekly for four weeks.

Results: No change was noticed throughout the dosage period.

Conclusions: No adverse effects were observed in this study.

Toxicity Study with 10% Fenbendazole Suspension in Cattle

Investigator: Hoechst Research Institute, South Africa, HAG #329

Methods: 50 Afrikaner and Afrikaner Simmentaler crossbred cattle were treated under extensive grazing conditions and hot and dry climatic conditions. The animals received a single oral dose of 20 mg fenbendazole/kg body weight in form of the 10% suspension. They were observed daily for one week and weekly for the four following weeks.

Results/Conclusions: No changes were observed, no toxicity was observed.

Five Day Subacute Oral Toxicity Study with Fenbendazole in Cattle

Investigator: Bio/dynamics Inc., East Millstone, NJ., U.S.A., AHC #075-B

Methods: Fenbendazole was administered orally to 4 groups of Aberdeen Angus cattle for 5 days at levels of 0, 10, 25 and 50 mg/kg/day. Each group consisted of 3 male and 3 female animals. The animals were examined before and after treatment for clinical signs of toxicity by observation, hematology and clinical chemistry tests. All animals were subjected to a postmortem 24 hours after the last dose. The untreated controls and the highest treatment group were also examined for histopathological signs of toxicity.

Results: Two animals, one control and one receiving 25 mg/kg/day died spontaneously prior to termination. Death of these animals was considered to have been caused by repeated trauma induced by the speculum used for intubation each day.

Conclusion: Evaluation of all in-life (physical observations, body weight, hematology and clinical chemistry) and postmortem parameters did not reveal any changes considered related to the administration of fenbendazole.

Long term safety studies included 30 day studies in rats (up to 2500 mg/kg), dogs (up to 250 mg/kg) and sheep (up to 45 mg/kg) and 90 day studies in rats (up to 2500 mg/kg) and dogs (up to 125 mg/kg). These studies provide only supporting evidence for the safety of the drug since lifetime studies in rats and mice reported in the Human Safety Section of this summary are a more sensitive measurement of possible toxicity.

No clinical signs of toxicity were found in any of the long term studies up to 90 days. The following postmortem lesions were found at the end of the studies:

In the 30 day dog study, a slight centrolobular fatty infiltration of the liver cells in one male dog of each of the groups which received 80 and 250 mg/kg.

In the 30 day sheep study, fenbendazole appeared to have caused the development of centrolobular fat free vacuoles in the liver parenchyma cells of a total of 12 sheep from all treated groups. In the group with the highest dosage of 45 mg/kg, two ewe lambs also developed fine powdery fatty degeneration and subendocardial necrosis of the heart muscles. These latter changes were also observed in another sheep in this group.

No drug related pathological lesions were found at postmortem in the other studies.

Teratogenicity studies were conducted in rats, rabbits, cattle and sheep. The studies in rats and rabbits are summarized in the Human Safety Section of this summary.

Two teratogenicity studies were conducted in cows.

Teratological Trial/Fenbendazole/Cattle

Investigator: Dr. C. A. Wilkins, Hoechst Research Institute, Malelane,Republic of South Africa, HAG #381

Methods: Twenty-seven cows were treated orally on days 12 & 21 of pregnancy, then at 3 week intervals until five months pregnant and thereafter at two monthly intervals until calving, at a dosage rate of 50 mg/kg livemass in form of the 10% suspension.

Results: There were no signs of any abnormalities in any of the 27 calves born, suckling capabilities of the calves were normal, milk yield and mothering capabilities were not affected

Teratological Trial/Fenbendazole/Cattle

Investigator: Drs. Grus, Humke: site of test: Mokrin, Yugoslavia, HAG #238

Methods: Thirty-five Friesian dairy cows aged from 4 to 13 years were treated individually with 20 mg fenbendazole/kg body weight in the form of the 10% suspension administered orally by means of a cattle drencher 9 to 10 days, 19, 39, 69, 99, 129, 159 and 189 days after the first insemination. In cows which only conceived after the second or third insemination, the times of administration were delayed accordingly. That is, these animals were treated two to three times before conception and instead of the compound being administered eight times, they received only five treatments after conception.

Results: Twenty-five cows were pregnant after one insemination, nine cows after two and one cow after three. The insemination index came to 1.31 and at this level lies within the normal range of conception rate to be expected with artificial insemination. Altogether, fifteen male calves, weighing 36-53 kg (average weight 43 kg) and twenty-one female calves weighing from 33-63 kg (average weight 43 kg) were born. One cow had twin calves. All calves were normally developed and healthy at birth.

The gestation period varied between 267 and 297 days and was on an average 284 days long.

Conclusions: Treatment with fenbendazole suspension 10% repeated 5 to 8 times in dairy cows during a 10 to 30 day period had no detrimental effect either on the conception rate of the cows or on the development and state of health of the calves that were born.

These data are supported by studies in sheep. Two teratogenicity studies in sheep were conducted. In one study, 15 mg fenbendazole/kg body weight were given 4 times at 3 week intervals to ewes known to be pregnant. All stages of pregnancy were represented. In another study, 5 groups of 10 ewes each were treated on day 8, 12, 16, 20 and 24 after service. The ewes were given 50 mg fenbendazole/kg body weight. No untoward effects of any kind were found in the ewes or their offspring. No teratogenic effects were found (Dr. C. A. Wilkins, Hoechst Research Institute, Malelane, Republic of South Africa).

Fertility studies were conducted in bulls, sheep (ewes and rams), and horses (mares and stallions).

Investigations on the Effect of the Anthelmintic Fenbendazole on the Quality of Semen in AI Bulls

Investigator: D. Krause, H. J. Reinhard, W. Koehler, B. Tiefenbach, Veterinary College, Hannover, Federal Republic of Germany, HAG #265

Methods: Fenbendazole suspension 10% was administered 3 times at intervals of four weeks at doses of 10 mg active agent per kg body weight in three Black Pied bulls of 2-1/2 years and 500-600 kg body weight each.

Results: No deleterious effects on the semen quality could be found in comparison with the ejaculates of three untreated control bulls of the same age and body weight.

Supportive evidence was collected in other animal species:

In one study, semen was collected from 4 rams, 2 times prior to dosing (50 mg fenbendazole/kg body weight), once on the day of dosing, 24 and 72 hours after, then weekly for two weeks and thereafter monthly for four months. The rams remained healthy throughout the period, no changes in semen samples were observed (Hoechst Research Institute, Malelane, Republic of South Africa).

In another study, 5 adult rams were kept on pasture and dosed with fenbendazole at the rate of 15 mg fenbendazole/kg body weight every month for four months. The rams were observed daily and semen was collected 12 hours after dosing. No effects attributable to the treatment were found throughout the study (Hoechst Research Institute, Malelane, Republic of South Africa).

Three fertility trials were conducted in ewes.

Ten ewes were administered fenbendazole at a dose of 50 mg fenbendazole/kg body weight 96 hours after service. No adverse reactions were observed and the offspring were normal (Hoechst Research Institute, Malelane, Republic of South Africa).

Nineteen ewes were kept as one flock and dosed every 4 weeks at a rate of 15 mg fenbendazole/kg. Rams of known fertility were introduced to the flock for a period of one month. The ewes were observed to lambing. No abnormal changes were found in the ewes or their offspring (Hoechst Research Institute, Malelane, Republic of South Africa).

In another study, 45 ewes were treated with fenbendazole at a dose rate of approximately 10 mg/kg on the 10th, 20-25th, 40th, 70th and 100th day of the mating season. Twenty-six animals served as controls. The ewes were mated by free service. One lamb of a treated ewe had a thumb sized umbilical hernia with an intestinal prolapse at birth. There were no other disease symptoms in the group of the treated ewes and their lambs as compared with the controls (Dr. Grote, Gross Burgwedel, West Germany).

Reproduction, teratogenicity and fertility were also evaluated in sows and boars after oral treatment with fenbendazole.

Groups of 10 sows each were treated with 3 mg fenbendazole/kg per day for 3 days prior to breeding, at 1, 2, 3, 4, 10, 13, and 14 weeks of gestation and compared to untreated controls. No abnormalities were found. (Lawrence E. Evans, D.V.M., Iowa State University, College of Veterinary Medicine, Ames, IA.).

In another study, fenbendazole was fed to 10 sexually mature boars at the rate of 3 mg/kg body weight for 3 consecutive days. Five boars were used in breeding trials and 5 boars were utilized in semen quality studies. Semen quality was assayed pretreatment, and 7, 28, and 62 days after feeding fenbendazole. Fertility was assessed by breeding each boar to 5 females prior to and approximately 1 week and 4 weeks after treatment. No pathological changes were found. (Lawrence E. Evans, D.V.M., Iowa State University, College of Veterinary Medicine, Ames, IA.).

VI. HUMAN SAFETY

A. Toxicity and Teratogenicity Tests

Toxicity and teratogenicity studies were done to determine potential hazards to human health when food derived from treated animals is ingested.

Six Month Oral Toxicity Study in Dogs
International Research & Development Corporation
Mattawan, ML, U.S.A.
Report IRDC #327-028 of 8/11/78

Thirty male and 30 female beagle dogs were equally distributed into five groups of six animals/sex/group and given 0 (control), 4, 8, 12 and 20 mg/kg fenbendazole daily in gelatin capsules for six months. Two of the animals in each sex/dose group were allowed an additional three-week recovery period (no compound administration) before sacrifice.

No compound-related changes were seen in general appearance and behavior, reflexes, teeth, mucous membranes, body weights, food consumption, ophthalmoscopy, or clinical laboratory tests in any of the treated groups. Differences in various absolute and relative organs weights were seen in treated dogs; however, since no histopathology was observed in these organs, the differences were not considered to be of biological significance. At six month sacrifice, an increased incidence of lymphocytic foci in the gastric mucosa and hyperplasia and congestion of the mesenteric lymph nodes were seen microscopically in dogs treated with higher dose levels.

In dogs sacrificed after the three week recovery period, the gastric lymphocytic foci were found in animals from both control and treated groups. However, no statistical difference could be demonstrated between the control and any drug group.

All microscopic findings which were considered possibly compound related were those which commonly occur in untreated dogs. The increased incidence and magnitude of these conditions in the fenbendazole-treated dogs suggested that the changes were treatment related.

The dose of 4 mg fenbendazole/kg body weight was established as the no effect level.

Lifetime Oral Toxicity Study in Rats
International Research & Development Corporation
Mattawan, ML, U.S.A.
Report IRDC #327-030 of 12/12/80

Fenbendazole was offered daily in the diet to male and female F1a rats (Charles River, derived from F0 rats of a 3 generation reproduction study) at dosage levels of 5, 15, 45 and 135 mg/kg/day. Fifty rats per sex per group were used. All surviving male rats were terminated at week 123 and all surviving female rats at week 125.

Apparent compound related changes in appearance and behavior noted for some of the treated rats included: diarrhea (45 mg/kg/day males and 135 mg/kg/day males and females), reddish brown urine (15, 45 and 135 mg/kg/day males and females) and red material in the feces (45 and 135 mg/kg/day males).

A statistically significant decrease in hemoglobin concentration, hematocrit values and erythrocyte counts was observed at some of the checkpoints for male rats administered 15, 45 and 135 mg/kg/day and for female rats administered the same dose levels but at fewer checkpoints. There were statistically significant differences (increases or decreases) in some of the biochemical parameters at some checkpoints. The most consistent of these changes were increases in alkaline phosphatase values for rats receiving 15, 45 and 135 mg/kg/day. With the exception of increased serum glutamic oxalacetic transaminase values at the 135 mg/kg/day dosage level, the other variations in the biochemical parameters were usually in the normal range of values for this laboratory. Although not always statistically significant there was a decrease in mean urine volume noted in both sexes for most sampling intervals in the 135 mg/kg/day group.

Relative liver weights were significantly increased for the males of the 45 mg/kg group and males and females of the 135 mg/kg group. Absolute kidney weights were significantly decreased for females of the 45 mg/kg group and for males and females of the 135 mg/kg group.

At necropsy, a slight increase in the incidence of the following lesions was observed: enlargement or cyst formation in the lymph nodes among male and female rats in the 45 and 135 mg/kg/dosage groups; liver masses and/or nodule formation among male and female rats in the 135 mg/kg/day dosage group; cyst formation among male and female rats in the 135 mg/kg/day dosage group; cyst formation in the liver among the female rats at this dosage level was also increased and, lastly, slightly increased incidence of testicular masses among male rats in the 135 mg/kg/day dosage level.

No meaningful differences were found in other observations including body weight, survival time and ophthalmoscopic examination.

The investigator reported dose related increased incidences of reactive hyperplasia in the mesenteric lymph nodes of animals that had received 15, 45 and 135 mg/kg/day.

Treatment related microscopic liver changes were seen in male and female rats in all dosage groups except the lowest. The incidence and extent of centrolobular hepatocellular hypertrophy, focal hepatocellular hyperplasia, hepatocellular cytoplasmic vacuolation, focal bile duct proliferation, and the formation of biliary cysts were increased for the 15, 45 and 135 mg/kg/day dosage levels. The 45 and 135 mg/kg/day female rats had increased incidences of nodular hepatocellular hyperplasia.

The formation of biliary cysts was increased for male and female rats of the 45 and 135 mg/kg/day dosage groups. No distinct or consistent compound related differences were observed in the total number of neoplasms, total benign neoplasms, or total malignant neoplasms in this study for rats in the treated groups compared to controls.

No distinct or consistent drug related microscopic changes were found in the rats administered fenbendazole at 5 mg/kg/day. It is concluded that this is the no effect level.

Fenbendazole was demonstrated not to be a carcinogen in rats in this study.

24-Month Oral Carcinogenicity Study in Mice with Fenbendazole
International Research & Development Corporation
Mattawan, MI., U.S.A.
Report IRDC #327-037 of 11/20/80 and 8/25/81

The two year dietary administration of fenbendazole to Charles River CD-1 mice (dosage levels of 45, 135 and 405 mg/kg/day) produced no definitive differences between treated and control mice. Sixty mice per sex per test group were used. The parameters studied included appearance and behavior, mortality, body weight, food consumption, and gross pathology and histopathology.

The no-effect level for this compound in this species is greater than 405 mg/kg/day.

Fenbendazole was demonstrated not to be a carcinogen in mice in this study.

Three Generation Reproduction Study in Rats
International Research & Development Corporation
Mattawan, MI., U.S.A.
Report IRDC #327-033 of 9/5/80

Fenbendazole was administered in the diet at dosage levels of 5, 15, 45 and 135 mg/kg/day to Charles River CD rats. The F_0 consisted of 20 males and 40 females per dosage group. The two lower dose levels (5 and 15 mg/kg/day) did not significantly affect reproductive parameters (live births, pup weights, pup sex or pup mortality) or parental body weight, food consumption or general appearance and behavior.

Fenbendazole administered in the diet at dosage levels of 45 and 135 mg/kg/day produced significant signs of toxicity. This was concluded from frequent observations in parental rats and pups such as soft stool, diarrhea and decreased size, the marked decreases in parental body weights and moderately reduced food consumption, the decreases in pup survival and marked decreases in mean pup body weights. It is concluded that the no effect level is 15 mg/kg/day.

Teratogenicity Study in Wistar Rats
Hoechst AG
Frankfurt, West Germany
Report #29 of 7/19/73

Fenbendazole (doses of 25, 250 and 2500 mg/kg) was given to groups of 20 pregnant female Wistar rats at the seventh to sixteenth day of gestation by gavage. Fetuses were delivered on the 21st day of pregnancy by Cesarean section. Behavior and general condition, food consumption and body weight were monitored for the dams. After opening of the uterus, the living and dead fetuses, resorptions, and placentas were counted, weighed, and examined microscopically. Implantation sites were counted. The fetuses were sexed, and examined for external appearance and detectable abnormalities. Half of the fetuses were processed for determination of skeletal abnormalities and half for organ abnormalities.

The administration of fenbendazole did not impair the general health of the mothers at any dosage level nor the intrauterine development of the fetuses at 25 and 250 mg/kg. An increased number of anomalies seen in one litter of the group which received 2500 mg/kg was not determined to be of sufficient significance to label fenbendazole a teratogen. The intrauterine absorption and death rate was not increased as compared to controls. Fenbendazole was not found to be a teratogen in the rat and the no-effect level for the parameters studied was 250 mg/kg.

Teratogenicity Study in Rabbits
Hoechst AG
Frankfurt, West Germany
Report #31 of 9/05/73

Fenbendazole (10, 25 and 63 mg/kg body weight) was given to groups of ten pregnant yellow silver rabbits at the 7th to the 19th day of gestation once a day via gavage. The administration of 10 mg/kg of fenbendazole impaired neither the general health of the mothers nor the intrauterine development of the fetuses. The parameters studied in this experiment were similar to those in the rat teratology study.

One out of 10 rabbits aborted after the administration of 63 mg fenbendazole. Two more rabbits at 63 mg/kg and one rabbit at 25 mg/kg had only implantation sites which resulted either from abortion or early absorption at the time of Cesarean section conducted on the 29th day of gestation. The survival rate of fetuses in the 63 mg/kg dosage group in the incubator 24 hours after delivery was decreased as compared to controls. The observations in the groups administered the highest dose (63 mg/kg) indicate a toxic effect on the fetus. The fetuses which were delivered by Cesarean section at the 29th day of gestation were developed normally and did not show any external organ or skeletal abnormalities caused by the substance. The survival rate of fetuses from 10 and 25 mg/kg groups in the incubator 24 hours after delivery by Cesarean section was within the physiological range.

Fenbendazole was not a teratogen in the rabbit but was found to be fetotoxic. The no-effect level for the parameters studied was 10 mg/kg.

The no-effect levels for the pertinent studies are:

1.	Chronic rat study	5 mg/kg/day
2.	Carcinogenicity study in mice	405 mg/kg/day
3.	Six month dog study	4 mg/kg/day
4.	Three Generation Reproduction Study in rat	15 mg/kg/day
5.	Rat teratology study	250 mg/kg/day
6.	Rabbit teratology study	10 mg/kg/day

B. Safe Concentration of Residues

The most sensitive species was the dog in which a no-effect level of 4 mg/kg/day was found. For chronic toxicity a 100 fold safety factor is applied for calculating a safe concentration.

$$\text{Safe concentration (ppm)} = \frac{\text{Average human wt (kg)} \times \text{NEL (mg/kg)}}{\text{Food factor for meat} \times \text{safety factor}}$$

$$= (60)(4) / (0.5) \times 100 = 4.8$$

The safe concentration for total fenbendazole residues in muscle is 5 ppm. After applying appropriate consumption factors the safe concentration in liver (factor of 2) is 10 ppm, kidney (3) 15 ppm and fat (4) 20 ppm.

C. Metabolism Studies

Metabolism studies were conducted in laboratory animals and in cattle to identify the metabolites of fenbendazole and to select a marker substance for a regulatory method in food producing animals.

Metabolites were identified in feces, urine, blood and tissues of treated animals. The metabolite profiles from the blood of treated laboratory animals (dogs, rabbits and rats) and the blood of treated cattle were similar. The major metabolites of fenbendazole that people will consume (NH_2 metabolite, SO-metabolite, SO_2-metabolite, p OH-metabolite) were found in blood of all species. Therefore, the metabolites of fenbendazole have been adequately tested for toxicity.

One day after a cow was administered 10 mg ^{14}C-fenbndazole/kg body weight, 95% of the total residue in liver was chloroform extractable. Of the extractable residue, 79-83% was identified as being fenbendazole, while 6% was characterized as the sulfoxide metabolite, and 8% as the pOH-metabolite. Seven days after a heifer was administered 10 mg ^{14}C-fenbendazole/kg body weight, 47% of the total residue in liver was chloroform:methanol extractable with 13% of the extractable residue being characterized as fenbendazole.

Six heifers and two cows were orally administered 10 mg/kg of ^{14}C-fenbendazole. Two animals were sacrificed at each time point. Below is a table of the total residue depletion (in ppm) from each tissue.

DAYS POST MEDICATION

	1	7	14	30
muscle	1.2	0.038	0.004	0.004
liver	17.6	11.26	2.50	1.01
kidney	4.7	1.93	0.40	0.094
fat	4.3	0.042	0.013	0.011

Cattle liver is the target tissue with parent fenbendazole being the marker substance. The R_m is 0.8 ppm parent fenbendazole for cattle receiving a single oral dose of 10 mg fenbendazole/kg body weight, i.e., when total fenbendazole residues are 10 ppm in liver, there is 0.8 ppm parent fenbendazole present as determined by the regulatory assay.

D. Regulatory Methods

A method has been developed for the determination of fenbendazole at 0.8 ppm concentration levels and above. Fenbendazole is extracted from homogenized tissues with ethyl acetate. The dried extract is then partitioned between hexane and acetonitrile to remove lipid components. The acetonitrile phase is evaporated, and the residue is dissolved in methanol and assayed quantitatively for fenbendazole by reverse phase high pressure liquid chromatography. The confirmatory test involves further purification by silica gel thin layer chromatography. The isolated fenbendazole is transformed to a benzyl derivative by phase transfer alkylation. The derivative is assayed by high pressure liquid chromatography.

Results from the method trial validation will be inserted here after these trials are complete.

E. Withdrawal Time

The regulatory method was used to measure the depletion of the marker residue, fenbendazole, in the target tissue, liver, from animals treated with fenbendazole at 10 mg/kg body weight.

These results are presented in the table below:

RESIDUE DEPLETION IN CATTLE AFTER TREATMENT

	Day 1	Day 3	Day 6	Day 7	Day 8
Number of animals	5	3	3	4	3
Average ppm	6.64	3.17	0.26	0.25	0.083

A statistical evaluation of these data indicated that a withdrawal time of 8-days is necessary for the marker residue to deplete to its safe concentrations in the target tissue.

F. Safety to Handler

The drug was also evaluated for untoward effects which might result from physical contact with it:

A 5% and 10% dilution in sesame oil were not irritating when applied to rabbit skin either directly or by patch test. The same concentration was tolerated on the mucosa of the rabbit eye without reactions.

The drug was evaluated for sensitizing properties by intracutaneous injection together with Freud's adjuvant in guinea pigs and no sensitizing effect was found.

The drug was introduced into the trachea of sheep at twice the therapeutic dose (5 x 2 = 10 mg/kg). An increased respiratory frequency, spontaneous cough and slight elevated temperatures were seen. The symptoms occurred for two to three days after the administration and disappeared without treatment within 24 to 48 hours.

The studies demonstrated that the drug would have no ill effects on persons handling it if it is used according to label recommendations.

7. Agency Conclusions:

The data submitted in support of this NADA comply with the requirements of section 512 of the ACT and demonstrate that fenbendazole when used under its proposed conditions of use is safe and effective.

The Agency concludes that adequate directions for lay use have been written for the proposed conditions of use of the drug which is indicated for the removal and control of parasites commonly occurring in cattle. Fenbendazole has a wide spectrum of activity and the safety margin in cattle is greater than 5 times the recommended dose.

FINDING OF NO SIGNIFICANT IMPACT

for

Fenbendazole 10% Suspension

NADA 128-620
American Hoechst Corporation

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

American Hoechst Corporation of Somerville, New Jersey has filed a new animal drug application (NADA 128-620) providing for the use of fenbendazole 10% suspension as an oral dewormer for cattle. The suspension is added to the water of cattle for one day at a dose level of 5 mg fenbendazole/kg body weight. Fenbendazole is active against gastrointestinal nematodes and lungworms. Retreatment with fenbendazole after 4-6 weeks may be necessary if the treated cattle continue to be exposed to worms. The treated cattle can be slaughtered eight days after treatment.

The chemical name of fenbendazole is methyl 5-(phenylthio)-2-benzimidazole carbamate. There are a number of widely used compounds which like fenbendazole, contain the benzimidazole nucleus. The use of fenbendazole in cattle is expected to displace some of the other benzimidazole compounds already used in this species. Therefore significant additional introductions of benzimidazoles into the environment are not expected to occur.

Approval Date: March 28, 1996

Freedom of Information Summary
NADA 132-872

I. GENERAL INFORMATION:

NADA	132-872
Sponsor:	Hoechst-Roussel Agri-Vet Co. P. O. Box 2500 Route 202-206 Somerville, NJ 08876-1258
Generic Name:	fenbendazole
Trade Name:	Safe-Guard® Paste 10%; Panacur® Paste 10%
Marketing Status:	Over the Counter (OTC)
Effect of Supplement:	This supplement provides for the use of fenbendazole for the removal and control of gastrointestinal parasites and lungworm in dairy cattle of breeding age.

II. INDICATIONS FOR USE AND LABEL DOSE:

BEEF AND DAIRY CATTLE- INDICATIONS DOSAGE

For the removal and control of: 5 mg/kg

Lungworm: (*Dictyocaulus viviparus*)

Stomach Worm (adults):
Brown Stomach worm *(Ostertagia ostertagi)*.

Stomach Worm (adults & 4th stage larvae):
Barberpole Worm *(Haemonchus contortus/placei)*,
Small Stomach Worm *(Trichostrongylus axei)*.

Intestinal Worms (adults & 4th stage larvae):
Hookworm *(Bunostomum phlebotomum)*,
Threadneck Intestinal Worm *(Nematodirus helvetianus)*,
Small Intestinal Worms *(Cooperia oncophora, Cooperia punctata)*,
Bankrupt Worm *(Trichostrongylus colubriformis)*,
Nodular Worm *(Oesophagostomum radiatum)*.

III. EFFECTIVENESS:

Efficacy was established in the original approval under NADA 132-872 and its supplements (46 FR 32018, June 19, 1981; 47 FR 15327, April 9, 1982; 49 FR 8433, March 7, 1984; and

50 FR 26358, June 26, 1985). No new studies were conducted to establish effectiveness associated with the use of fenbendazole in dairy cattle of breeding age.

IV. TARGET ANIMAL SAFETY:

Animal safety was established in the original approval under NADA 132-872 and its supplements (46 FR 32018, June 19, 1981; 47 FR 15327, April 9, 1982; 49 FR 8433, March 7, 1984; and 50 FR 26358, June 26, 1985). No new studies were conducted to establish animal safety associated with the use of fenbendazole in dairy cattle of breeding age.

V. HUMAN FOOD SAFETY:

A. Toxicity Tests:

Toxicity and teratogenicity studies were presented in the original NADA 128-620 and were conducted in Hoechst Research Laboratories in Frankfurt, Germany and in the United States. Fenbendazole was determined to be safe to human health when food derived from treated animals is ingested (48 FR 42809, September 20, 1983).

B. Safe Concentrations and Tolerances

Safe concentrations for fenbendazole total residues in cattle tissues were established with the original NADA 128-620 and are listed below along with the tissue consumption factors that were used.

Tissue	*Safe Concentration*
muscle	5 ppm
liver	10 ppm (factor of 2)
kidney	15 ppm (factor of 3)
fat	20 ppm (factor of 4)

The tolerance and marker residue for fenbendazole in cattle also were assigned with the original NADA 128-620. The tolerance in cattle liver (the target tissue) is 0.8 ppm parent fenbendazole (the marker residue) as measured by the regulatory assay.

Newly established with this supplement to NADA 132-872 are a safe concentration and a tolerance for residues of fenbendazole in milk. The safe concentration for fenbendazole total residues in milk is set at 1.67 ppm (1/3 of the 5 ppm safe concentration in muscle tissue). The 1.67 ppm value was determined using FDA's approach to assigning safe concentrations based on food factors (44 FR 17070, March 20, 1979).

As explained in Part C below, the marker residue for fenbendazole in milk is the sulfoxide of parent fenbendazole. The tolerance is assigned at 0.6 ppm, although the marker residue never reaches that level in the milk from cattle treated at the approved dosing rate of 5 mg/kg body weight. The tolerance value was calculated from the marker residue to total residue percentage when total fenbendazole residues are at a maximum in milk. That maximum occurs in the range of 24 to 36 hours following dosing, and at that time, the sulfoxide

represents approximately 35% of the total residue present. Accordingly, the tolerance for the fenbendazole sulfoxide is set at 0.6 ppm (35% of the 1.67 ppm safe concentration).

C. Total Residue and Metabolism Studies

Tissue residue depletion and metabolism studies in cattle were presented in the original NADA 128-620. Based on data from those studies, an eight (8) day withdrawal time in edible tissues (muscle, liver, fat, kidney) was established (48 FR 42809, September 20, 1983). The total residue studies summarized below were submitted with this supplement to describe fenbendazole residues in milk.

Milk Total Residue Study.

A study designed to measure residues in milk from one untreated and five ^{14}C fenbendazole-treated lactating dairy cattle was conducted to determine the total residue profile as a function of time, to identify metabolites of fenbendazole in milk, and to select a marker substance to monitor residues in milk of lactating dairy cattle.

Study No.	U.S. Dairy Cow Milk Residue Study LAV #1506 SVM (LSU Account # 166-60-6166)
Starting Date	January 31, 1992
End Date	August 5, 1993
Study Director	Dr. Steven A. Barker School of Veterinary Medicine Louisiana State University Baton Rouge, LA 70803
Identification of Substance and Dosage Form	14C-fenbendazole 1.89 mCi/g in aqueous suspension
Species and Age	Holstein, 33 months to 7 years
Number of Animals/weight	Six lactating dairy cows; average 603 kg
Drug Level Tested	5.0 mg/kg body weight
Route of Administration	Oral, administered once

After an acclimatization period, a morning milk sample was taken from each cow prior to treatment. This sample served as a blank control for the study. Following this milking, five cows received fenbendazole suspension by stomach tube. This aqueous suspension of labeled (^{14}C) fenbendazole and unlabeled fenbendazole contained approximately 2 mCi activity/g. The amount of fenbendazole administered to each cow by stomach tube was calculated to equal 5.0 mg/kg body weight. The stomach tube was flushed with suspension solution to assure complete delivery. A control cow received suspension solution which did not contain

fenbendazole. Morning and afternoon milk samples were collected for six days following drug administration. After six days the residues were below the level of detection.

Total residues for each whole milk sample from each cow were determined by scintillation counting. Each sample was assayed in triplicate by dissolving 0.5 mL aliquots of blended sample in 12 mL of scintillation cocktail. Before counting, each sample was placed in the dark for one hour to reduce contributions from chemiluminescence. Selected whole milk samples were also centrifuged, and 0.5 mL aliquots of fat and water portions were counted to determine label distribution.

For metabolic profiling, milk samples were extracted by matrix solid phase dispersion (MSPD) techniques, and the absolute recovery of total label was determined by scintillation counting of the extracts. The distribution of the extracted label between remaining parent drug and metabolites was determined by HPLC analyses using UV diode array and in-line radiolabel detection. The identity of radiolabeled peaks was matched with known standards for the metabolites of fenbendazole based on retention time and UV-diode array spectra. Samples were also assayed quantitatively by HPLC using an internal (mebendazole) standard and correcting for recovery.

The results from the radiolabel assay for total residues in whole milk and the HPLC analyses of metabolites in whole milk averaged for the five cows as a function of time are presented in Table 1.

Table 1. Average Concentrations of Total Residues and Metabolites of Fenbendazole in Whole Milk as a Function of Time Following Oral Administration of 5.0 mg Fenbendazole/kg Body Weight to Five Lactating Dairy Cows*.

Day, Milking	Total Residue (± SD) µg/mL n = 5	FBZ-SO (± SD) µg/mL n = 5 (Sulfoxide)	FBZ-SO2 (± SD) µg/mL n = 5 (Sulfone)	FBZ/Total Residue x 100 Ratio %
1, am (Time 0)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000
1, pm	0.060 ± 0.043	0.026 ± 0.025	0.000 ± 0.000	43.333
2, am	0.482 ± 0.076	0.232 ± 0.045	0.018 ± 0.011	48.133
2, pm	0.526 ± 0.111	0.186 ± 0.005	0.024 ± 0.013	35.361**
3, am	0.408 ± 0.102	0.158 ± 0.026	0.062 ± 0.016	38.725
3, pm	0.298 ± 0.086	0.088 ± 0.034	0.046 ± 0.033	29.530
4, am	0.186 ± 0.080	0.030 ± 0.030	0.046 ± 0.024	16.129
4, pm	0.108 ± 0.044	0.006 ± 0.013	0.014 ± 0.017	5.555
5, am	0.054 ± 0.030	0.000 ± 0.000	0.010 ± 0.017	0.000
5, pm	0.024 ± 0.015	0.000 ± 0.000	0.000 ± 0.000	0.000
6, am	0.012 ± 0.008	0.000 ± 0.000	0.000 ± 0.000	0.000
6, pm	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000

*All residue levels were below the target of 0.83 ppm for the 1X tracer study (one-half the 1.67 ppm established safe concentration). No residues were

detected in milk from the placebo (control) cow.
**Ratio percent used to calculate tolerance level.

At all times following administration of fenbendazole to lactating dairy cattle, residues in milk of fenbendazole and its metabolites were below the established safe concentration, and the total residue was evenly distributed between the fat and aqueous fractions of the whole milk.

Metabolic profiling of the total residues indicated that the concentration of parent drug in milk was negligible. The sulfoxide and sulfone metabolites of fenbendazole were the compounds that contributed to milk residues. The sulfoxide metabolite of fenbendazole was established to be the marker residue as it was present at levels significantly higher than parent fenbendazole or its sulfone metabolite. No other metabolites of fenbendazole were found in milk.

Milk Tolerance Calculation.

In Table 1 above, the ratio percent value of fenbendazole sulfoxide, the marker residue, to total residues was 35.4% at 36 hours following fenbendazole administration. At this time total residues in milk were greatest. The tolerance was calculated by multiplying the ratio percent of fenbendazole sulfoxide to total residues by the safe concentration (1.67 ppm). The tolerance was established to be 0.6 ppm (600 ppb).

D. Calf Tissue Total Residue Study.

A study was conducted to measure residues in calves born to ^{14}C fenbendazole-treated dairy cattle. Total residue profiles in calf liver, kidney, fat and muscle were measured to provide data demonstrating the extent to which fenbendazole and its metabolites are transferred to and retained by the tissues of calves born to fenbendazole-treated cows.

Study No.	U.S. Dairy Calf Tissue Residue Study LAV #1507 SVM (LSU Account # 166-60-6167)
Starting Date	April 21, 1992
End Date	August 5, 1993
Study Director	Dr. Steven A. Barker School of Veterinary Medicine Louisiana State University Baton Rouge, LA 70803
Identification of Substance and Dosage Form	14C-fenbendazole, 1.89, 1.95 and 2.09 mCi/g in aqueous suspension
Species and Age	Holstein, 3 years old or older
Number of Animals/weight	Six pregnant dairy cows; average 616 kg

Drug Level Tested 5.0 mg/kg body weight

Route of Administration Oral, administered once to the cow

Eight days prior to anticipated calving, six pregnant dairy cows were moved to an approved facility for acclimation and for study conduct. Three days after the start acclimation, each cow was administered fenbendazole by stomach tube at a dose calculated to equal 5.0 mg/kg body weight. The drug was administered as an aqueous suspension of labeled (^{14}C) and unlabeled fenbendazole and contained approximately 2 mCi activity/g. One of the six cows was administered carrier only and was the control for the study. The calf from the control cow and calves from three cows receiving fenbendazole were delivered by cesarean surgery approximately 70 hours after dosing; the other two calves from treated cows were delivered by natural birth at 4 and 25 hours post-dosing. One calf died 5 hours after delivery, three treated calves and the control calf were sacrificed 24 hours after delivery, and one treated calf was sacrificed 48 hours after delivery. Surviving calves received colostrum from treated dams and milk replacer as needed for 24 to 48 hours after birth and prior to sacrifice.

Total residues for the described tissues were determined by oxidation of 0.5 g tissue samples in triplicate and scintillation counting (Table 1). Each sample was placed in the dark for one hour to reduce contributions from chemiluminescence.

Table 1. Concentrations of Total Residues of Fenbendazole and Metabolites in Calf Tissues Following Administration of 5.0 mg Fenbendazole/kg Body Weight to Five Pregnant Dairy Cows.

Tissue	*Total Residue (µg/g)*
liver	1.398 ± 0.998*
kidney	0.528 ± 0.383
fat	0.386 ± 0.400
muscle	0.306 ± 0.236

*mean ± SD; n = 5

For metabolic profiling, liver tissue from one calf was extracted by matrix solid phase dispersion (MSPD) techniques, and the absolute recovery of total label was determined by scintillation counting of the extracts. The distribution of the extracted label between parent drug and metabolites was determined by HPLC analyses using UV diode array and in-line radiolabel detection. The identity of radiolabeled peaks was matched with known standards for the metabolites of fenbendazole based on retention time and UV-diode array spectra.

Results indicated that the label was distributed between the sulfone (34%) and sulfoxide (58%) metabolites of fenbendazole and parent fenbendazole (8%). No other radiolabeled metabolites were observed in the liver. Profiles of kidney, fat and muscle tissue from all calves using HPLC indicated the presence of the sulfoxide and sulfone metabolites. The

parent drug, fenbendazole, was present in trace quantities. No other metabolites were indicated.

It was concluded from the residue data above, that in calves born to and consuming colostrum from fenbendazole-treated dams, residues of fenbendazole in liver, kidney, fat and muscle were below the established safe concentrations. Residue levels in liver, kidney, fat and muscle as a percent of the safe concentrations were 13.98%, 3.52%, 1.93% and 6.12%, respectively. Therefore, meat from calves born to fenbendazole-treated dams is safe even when fenbendazole is administered prior to parturition.

E. Milk Residue Tolerance Study.

A study with non-radiolabeled fenbendazole was conducted to determine the total quantity of fenbendazole and its metabolites in whole milk as a function of time and to expand the examination to include use of the actual market formulation. A further objective was to determine whether incurred fenbendazole residues or its metabolites demonstrate activity in three commonly used milk antibiotic screening tests, Charm II assay, Delvotest P, and *Bacillus stearothermophilis* disc assay. For this study fenbendazole paste 10% at a rate of 5 mg/kg body weight was administered to ten lactating dairy cows; an additional cow served as a control.

Study No.	U.S. Dairy Cow Milk Residue Study LAV #1591 SVM (LSU Account # 166-60-6172)
Starting Date	November 3, 1992
End Date	July 16, 1993
Study Director	Dr. Steven A. Barker School of Veterinary Medicine Louisiana State University Baton Rouge, LA 70803
Identification of Substance and Dosage Form	Fenbendazole, Safe-Guard® Paste 10% (100 mg/g)
Species and Age	Holstein, > 20 kg milk per day
Number of Cows/weight	Eleven lactating dairy cows; average 530 kgs
Drug Level Tested	5.0 mg/kg body weight
Route of Administration	Oral, administered once

Animals used in this study were selected from the Holstein herd maintained at the LSU Agricultural Experimental Station. All lactating dairy cows were at least thirty days postpartum and were producing a target minimum of 20 kg of milk per day. Animals were managed in the same manner as the remaining cow herd. The ration consisted of concentrate

and corn silage, and the cows grazed Bermuda and rye grass. Cows were monitored for reproductive function, were bred by artificial insemination, and were treated for reproductive dysfunction according to standard herd practices.

Cows were weighed within twenty-four (24) hours of drug administration. The ten treated cows received Safe-Guard® Paste 10% in an amount to equal delivery of 5.0 mg fenbendazole/kg body weight. The control cow was untreated.

Cows were machine milked in the morning prior to treatment. Milk samples were collected at that milking and were used as blank controls for the study. Milk samples (100 mL) were then collected at the 4:00 AM and 4:00 PM milkings for seven days following fenbendazole treatment.

For metabolic profiling, milk samples were extracted by matrix solid phase dispersion (MSPD) technique. The amount of parent drug and metabolites was determined quantitatively by HPLC analyses using UV diode array detection. The identity of peaks was matched with known standards for the metabolites of fenbendazole based on retention time and UV-diode array spectra.

The administration of fenbendazole at a target dose of 5.0 mg/kg body weight as paste 10% to lactating dairy cows produced residues in whole milk identifiable as fenbendazole sulfoxide, fenbendazole sulfone and trace quantities of fenbendazole. Peak residue time in milk was twenty-four (24) hours after administration, and the peak fenbendazole sulfoxide marker level was 0.24 ± 0.03 µg/mL (Table 1). No residues of fenbendazole were detected in the control cow.

Table 1. Concentrations of Fenbendazole and Marker Metabolites of Fenbendazole in Whole Milk as a Function of Time Following Oral Administration of Paste 10% (100 mg/gm) at a Rate of 5.0 mg Fenbendazole/kg Body Weight to Ten Lactating Dairy Cows.

Time after FBZ Administration	Fenbendazole (± SD), µg/mL, n= 10	FBZ-SO (± SD), µg/mL, n= 10 (Sulfoxide)**	FBZ-SO2 (± SD), µg/mL, n= 10 (Sulfone)
0	nd*	nd	nd
12	nd	0.15 ± 0.06	0.01 ± 0.00
24	nd	0.24 ± 0.03	0.08 ± 0.01
36	nd	0.19 ± 0.03	0.11 ± 0.01
48	nd	0.10 ± 0.02	0.11 ± 0.01
60	nd	0.03 ± 0.01	0.08 ± 0.01
72	nd	0.00 ± 0.00	0.03 ± 0.00

*No residues detected.
**Marker residue

Antibiotic residue test screening was conducted on milk samples from three (3) treated cows chosen randomly. The samples were collected at 12 hour intervals for 72 hours post-dose .

Tests performed included the Charm II assay, Delvotest P, and *Bacillus stearothermophilis* disc assay. Zero time samples were included in all antibiotic screening tests; Delvotest P and *B. stearothermophilis* disc assay also included milk collected from the control animal at 12 hour intervals for 72 hours post-dose. Examinations indicated that the incurred residues from cows receiving fenbendazole suspension 10% at a rate of 5.0 mg/kg body weight had no discernible or consistent effect on the assays in term of producing false positive or suspect sample results. No sample from any cow examined gave a "positive" response to the Delvotest P and *Bacillus stearothermophilis* disc assay. Assay results of ten antibiotic classes indicated that fenbendazole and its metabolites do not interfere or cross-react with any consistency in the Charm II assay.

It was concluded that the fenbendazole sulfoxide marker residue level was below the tolerance level, and therefore, total residues were below the established safe concentration for milk. A zero-day withdrawal period was approved for use of fenbendazole paste 10% in dairy cattle of breeding age. It was further concluded that use of fenbendazole does not interfere with the practice of antibiotic drug screening.

F. Milk Discard and Slaughter Time

A zero (0) milk discard time is established for fenbendazole in dairy cattle of breeding age. The milk residue depletion studies described in Parts C and E above demonstrate that the maximum levels of fenbendazole residues in milk are well below the 1.67 ppm safe concentration and 0.6 ppm tolerance when lactating dairy cows are treated at the approved dosing rate of 5 mg/kg body weight. Accordingly, no discard of milk is required following treatment with fenbendazole.

An eight (8) day withdrawal time in edible tissues (muscle, liver, fat and kidney) was established in the original NADA 128-620 (48 FR 42809, September 20, 1983) and applies to dairy cows treated with fenbendazole.

G. Regulatory Methods:

A regulatory milk assay method is not required because of the establishment of a zero (O) milk withdrawal period in lactating dairy cattle. However, an HPLC assay method is on file at FDA/CVM in Rockville, MD.

A regulatory tissue method was developed as part of the original fenbendazole approval. The method, entitled, "Determination Procedure for the Measurement of Fenbendazole in Bovine Liver Tissue", is on file at the FDA's Freedom of Information Office, 5600 Fishers Lane, Rockville, MD 20857.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this supplement satisfy the requirement of Section 512 of the Federal Food, Drug, and Cosmetic Act (FFDCA). The toxicology data on fenbendazole that were submitted with the original NADA 128-620 have allowed the establishment of a

safe concentration of 1.67 ppm for total residues of fenbendazole in milk. From the residue and metabolite data on fenbendazole in dairy cattle that was submitted with this supplement, a tolerance of 0.6 ppm is established as the tolerance for residues in milk of the fenbendazole sulfoxide metabolite (the marker residue). Because the maximum levels of residues found in milk of fenbendazole-treated cattle are well below the safe concentration and tolerance noted above, no discard of milk (zero milk withdrawal) is required. The slaughter withdrawal time of 8 days required for treated dairy cattle is the same as established for beef cattle under the original NADA 132-872.

Under the Center's supplemental approval policy [21 CFR 514.106(b)(2)(v and x)], the addition of dairy cattle to the claim is a Category II change. The approval of this change is not expected to have any adverse effect on the safety or effectiveness of this new animal drug. Accordingly, this approval did not require a reevaluation of the safety and effectiveness data in the parent application.

Under section 512(c)(2)(F)(iii) of the FFDCA, this approval for food producing animals does not qualify for exclusivity because the supplemental application does not contain new clinical or field investigations (other than bioequivalence or residue studies) and new human food safety studies (other than bioequivalence or residue studies) essential to the approval and conducted or sponsored by the applicant.

VII. LABELING (Attached)

Supplement labels: #1. Safe-Guard® (fenbendazole) Paste 10% syringe label for cattle including dairy cattle of breeding age. 3.2 oz.

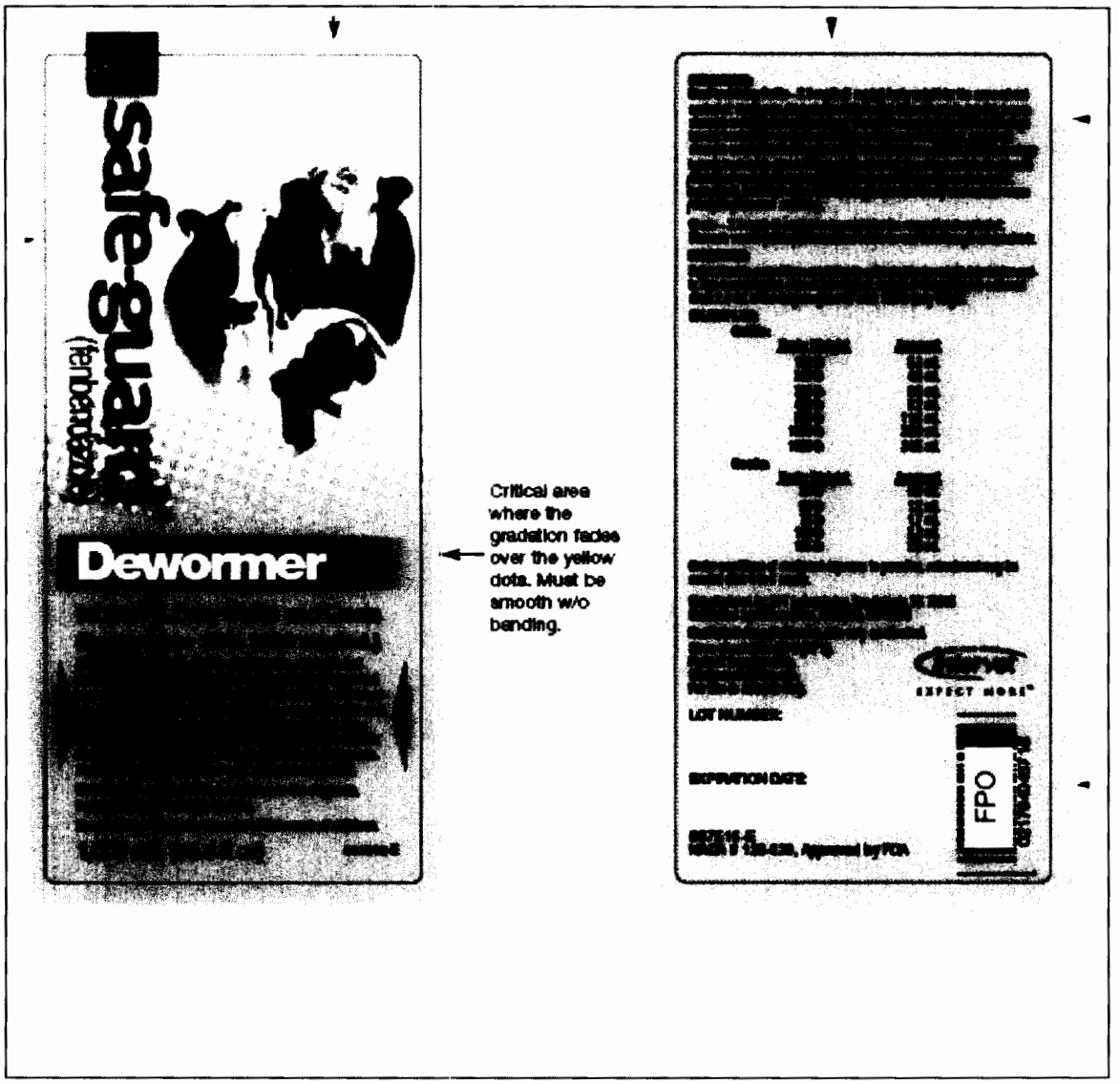
#2 Panacur® (fenbendazole) Paste 10% syringe label for cattle including dairy cattle of breeding age. 3.2 oz.

Copies of applicable labels may be obtained by writing to the:

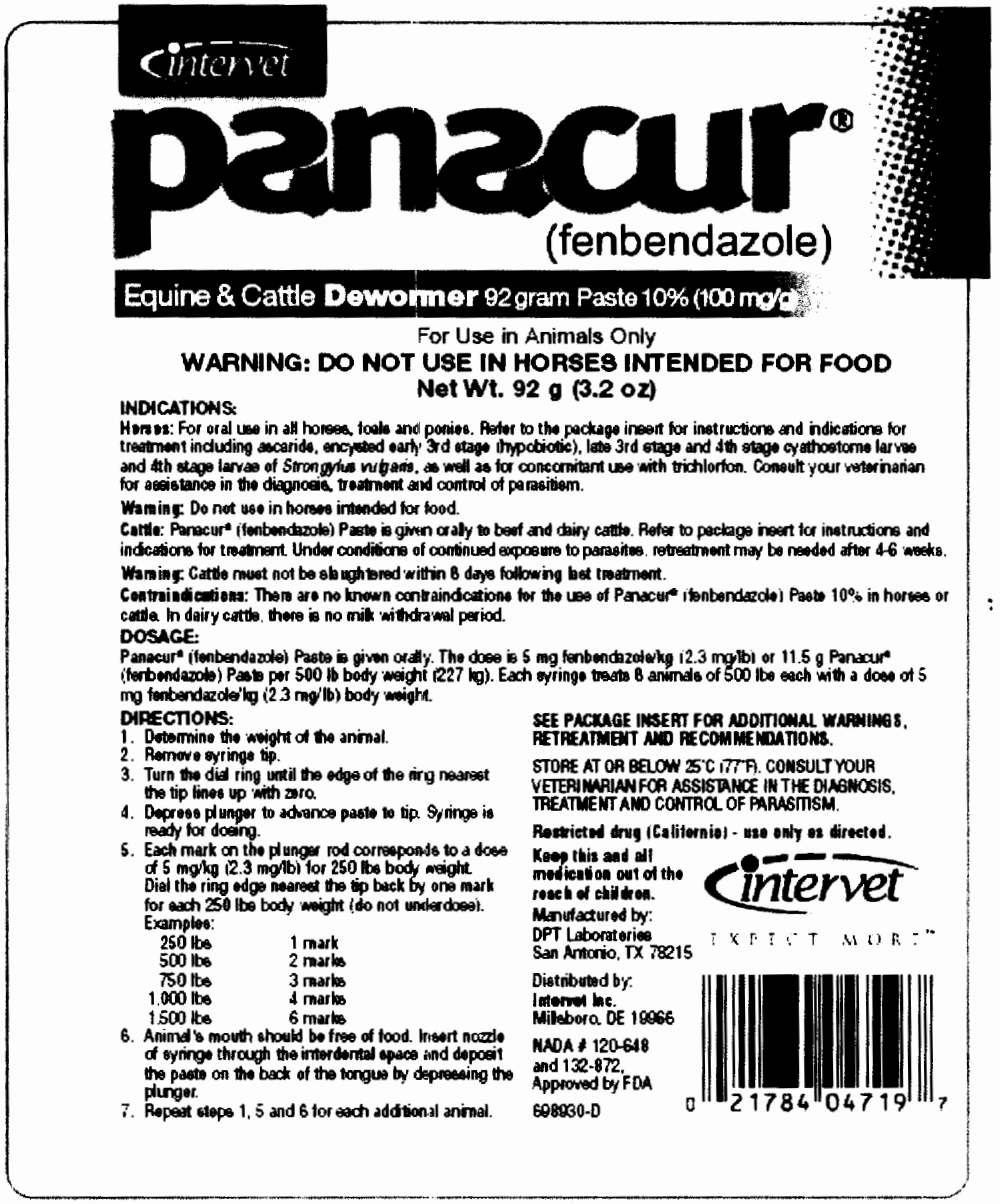
Food and Drug Administration
Freedom of Information Staff (HFI-35)
5600 Fishers Lane
Rockville, MD 20857

Or requests may be sent via fax to: (301) 443-1726. If there are problems sending a fax, call (301) 443-2414.

Attachment C
Product Labels
Safeguard drench: NADA # 128-620



Attachment C
Product Labels
Panacur drench: NADA #s 104-494 and 128-620



Intervet

panacur®

(fenbendazole)

Equine & Cattle **Dewormer** 92 gram Paste 10% (100 mg/g)

For Use in Animals Only

WARNING: DO NOT USE IN HORSES INTENDED FOR FOOD

Net Wt. 92 g (3.2 oz)

INDICATIONS:

Horses: For oral use in all horses, foals and ponies. Refer to the package insert for instructions and indications for treatment including ascaride, encysted early 3rd stage (hypobiotic), late 3rd stage and 4th stage cyathostome larvae and 4th stage larvae of *Strongylus vulgaris*, as well as for concomitant use with trichlorfon. Consult your veterinarian for assistance in the diagnosis, treatment and control of parasitism.

Warning: Do not use in horses intended for food.

Cattle: Panacur® (fenbendazole) Paste is given orally to beef and dairy cattle. Refer to package insert for instructions and indications for treatment. Under conditions of continued exposure to parasites, retreatment may be needed after 4-6 weeks.

Warning: Cattle must not be slaughtered within 8 days following last treatment.

Contraindications: There are no known contraindications for the use of Panacur® (fenbendazole) Paste 10% in horses or cattle. In dairy cattle, there is no milk withdrawal period.

DOSAGE:

Panacur® (fenbendazole) Paste is given orally. The dose is 5 mg fenbendazole/kg (2.3 mg/lb) or 11.5 g Panacur® (fenbendazole) Paste per 500 lb body weight (227 kg). Each syringe treats 8 animals of 500 lbs each with a dose of 5 mg fenbendazole/kg (2.3 mg/lb) body weight.

DIRECTIONS:

1. Determine the weight of the animal.
2. Remove syringe tip.
3. Turn the dial ring until the edge of the ring nearest the tip lines up with zero.
4. Depress plunger to advance paste to tip. Syringe is ready for dosing.
5. Each mark on the plunger rod corresponds to a dose of 5 mg/kg (2.3 mg/lb) for 250 lbs body weight. Dial the ring edge nearest the tip back by one mark for each 250 lbs body weight (do not underdose). Examples:

250 lbs	1 mark
500 lbs	2 marks
750 lbs	3 marks
1,000 lbs	4 marks
1,500 lbs	6 marks

6. Animal's mouth should be free of food. Insert nozzle of syringe through the interdental space and deposit the paste on the back of the tongue by depressing the plunger.
7. Repeat steps 1, 5 and 6 for each additional animal.

SEE PACKAGE INSERT FOR ADDITIONAL WARNINGS, RETREATMENT AND RECOMMENDATIONS.

STORE AT OR BELOW 25°C (77°F). CONSULT YOUR VETERINARIAN FOR ASSISTANCE IN THE DIAGNOSIS, TREATMENT AND CONTROL OF PARASITISM.

Restricted drug (California) - use only as directed.

Keep this and all medication out of the reach of children.

Intervet

EXPECT MORE™

Manufactured by:
DPT Laboratories
San Antonio, TX 78215

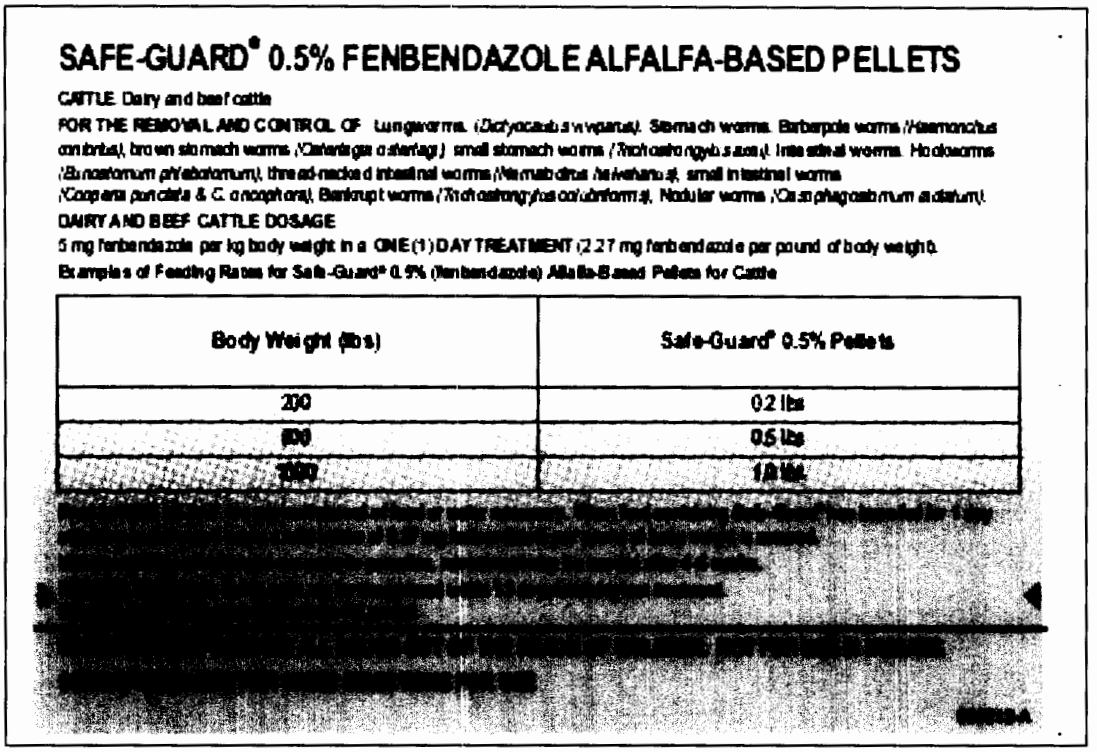
Distributed by:
Intervet Inc.
Millsboro, DE 19966

NADA # 120-648
and 132-872,
Approved by FDA

698930-D

0 21784 04719 7

Attachment C
Approved Product Labels
Safeguard .5% (top dress pellets): NAC No.: 11061772



SAFE-GUARD® 0.5% FENBENDAZOLE ALFALFA-BASED PELLETS

CATTLE: Dairy and beef cattle

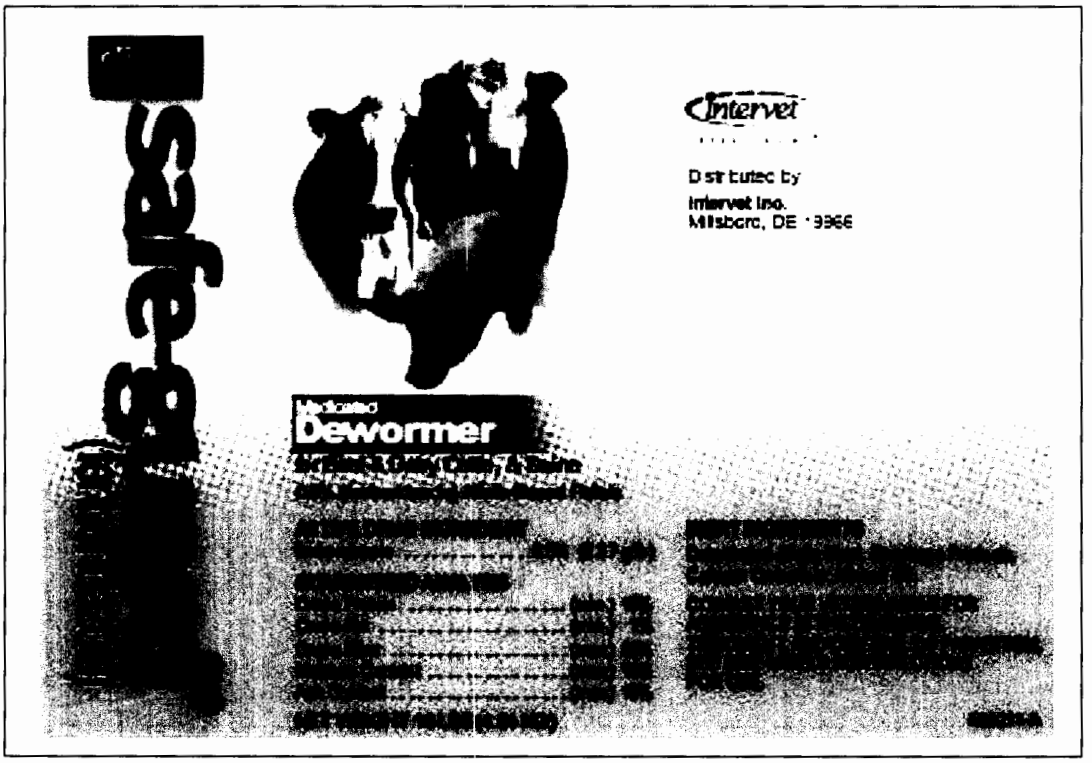
FOR THE REMOVAL AND CONTROL OF: Lungworms (*Dictyocaulus viviparus*), Stomach worms, Barberpole worms (*Haemonchus contortus*), brown stomach worms (*Ostertagia ostertagi*), small stomach worms (*Trichostrongylus* spp.), Intestinal worms, Hookworms (*Bunostomum phlebotomum*), thread-necked intestinal worms (*Nematodirus helvetianus*), small intestinal worms (*Cooperia punctata* & *C. oncophora*), Bankrupt worms (*Trichostrongylus colubriformis*), Nodular worms (*Oesophagostomum radiatum*).

DAIRY AND BEEF CATTLE DOSAGE

5 mg fenbendazole per kg body weight in a ONE (1) DAY TREATMENT (2.27 mg fenbendazole per pound of body weight).

Examples of Feeding Rates for Safe-Guard® 0.5% (fenbendazole) Alfalfa-Based Pellets for Cattle

Body Weight (lbs)	Safe-Guard® 0.5% Pellets
200	0.2 lbs
400	0.5 lbs
800	1.0 lbs



Safe-Guard

Dewormer

Intervet

Distributed by
Intervet Inc.
Millsboro, DE 19966

Attachment C
Approved Product Labels
Safeguard 1.96%: (flaked meal) NAC No.: 11061781

[redacted]

PMS 341 PMS 109

intervet

safe-guard

(fenbendazole)

Medicated Dewormer

for Beef & Dairy Cattle

Flaked Meal (1.96% fenbendazole)
(Scoop Included)

ACTIVE DRUG INGREDIENT:
Fenbendazole ..... 19.6 grams per kilogram (8.9 grams per pound)

OTHER INGREDIENTS:
Rice Hulls, Calcium Carbonate and Mineral Oil.

GUARANTEED ANALYSIS:

Calcium (Ca) ..... (min) 19.0%
Calcium (Ca) ..... (max) 22.5%

For dairy cattle, there is no milk withdrawal period.

intervet
EXPECT MORE

Distributed by:
Intervet Inc.
Millsboro, DE 19966

585300-B

Type B Medicated Feed
Net Weight 25 pounds (11.34 kg)

PMS 341 PMS 109

safe-guard®

(fenbendazole)

Type B Medicated

[redacted]

for Beef & Dairy Cattle

[redacted] (1.98% fenbendazole)

DAIRY CATTLE AND BEEF CATTLE DOSAGE:

5 mg [redacted] per kg body weight in one (1) day treatment (2.27 mg per pound body weight).

DAIRY CATTLE MUST NOT BE SLAUGHTERED WITHIN 13 DAYS FOLLOWING LAST TREATMENT.

[redacted] DOSAGE:

Feed one (1) [redacted] Safe-Guard® Dewormer to the daily ration of the [redacted] 1,000 pounds of body weight (one (1) scoop per 1,000 pounds body weight).

THIS PACKAGE WILL TREAT THE FOLLOWING NUMBER OF CATTLE.

WEIGHT OF CATTLE	NO. HEAD TREATED PER PACKAGE
[redacted] lbs	195
1,000 lbs	98
1,300 lbs	65

Feed for one (1) day. No prior withdrawal of feed or water necessary. When feed containing Safe-Guard® has been fed for 1 day and blended according to the directions based on weight and number of cattle treated, a [redacted] 2.27 mg fenbendazole per pound of body weight is assured.

Under conditions of continued exposure to parasites, retreatment may be needed after 4-6 weeks.

For dairy cattle, there is no milk withdrawal period.

GENERAL USE DIRECTIONS:

Safe-Guard® Dewormer must be spread uniformly on top of the daily ration. Sufficient bunk space must be available so all animals can eat at the same time to ensure uniform consumption. All cattle must be group managed to ensure that each animal consumes an adequate amount. Safe-Guard® Dewormer can also be uniformly blended with the complete ration at the feed mill.

CONSULT YOUR VETERINARIAN FOR ASSISTANCE IN THE DIAGNOSIS, TREATMENT AND CONTROL OF PARASITISM.

FOR THE REMOVAL AND CONTROL OF:

Lungworms *(Dictyocaulus viviparus)*, Stomach worms: Barberpole worms *(Haemonchus contortus)*, Brown stomach worms *(Ostertagia ostertagi)*, Small stomach worms *(Trichostrongylus axei, Trichostrongylus colubriformis)*, Hookworms *(Bunostomum phlebotomum)*, Thread-necked intestinal worms *(Nematodirus helvetianus)*, Small intestinal worms *(Cooperia punctata, C. oncophora)*, Bankrupt worms *(Trichostrongylus colubriformis)*, Nodular worms *(Oesophagostomum radiatum)*.

STORE AT ROOM TEMPERATURE

MUST BE MIXED BEFORE FEEDING ACCORDING TO DIRECTIONS AND PERMITTED CLAIMS.

FOR USE IN MANUFACTURED FEEDS ONLY.

Intervet

EXPECT MORE

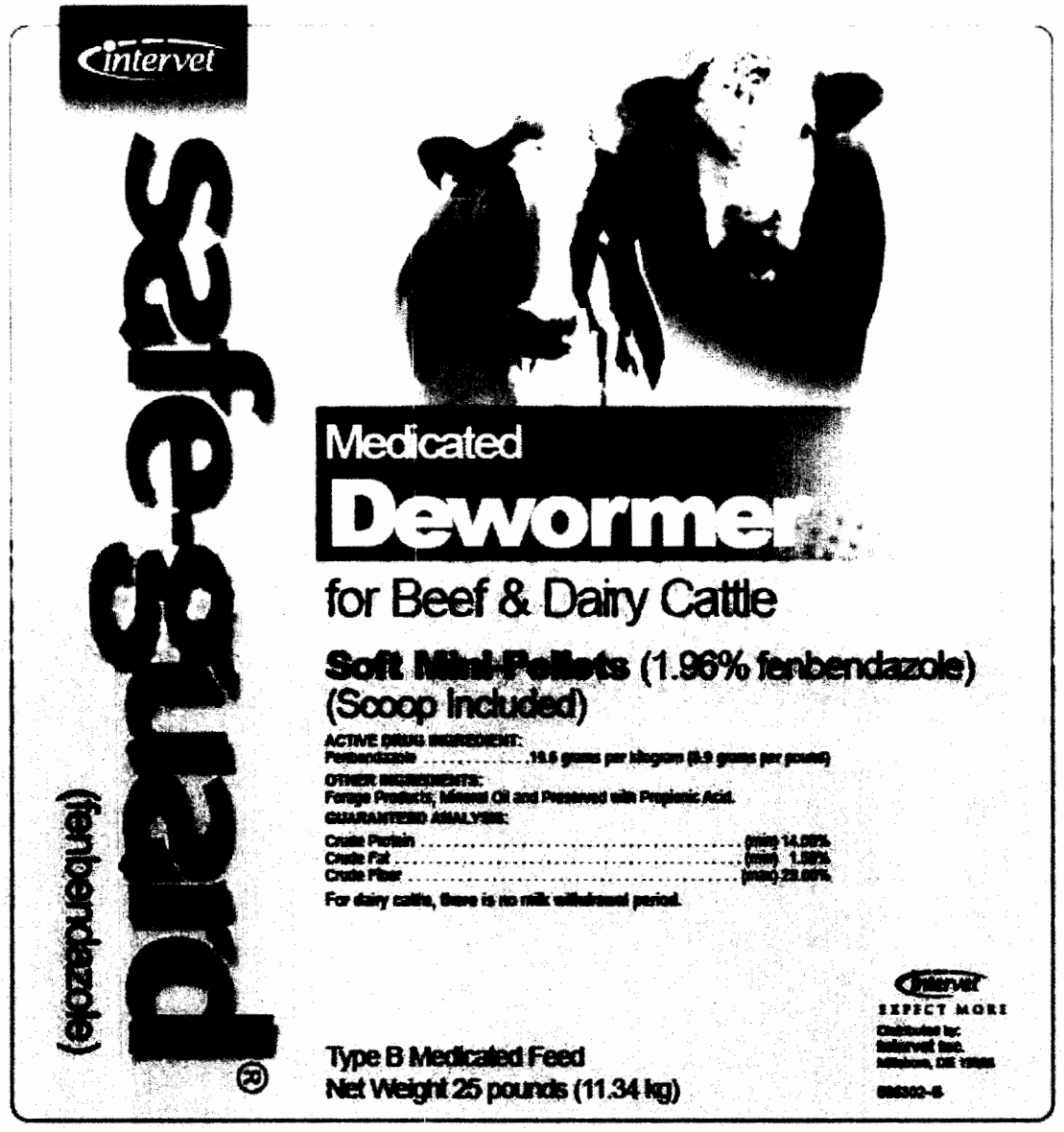
Distributed by:
Intervet Inc.
Millsboro, DE 19966

585301-B

FPO

NET WT 25 pounds (11.34 kg)

Attachment C
Approved Product Labels
Safeguard 1.96%: (soft mini pellet) NAC No.: 11061791



safe-guard®

(fenbendazole)

Type B Medicated

Dewormer

for Beef & Dairy Cattle

Safe While Pregnant (1.90% fenbendazole)

DAIRY CATTLE AND BEEF CATTLE DOSAGE:

▶ WARNING: CATTLE MUST NOT BE SLAUGHTERED WITHIN 13 DAYS FOLLOWING LAST TREATMENT. ◀

DURATION OF DOSING AND FEEDING RATE:

WEIGHT OF CATTLE	NO. HEAD TREATED PER PACKAGE
500 lbs	196
1,000 lbs	98
1,500 lbs	65

GENERAL USE DIRECTIONS:

CONSULT YOUR VETERINARIAN FOR ASSISTANCE IN THE DIAGNOSIS, TREATMENT AND CONTROL OF PARASITISM.

FOR THE REMOVAL AND CONTROL OF:

STORE AT ROOM TEMPERATURE.

MUST BE MIXED BEFORE FEEDING ACCORDING TO DIRECTIONS AND PERMITTED CLAIMS.

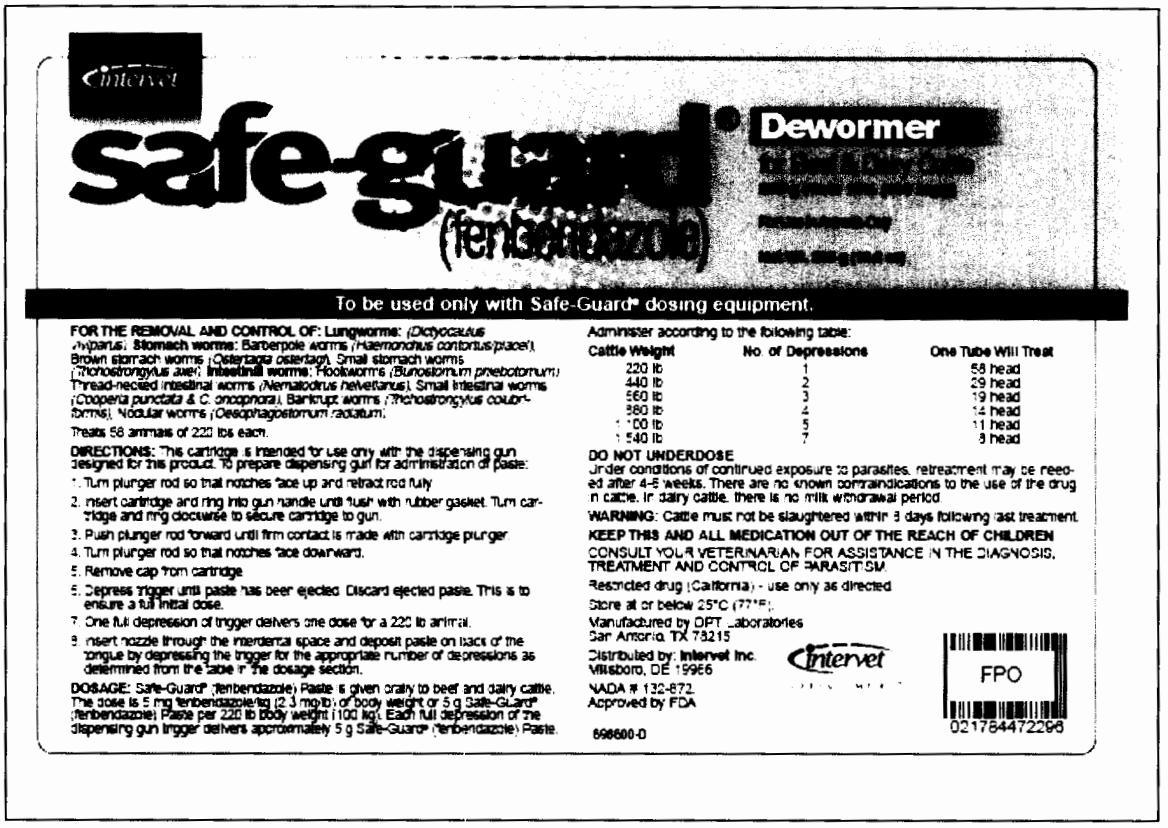
FOR USE IN MANUFACTURED FEEDS ONLY.

EXPECT MORE

FPO

NET WT 25 pounds (11.34 kg)

Attachment C
Approved Product Labels
Safeguard 290 gm paste: NADA # 132-872



Intervet

safe-guard® Dewormer

(fenbendazole)

To be used only with Safe-Guard® dosing equipment.

FOR THE REMOVAL AND CONTROL OF: Lungworms: *(Dictyocaulus viviparus);* **Stomach worms:** Barberpole worms *(Haemonchus contortus/placei),* Brown stomach worms *(Ostertagia ostertagi),* Small stomach worms *(Trichostrongylus axei);* **Intestinal worms:** Hookworms *(Bunostomum phlebotomum),* Thread-necked intestinal worms *(Nematodirus helvetianus),* Small intestinal worms *(Cooperia punctata & C. oncophora),* Bankrupt worms *(Trichostrongylus colubriformis),* Nodular worms *(Oesophagostomum radiatum).*

Treats 58 animals of 220 lbs each.

DIRECTIONS: This cartridge is intended for use only with the dispensing gun designed for this product. To prepare dispensing gun for administration of paste:

1. Turn plunger rod so that notches face up and retract rod fully.
2. Insert cartridge and ring into gun handle until flush with rubber gasket. Turn cartridge and ring clockwise to secure cartridge to gun.
3. Push plunger rod forward until firm contact is made with cartridge plunger.
4. Turn plunger rod so that notches face downward.
5. Remove cap from cartridge.
6. Depress trigger until paste has been ejected. Discard ejected paste. This is to ensure a full initial dose.
7. One full depression of trigger delivers one dose for a 220 lb animal.
8. Insert nozzle through the interdental space and deposit paste on back of the tongue by depressing the trigger for the appropriate number of depressions as determined from the table in the dosage section.

DOSAGE: Safe-Guard® (fenbendazole) Paste is given orally to beef and dairy cattle. The dose is 5 mg fenbendazole/kg (2.3 mg/lb) of body weight or 5 g Safe-Guard® (fenbendazole) Paste per 220 lb body weight (100 kg). Each full depression of the dispensing gun trigger delivers approximately 5 g Safe-Guard® (fenbendazole) Paste.

Administer according to the following table:

Cattle Weight	No. of Depressions	One Tube Will Treat
220 lb	1	58 head
440 lb	2	29 head
660 lb	3	19 head
880 lb	4	14 head
1100 lb	5	11 head
1540 lb	7	8 head

DO NOT UNDERDOSE

Under conditions of continued exposure to parasites, retreatment may be needed after 4-6 weeks. There are no known contraindications to the use of the drug in cattle. In dairy cattle, there is no milk withdrawal period.

WARNING: Cattle must not be slaughtered within 8 days following last treatment.

KEEP THIS AND ALL MEDICATION OUT OF THE REACH OF CHILDREN

CONSULT YOUR VETERINARIAN FOR ASSISTANCE IN THE DIAGNOSIS, TREATMENT AND CONTROL OF PARASITISM.

Restricted drug (California) - use only as directed.

Store at or below 25°C (77°F).

Manufactured by DPT Laboratories
San Antonio, TX 78215

Distributed by: **Intervet Inc.**
Millsboro, DE 19966

NADA # 132-872,
Approved by FDA

698600-0

FPO

021784472298

Attachment D
Material Safety Data Sheet

MATERIAL SAFETY DATA SHEET

Intervet — SAFE-GUARD(R) 0.5% TOP DRESS PELLETS

VERSION DATE: 6/2002

1. CHEMICAL PRODUCT and COMPANY IDENTIFICATION

Product Name: SAFE-GUARD(R)
Product Family: PHARMACEUTICALS

PRODUCT:	PRODUCT CODE:
Safe-Guard(R) 35% Salt: Free Choice Mineral Mix	SG-474-20
Safe-Guard(R) 0.5% Top Dress Pellets	SG-469-10
Safe-Guard(R) 290GM Cartridge	SG-472-290GM
Safe-Guard(R) 20% Salt: Free Choice Mineral Mix	SG-459-25
Safe-Guard(R) 1.96% Soft Mini Pellets	SG-458-25
Safe-Guard(R) 1.96% Flaked Meal	SG-457-25
Safe-Guard(R) Liters	SG-467-1LT
Safe-Guard(R) Gallons	SG-467-1GAL
Safe-Guard(R) Paste-25 Gram	SG-471-25-12
Safe-Guard(R) Paste-92 Gram	SG-471-92-12
Safe-Guard(R) EZ Scoop	SG-463-10
Safe-Guard(R) En-pro-al blocks	SG-465-25
Safe-Guard(R) Sweetlix(R) 20% Protein blocks	SG-464-25
Safe-Guard(R) Premix 20%	SG-473-25
Safe-Guard(R) Granules	SG-470-5.2-10, 48

SYNONYMS: FENBENDAZOLE
PRODUCT USE: Refer to product packaging or insert for proper usage.
COMPANY ADDRESS- Intervet Inc - 405 State Street - Millsboro, DE. 19966

2. COMPOSITION / INFORMATION on INGREDIENTS

HAZARDOUS COMPONENT:	CONCENTRATION:	CAS NUMBER:
FENBENDAZOLE	0.5%-20%	43210-67-9

3. HAZARDS IDENTIFICATION

ROUTES OF ENTRY: Dermal, Injection, Inhalation, Ingestion
ACUTE EFFECTS OF EXPOSURE: May cause irritation at site of contact.
CHONIC EFFECTS OF EXPOSURE: None known
CARCENOGINIC EFFECTS: This product is not considered a carcinogen and is not listed by OSHA, IRAC or NTP.

4. FIRST AID MEASURES

SKIN: Wash immediately affected area with soap and water. Contact a physician.
EYES: Immediately flush with plenty of water for fifteen minutes. Contact a physician
INHALATION: Remove to fresh air. If not breathing, give artificial respiration and call for medical help immediately.
INGESTION: Seek medical attention immediately.

5. FIRE FIGHTING MEASURES

EXTINGUISHING METHODS: Use Water, Water Mist, Foam or Dry Chemical to extinguish fire.
FIRE FIGHTING INSTRUCTIONS: Wear full bunker gear, including SCBA, for fighting fires involving large quantities of this material. Keep upwind.

6. ACCIDENTAL RELEASE MEASURES

PROCEDURES IN CASE OF SPILL OR LEAK: Clean up spilled material. Place in a secure container for disposal.

7. HANDLING and STORAGE

STORAGE: Store at room temperature. Keep material dry. Protect containers from damage.
SHELF LIFE: See expiration date on product label.
HANDLING PRECAUTIONS: Do not empty contents of sacks into vessels containing a combustible mixture of gasses. Static discharge may ignite vapors or gasses. Equipment used in handling this product should be electrically grounded to prevent possible dust explosion.

8. EXPOSURE CONTROL / PERSONAL PROTECTION

EYES: Prevent eye contact by wearing appropriate eye protection for handling tasks.
SKIN: Avoid skin contact. Wear chemical resistant gloves, long-sleeves and trousers to prevent dermal contact.
RESPIRATOR PROTECTION: Under normal conditions of use, as stated in the product insert, no respiratory protection is necessary. However, if ventilation is inadequate wear a NIOSH approved respirator.

9. PHYSICAL and CHEMICAL PROPERTIES

APPEARANCE: Dry mixture with inert edible excipients. See product label for details.
pH: 5.0-7.0

10. STABILITY and REACTIVITY

CHEMICAL STABILITY: Stable
CONDITIONS TO AVOID: None known
INCOMPATABILITY: None Known
HAZARDOUS POLYMERIZATION: Will not occur

11. TOXICOLOGICAL INFORMATION

Oral LD 50 Rat: Greater than 10,000 mg/kg
Intraperitoneal LD50 (rat): Not available
Intraperitoneal LD50 (mouse): Not available

12. ECOLOGICAL INFORMATION

ECOTOXITY: LC 50 greater than 500 mg/L (48 and 96 hrs) Zebrafish

13. DISPOSAL CONSIDERATIONS

Waste should be incinerated.

14. TRANSPORTATION

DOT SHIPPING INFORMATION: Not regulated by the DOT

15. REGULATORY INFORMATION

STATE REGULATIONS: The following chemicals associated with the product are subject to the Right-To-Know regulations in these states:
Mineral Oil (8012-95-1): IL, LA, MA, RI
U.S. FEDERAL REGULATIONS: Sara 313: No components listed
This product is listed with the FDA for use in animals.

16. OTHER INFORMATION

DISCLAIMER: The information contained herein is true and accurate to the best of the knowledge of Intervet Inc. However, all data, instructions and/or recommendations are made without guarantee. The buyer and handler assume all risk and liability of use, storage and/or handling of this product not in accordance with the terms of the product label.

Attachment D (Cont.)
Material Safety Data Sheet

EMERGENCY:

HUMAN, FIRE, SPILL OR ENVIRONMENTAL: 1-800-228-5635
EXT. 132 24 HRS.
ANIMAL: 1-800-345-4735 EXT. 104 24 HRS.
CHEMTREC(R) FOR CHEMICAL EMERGENCY SPILL, LEAK, FIRE: 1-800-424-9300

PRODUCT INFORMATION: 1-800-441-8272 OR 1-302-934-8051
8:00 A.M. - 5:00 P.M. EST

DISCLAIMER OF EXPRESSED AND IMPLIED WARRANTIES: Although preparer and owner have taken reasonable care in the preparation of this document, we extend no warranties and make no representation as to the accuracy or completeness of the information contained herein, and assume no responsibility regarding the suitability of this information for the user's intended purposes or for the consequences of its use. Each user should make a determination as to the suitability of the information for their particular purpose(s). This MSDS is the most recent received from the product manufacturer or distributor.

COMPAS Code: 46501605

Attachment E
Research Information

BARKER, S; KAPPEL L (1997) Drug residues in milk from cows administered fenbendazole as a paste, drench, or feed top-dressing, Journal of Veterinary Pharmacology and Therapeutics, Volume 20 Issue 2 Page 160 - April 1997

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BROWN, W.R. (1982) Lifetime oral toxicity study of fenbendazole in rats histopathology - liver. Research Pathology Services Inc., New Britain, Penna. USA unpublished report. Submitted to WHO by Hoechst AG, Frankfurt am Main, Germany.

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Anthelmintic Resistance: An Examination of its Growing Prevalence in the U.S. Cattle Herd

Executive Summary of the 2005 Anthelmintic Resistance Roundtable

INTRODUCTION

When livestock producers use anthelmintic parasite-control products in their herd and fail to see a response, there are a number of factors to consider. Was the timing of use appropriate to minimize re-infection? Did the dosage match the weight of the animals? Or did the product fail to achieve a response because the parasite population has become resistant to the dewormer of choice?

The demands of maximizing production have led today's beef and dairy producers to adopt preventive control measures that include regular treatment of their cattle with an anthelmintic parasite-control product during the grazing season. This intensive management approach combined with environmental factors and dosing practices is believed to have resulted in the selection of parasites resistant to some classes of anthelmintic products.

Parasite resistance is a potentially costly problem and will likely continue to be a concern within the United States beef and dairy industries.

Anthelmintic resistance in cattle is becoming a worldwide problem. But, until recently, there was little or no documented research on parasite resistance to commonly used bovine anthelmintics in the United States. Meanwhile, countries with livestock numbers comparable to the United States have reported occurrences of resistance in areas where cattle producers extensively used dewormers. Parasite resistance is a potentially costly problem and will likely continue to be a concern within the United States beef and dairy industries.

To address this information void, Intervet, a leading global animal-health company, brought together the top experts in the field of parasitology for an in-depth, roundtable discussion on anthelmintic resistance in the United States.

As host of the 2005 Anthelmintic Resistance Roundtable, Intervet sought to open a dialog about resistance, its diagnosis, its economic effect on the U.S. cattle industry and solutions to prevent it.

This executive summary was generated from comments made during the five-hour roundtable discussion held in conjunction with the 2005 American Association of Veterinary Parasitologists (AAVP) annual convention in Minneapolis, Minn.

PARTICIPANTS:

Dwight Bowman, Ph.D. — *professor of parasitology, Cornell University*

Don Bliss, Ph.D. — *parasitologist at the MidAmerica Agricultural Research Center, Madison, Wis.*

Tom Craig, D.V.M. — *professor of parasitology, Texas A&M*

Louis C. Gasbarre, Ph.D.— *immunologist and research lead with USDA-ARS*

Bill Kvasnicka, D.V.M. — *parasitologist (retired Extension Veterinarian University of Nevada)*

Gil Myers, Ph.D. — *Myers Parasitology Services, Magnolia, Ky.*

Jim Miller, D.V.M. — *professor of parasitology, Louisiana State University*

Cliff Monahan, D.V.M., Ph.D. — *professor of parasitology, The Ohio State University*

MODERATOR:

Bert Stromberg, Ph.D. — *professor and associate dean for research and graduate programs, University of Minnesota College of Veterinary Medicine*

RESISTANCE IN THE U.S CATTLE HERD

To date, research of anthelmintic resistance around the world has focused primarily on sheep and goats. Researchers in the United States have documented resistance in horses as well. But only in the past couple of years have reports of resistance in U.S. cattle herds been documented and presented to the veterinary community.

One of the first cases documented in the United States was described by Dr. Lou Gasbarre, research leader with USDA's Agricultural Research Service, and Larry Smith, DVM, Smith Research and Development, Inc., Lodi, Wis., who studied a Wisconsin background operation where the owner noticed an apparent decrease in the effectiveness of his strategic anthelmintic program. Upon evaluating parasite loads, it was noted that treatment with ivermectin injectable, moxidectin pour-on, doramectin injectable, eprinomectin pour-on or albendazole oral did not result in parasite burden reductions of at least 80 percent.

"In the past two to three years, it has become evident that the modern anthelmintics upon which the American cattle industry relies have begun to show diminished efficacy," said Dr. Don Bliss, parasitologist at the MidAmerica Agricultural Research Center, Madison, Wis., who has monitored thousands of fecal samples from cattle throughout the United States over the past 20 years. Bliss said he sees examples of anthelmintic resistance on a daily basis.

HOW TO DIAGNOSE RESISTANCE

During the roundtable, a general consensus was developed that the fecal egg count reduction test (FECRT) remains the most practical tool to help parasitologists identify resistance to anthelmintics by nematodes.

The fecal egg count reduction test remains the most practical tool to help parasitologists identify resistance to anthelmintics by nematodes.

"The FECRT can be used to examine whether or not we are seeing loss of efficacy with these drugs," said Dr. Dwight Bowman, professor of parasitology at Cornell University.

He pointed out that when they came onto the market, the drugs had to prove their efficacy in order to get approval.

Gasbarre noted that while fecal egg counts are a good measure of what is happening in groups of animals, it is not reliable in an individual animal. "If you are going to evaluate a failure based on just a couple of animals, I think you would be misusing the technology," said Gasbarre. "If you are going to look at a single animal, it should be tested by multiple samples, not just once."

In addition to what tests are used, the panel noted that a consistent method of testing is also important.

"Like a lot of parasitologists, I felt that if we got our samples seven days after treatment, we had a representative sampling," explained Dr. Gil Myers, owner of Myers Parasitology Services in Magnolia, Ky.

"We now know that with the avermectins, for example, we really need to be sampling about 14 days after treatment. We don't understand all the reasons for that, but in studies from Ohio, the results at 14 days were entirely different than at seven days. There were many more parasitized animals and many more parasite eggs found. In contrast, the cattle that were treated with the Safe-Guard® (fenbendazole) or Panacur® product, there's no difference between day 7 and day 14 egg counts."

It appears that avermectins do not kill adult worms as fast as benzimidazoles.

It was noted that USDA statisticians believe 17 animals, no matter what the herd size, is a good sample number. The test group, however, should consist of animals of similar age. Focusing on younger animals also provides the most accurate measure of parasite worm load, according to the expert panel.

IDENTIFYING RESISTANCE

While the FECRT is a valuable tool to measure the parasite burden within an animal, the test itself doesn't define resistance. The panelists gathered at the Anthelmintic Roundtable discussed in detail not only what resistance is, but also what it is not.

"Resistance is selected for because it is a genetic switch. You are not changing something in the worm," explained Bowman. "By giving dewormers, we are not inducing resistance, we are simply providing an environment for the resistant [parasites] to flourish and multiply."

Dr. Cliff Monahan, professor of parasitology at The Ohio State University, further explained that users of dewormers are not creating mutants. "We are merely creating an environment where that particular genetic makeup survives," he said. "If we look at some of the drug treatments where it is 99.9 percent effective, .1 percent of the [parasites] survive, and they now have carte blanche to reproduce. So their genetic pool becomes the dominant pool."

The panel noted that resistance is not the development of "superworms."
"I define resistance as the failure of an anthelmintic to adversely affect helminthes in a specific host as efficiently as it formerly had done so," added Dr. Tom Craig, veterinarian at Texas A&M University.

Resistance is selected because it is a genetic switch. You are not changing something in the worm... We are simply providing an environment for the resistant [parasites] to flourish and multiply.

When a producer administers a parasite-control product and fails to see a result, the efficacy of that product is in question. To the producer, it was a product failure. The question becomes, why was the product not efficacious? Was it excessive parasite burden? Was the proper dose given for the animal's body weight? Was the timing wrong, allowing for re-infection through grazing? Or had the resistant parasites in the herd reached damaging levels?

CAUSES OF RESISTANCE DEVELOPMENT

Nematodes with the genetic makeup to withstand treatment are given the opportunity to flourish when susceptible nematodes are eliminated. It appears, however, that several environmental factors affect how quickly or how often resistant parasites flourish.

"We see a great deal of herd variation, and this stands to reason because no two cattle operations are managed the same or is their history of dewormer use the same," said Myers.

How producers handle the cattle, their stocking rate, age of cattle, pasture contamination level at the start of grazing and weather conditions all help determine parasite challenges more than location. It is generally accepted that the greater the parasite challenge, the harder it will be to have successful treatment. Preventing seasonal buildup of parasite contamination is important to preventing parasite resistance from developing within the animals.

Adding to the discussion on environmental factors, Dr. Jim Miller, professor of parasitology at Louisiana State University, noted that anthelmintic resistance is not contained to a certain region or area of the country. He noted that when dealing with resistance, one must look at the individual farm involved because what goes on there can be different from what goes on right down the road.

Of concern to the livestock industry when it comes to resistance is that there are limited tools in the parasite control toolbox. Only three major anthelmintic families are used in the United States, the endectocides, which are ivermectin, doramectin, eprinomectin and moxidectin; the benzimidazoles, which primarily include fenbendazole, oxfendazole and albendazole; and the imidazoles, which include levamisole and morantel tartrate. Use of imidazoles is very limited leaving only two classes to be used extensively.

Frequent and repeated use of the same drug class of anthelmintic was determined to be a considerable risk factor for development of resistance. It was noted that producers are missing opportunities for improved production by deworming just with endectocides because of poor efficacy and the increased opportunity for resistance.

Another risk factor discussed by the panel was the effect of subtherapeutic drug levels on the survivability of resistant worms. It was reasoned that some parasites survived subtherapeutic levels of treatment that would have been eradicated by exposure to a full dose of the drug. The panel had concerns about endectocides that persist in the animal and pasture environment at low levels for an extended duration of time.

It was also noted that endectocide pour-ons have created a phenomenon of low and variable blood-serum levels compared with injectable formulations, which may allow resistant parasites to flourish. Inconsistent absorption rates and improper dosing compound the problem.

Myers pointed out that there are two simple practices that producers can do to decrease the risk of anthelmintic resistance. "Know the weights of the animals you are treating, and make sure you are treating them with the proper amount of the drug," he said. "The literature clearly indicates that under-dosing can lead to drug resistance."

Frequent and repeated use of the same drug class of anthelmintic was determined to be a considerable risk factor for development of resistance.

The panel also sought to address what effect persistent activity — when a drug is present in the animal or environment for a prolonged period of time — had on the potential development of resistance.

Dr. Bill Kvasnicka, parasitologist and retired extension veterinarian from the University of Nevada, noted that persistent anthelmintic activity trials published as far back as 1995 discussed prolonged decreasing of blood levels. "This feature may protect animals from re-infection from some nematodes for up to four weeks or longer," he noted, "but slowly decreasing concentrations of anthelmintic in an animal can select for resistance."

"I have had the opportunity to test nearly every pour-on product sold today," said Bliss. "Based on what I've found and what the World Association for the Advancement of Veterinary Parasitology (WAAVP) standards are for a dewormer, I believe we are seeing true parasite resistance with the endectocide pour-ons.

"We've had the persistent doramectin (Dectomax®) products out in the market now for maybe 10 years or less," continued Bliss. "We are seeing a number of reports of worldwide potential resistance product failure. Persistent products may very well trigger resistance more quickly.

In contrast, fenbendazole (Safe-Guard) has been on the market for over 20 years with only one or two reports of anthelmintic resistance. Safe-Guard works fast and doesn't linger in the animal or the environment for a prolonged period of time.

Gasbarre noted that from a theoretical standpoint, the more selective pressure you place on a population, the more likely you are to select for traits that confer an advantage. So if the loss of efficacy is due to selection of a different population, any enhancement of persistence or long-lasting selection theoretically would result in resistance arising even faster.

PROJECTING AN IMPACT

As producers increase their use of anthelmintics and expose them to more parasites, more resistant parasites likely will evolve.

"We find parasites are present on nearly all operations and are one of the most important deterrents to efficient production that producers have to deal with," noted Bliss. "The most recent production-based deworming trials show that as cattle become more efficient in terms of their genetic potential, the more important parasites become in terms of the economic loss sustained."

Resistance takes a long time to develop, and it is a gradual process, said Myers. "The process takes time, and producers are not going to have a real clear-cut red light go on and say 'Bingo, the worms are resistant.' That's not going to happen. What we will see, and what producers are beginning to report, is 'The calves are just not gaining.'"

Monahan said he believes the spread of the drug resistance across the country will be under the radar for most producers. "But when it hits an individual producer it won't be a gradual loss of productivity," he said. "It's going to hit the individual producer extremely hard."

The most recent production-based deworming trials show that as cattle become more efficient in terms of their genetic potential, the more important parasites become in terms of the economic loss sustained.

"I believe producers are using anthelmintics more and more because they are convinced of the profitability of using them, added Gasbarre. "Any increased number of times that parasites are exposed to drugs should select for more resistance."

The panel agreed that as the trend toward more intensive operations continues, the industry could expect to see the problem of anthelmintic resistance grow.

Also of concern is that parasites have a negative effect on immunity, according to research done by Gasbarre and his team at the USDA Bovine Functional Genomics Laboratory.

Research has shown that while parasite burdens can trigger an immune response in cattle, response can ultimately shut down immune response to other infectious agents. The roundtable participants recognized that the economic value of having a strong immune system in animal production is extremely high for today's high-performance cattle. The effect of parasites on the immune system is another example of how important it is to have the dewormers working properly.

LOOKING AHEAD

Livestock producers have become accustomed to being able to control parasites with easy-to-use and inexpensive anthelmintic dewormers, so the economic impact of uncontrolled parasites in livestock does not register as a top priority. Meanwhile, modern management practices that include higher stocking densities and intensive grassland management mean parasites potentially represent a more serious threat than they did before the introduction of broad-spectrum anthelmintics.

It is unlikely that any new anthelmintics will be introduced in the near future, so loss of efficacy of existing products could pose severe problems for the cattle industry. Anthelmintic resistance is already a serious problem in some parts of the world, not just in sheep and goats but also in cattle. With that in mind, the participants of the Anthelmintic Resistance Roundtable identified important next steps necessary to address and slow the advancement of resistance in the U.S. cattle herd.

First, the panel determined that a standardized protocol for testing parasite burden should be established within the cattle industry against which all deworming practices can equally be measured and evaluated. The panelists agreed that a committee should be set up to identify the best testing protocol.

Second, the panel recommended that a shared database is needed to effectively monitor resistance development.

"I think it is important that we understand and we get some kind of handle on exactly how widespread the issue of drug resistance is in this country," said Gasbarre. "There have been a couple of published reports and other anecdotal reports, but we really don't have any idea of what is really out there. We don't know what drugs work where, and why they still work, and why the ones that don't work don't work."

It is unlikely that any new anthelmintics will be introduced in the near future, so loss of efficacy of existing products could pose severe problems for the cattle industry.

Third, it was agreed that increased awareness is necessary to encourage producers to work with their veterinarians to monitor what their dewormer is doing by testing their herd regularly in a consistent manner.

Participants noted that producers within the industry may not recognize the significance of resistance problems until it hits them in the pocketbook, but producers need to be educated and made aware of the warning signs for anthelmintic resistance.

"I don't think anything is going to happen until they recognize the problem themselves," explained Gasbarre. "But I think we can make them aware that this is a potential problem that they ought to look for. Otherwise, they won't notice it until it's too late."

Fourth, the panel concluded that an updated protocol for strategic deworming is needed. It was noted that a parasite control program that seeks total suppression leads to resistance development at a rapid pace. A program to reduce pasture contamination and keep the parasite population at an economically acceptable level would extend product efficacy and maintain profitability in the long run.

A second meeting of leading parasitologists and veterinary researchers will be held in August 2006 to serve as a workshop for addressing resistance issues identified by the Anthelmintic Resistance Roundtable.

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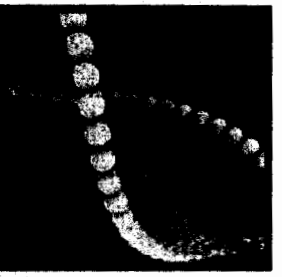
Finding The Genetic Link
To Parasite Resistance In Grazing Cattle



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Dr. Lou Gasbarre stands with the herd of Angus cattle he manages as research leader for the ARS bovine functional genomic laboratory in Beltsville, Md. (Photo by Dave Lefever)

BELTSVILLE, Md. — Lou Gasbarre knows how much of a toll internal parasites can take on grazing beef and dairy cattle.

As a regular part of his work, Dr. Gasbarre records the effects these worms can cause in the beef cattle he manages on USDA's Agricultural Research Service (ARS) farm in Beltsville, Md.

Over the years, he has observed these organisms developing widespread resistance to worming drugs, making parasites increasingly worrisome to graziers across the country.

The most promising part of Gasbarre's work involves pinpointing genes that are linked to natural parasite resistance in cattle. One of the practical results of this research is that in the near future, graziers will likely have the option to select cattle that are genetically resistant to parasites.

Gasbarre is research leader in the bovine functional genomics program at Beltsville. He was trained as an immunologist, but it became clear to him early on that he would not be able to use that knowledge to develop a way to inoculate cattle against parasites. The complexity of the worms made achieving this goal unlikely, if not impossible.

"It became apparent to me that I was going to spend my career futilely because I couldn't create a (vaccine) for parasites," Gasbarre said last November during a tour of the beef operation and laboratory he manages at Beltsville.

Instead, Gasbarre sees that genetics holds the key to parasite resistance. So that's where he decided to devote his talents and energy.

The work has been paying off. The research done by Gasbarre and his team of 11 scientists, along with new gene-mapping technologies, have helped identify why some cattle are resistant to parasites while others are not.

"We know right now that in cattle there are at least eight locations (in the genome) that have genes or a group of genes that will tell whether that animal will be parasite resistant," Gasbarre said.

The most dominant and ***economically significant types of internal parasites in cattle in the U.S. are nematodes — the brown stomach worm Ostertagia and intestinal worms Cooperia and Nematodirus.***

The dominance of these parasites is "pretty consistent throughout the U.S.," Gasbarre said. He noted that some other parasites can also have an impact, mainly in the Southeast.

The worms' unhealthful effects include interfering with protein digestion, causing bleeding in the gut, and throwing off salt and protein balance in tissues. ***An often overlooked effect is that the worms can make animals lose their appetite and the cattle can actually become anorexic,*** Gasbarre said.

continued on back

Researchers have documented the increasing resistance of these parasites to medications (wormers) in recent years.

"What's happening in the U.S., unfortunately, is resistance to ivermectin products," Gasbarre said.

Ivermectin, which goes by various chemical and brand names, is the most common class of wormers used for cattle. There are few treatment alternatives to ivermectin products.

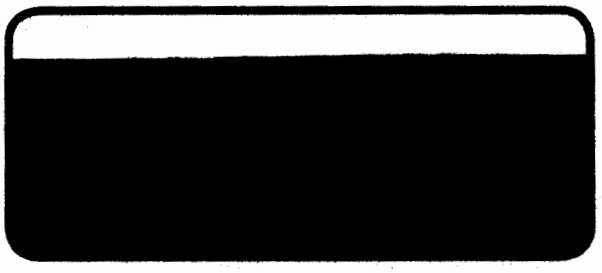
Overuse of ivermectin, along with intensive grazing, are practices that have contributed to the parasites' adapting to the point where they are no longer controlled by the drugs.

"We know that in the U.S. there are parasites that none of the drugs are effective against," Gasbarre said.

Because the nematodes' life cycle requires a grass environment, producers who keep their animals entirely in confinement operations generally don't have to worry about the parasites.

Instead of working on developing new chemicals to treat worms, Gasbarre is excited about using the burgeoning knowledge of cattle genetics to help select parasite-resistant animals.

Scientists at Baylor University in Texas are expected to soon complete sequencing of the bovine genome, mapping the complete array of genes found within cattle.



"Once that's established, it's going to help us tremendously with all this data we've backlogged," Gasbarre said.

Gasbarre and his crew collect performance data on calves that are raised on pastures heavily infested with parasites. The calves are timed to wean in mid-April to correspond with the grazing season. Weight gain and other data is collected on the animals each week through October.

In calves that have the least protection built into their genetic code to withstand parasites, the effects can often be observed by the eye in the thin, lackluster appearance of the animals.

Those that are able to withstand the heavy worm load and continue to perform well are noted as animals that are genetically superior when it comes to parasite resistance.

High-tech tools found in Gasbarre's laboratory to decipher the genetic makeup of cattle include a DNA analyzer for sequencing DNA fragments and a bead array reader (developed during the human genome project) used for identifying DNA bases.

The computer stack used to process genetic information in the laboratory is enormous, consisting of 30 high-powered processors, operating about 7,000 times as fast as a top-of-the-line home computer, Gasbarre noted. All of this research has wide implications for the livestock and dairy industries far beyond just parasite resistance. The days of relying on phenotypes (observed traits) in animals to develop bull proofs, for example, will soon be replaced by "molecular markers" that will be able to show exactly which traits are superior, Gasbarre said.

The herd Gasbarre works with on the research farm are derived from the Wye Angus originally developed on the Eastern Shore of Maryland. He also works with a dairy grazier in Somerset County, Pa. and with an organic beef grazier in Maryland on targeting parasite management.

Fenbendazole (Safe-Guard®) and it's ecological implications

(or, more than you ever wanted to know about cow manure)

Pasture management

Tom Shelton DVM

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Dung Beetle Benefits to the Pasture Ecosystem

1. Feed on manure
2. Improve nutrient cycling, soil structure and forage growth
3. Reduce nutrient contamination of waterways from runoff.

Information from ATTRA (Appropriate Technology Transfer for Rural Areas), under a grant from the USDA. October 2001

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Order: Coleoptera
Family: Scarabaeidae

- >90 species in U.S.
  - "Tumblers"
  - "Tunnelers"
  - "Dwellers"
    - Less than a dozen significant in dung burial

Appearance and Behavior

- Size
  - .1 to 2.5 inches
- Colors
  - black to brown to red with some "metallic" in appearance
- Drawn to manure by odor
  - (up to 10 miles)
- Utilize liquid contents of manure for nourishment

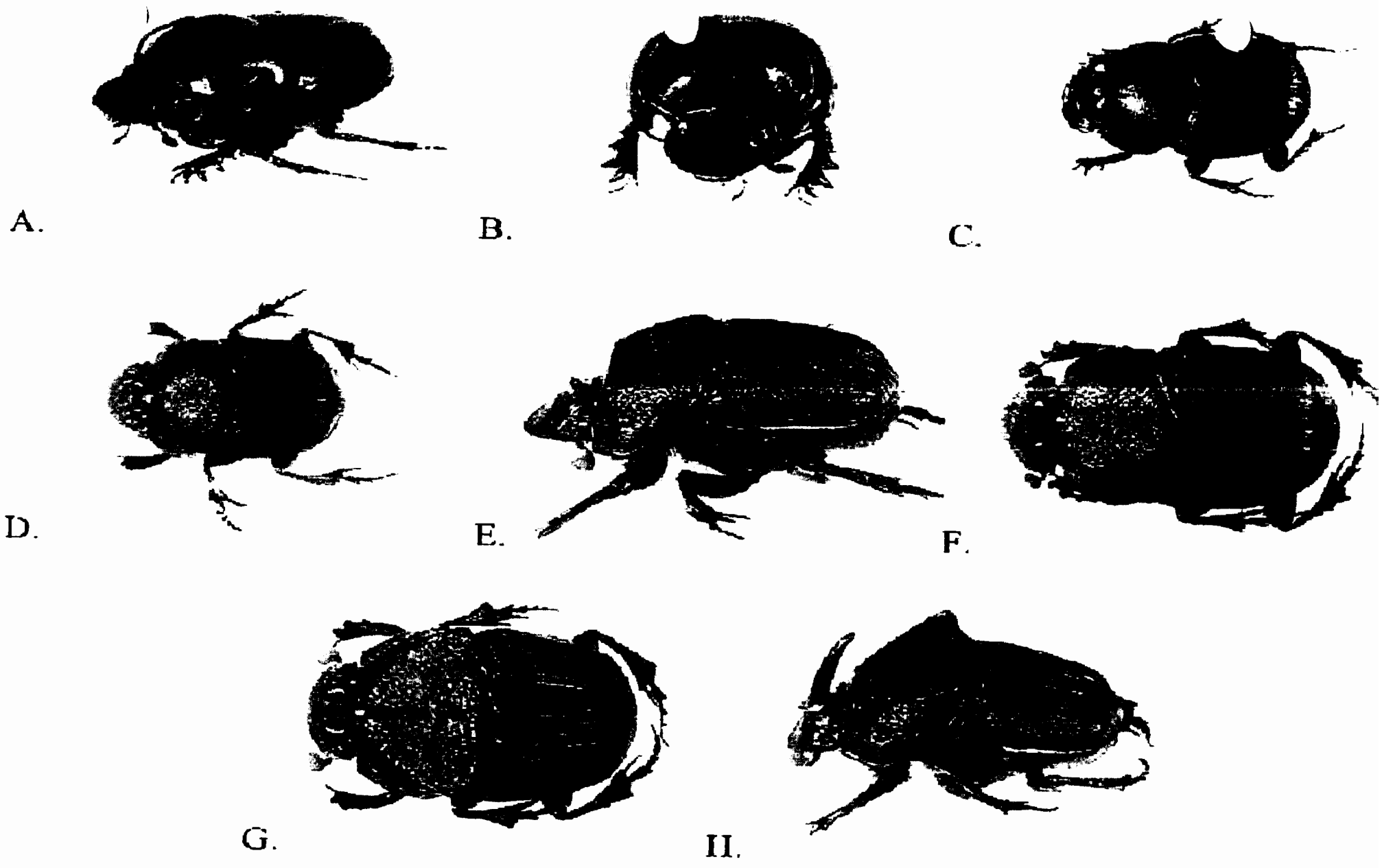
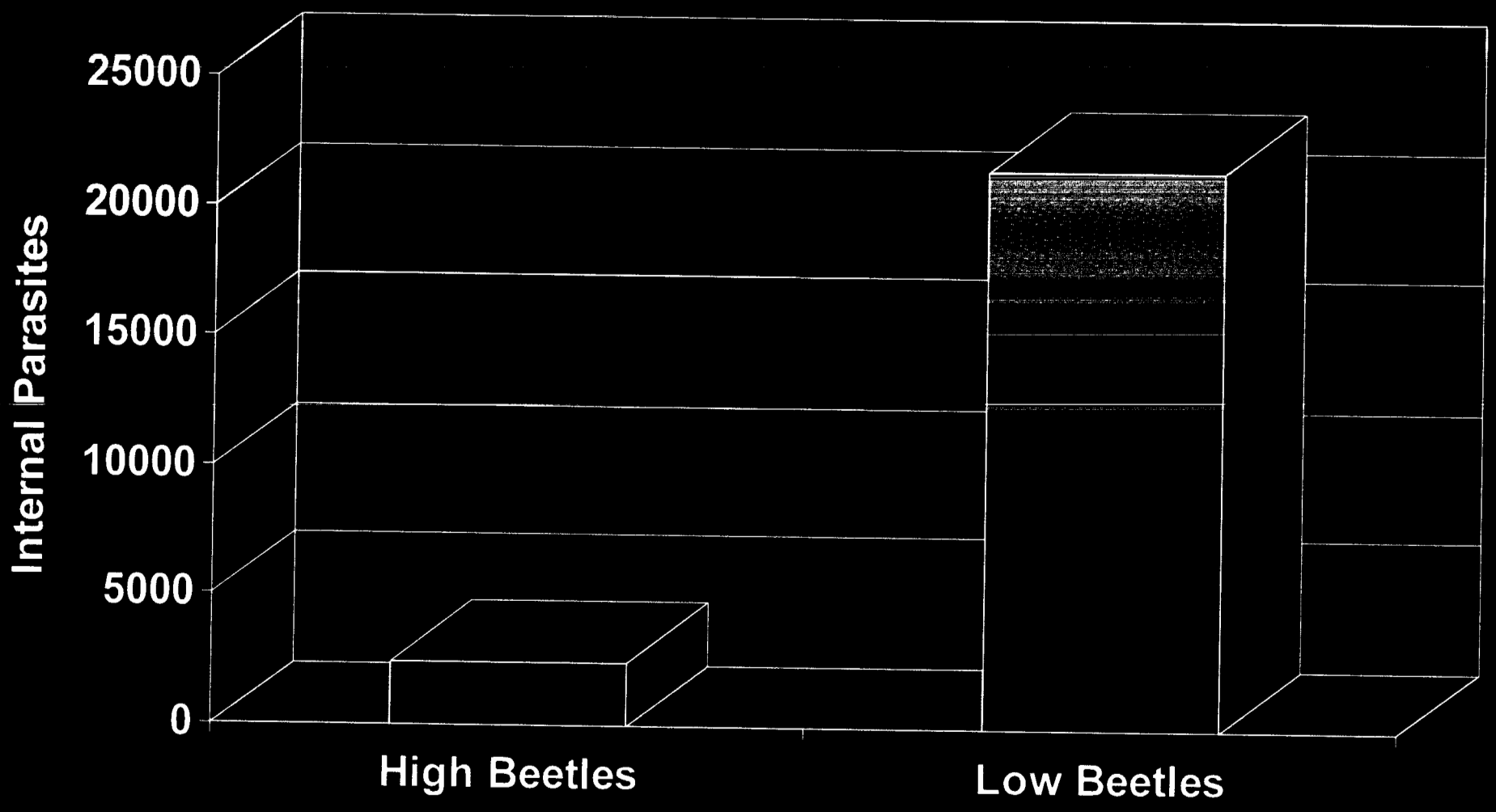


Figure 4. A. *O. taurus* (♂ lateral); B. *O. taurus* (♂ head); C. *O. tuberculifrons*; D. *Phanaeus vindex* (♀ dorsal); E. *P. vindex* (♀ lateral); F. *P. vindex* (minor ♂ dorsal); G *P. vindex* (♂ dorsal); H. *P. vindex* (♂ lateral)

Benefits to the Pasture System

- ↓ face flies and horn flies
  - = up to 95%
  - – compete for dung
    - – single pat = 60-80 adult horn flies
- Biological control agents for gastrointestinal parasites
  - – Removes manure/egg incubator



- 10× reduction of internal parasites
  - high numbers of beetles vs. low numbers
  - 21,513 vs. 2,404 worms/calf
  - *Matt Bertone, NCSU, 2004*

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- Dung pat degradation improves pasture utilization
  - Ruminants hesitant to graze around pats
  - Can make 5-10% of pasture unavailable
  - Under proper conditions manure disappearance occurs in 36 hours
- ↑ soil's capacity to absorb and retain water
- ↑ nitrogen availability to plants
  - w/o degradation up to 80% of nitrogen is lost to volatilization

Economic value of manure and urine to pasture preservation

- 1000# cow produces 50-60#/day
  - N=.35#'s @ .24/#=$0.08
  - P=.23#'s @ .22/#=$0.05
  - K=.28#;s @ .14/#=$0.04

    $0.17
- Therefore:
  - 100 cows=$17.00/day or $2380.00 for 5 months grazing

Effects of Wormers

- Avermectin injectable at recommended doses can reduce survival of larvae
  - Ivermectin and doramectin
- Ivermectin pour-on was similar
- Sustained release Ivermectin boluses most detrimental

- Alternatives: little/no effect
  - backrubbers
  - ear tags
  - occasional use of insecticide dusts and sprays
- Treat with larvacidals
  - During coolest months
- Determine egg counts in cattle to maximize anthelmintic benefit

- Benzamidazoles bind to tubulin which is specific for nematodes (starvation)
- Avermectins and Milbemycins have broad range of activity in nematodes and arthropods
  - Activate glutamate-gated chloride channels
  - Activate GABA-gated chloride channels
    - Non-specific to nematodes and arthropods

- 10 grams (28.4 gms/oz) dry weight of dung have several hundred insect larvae.
- 200 *ug/kg* Ivomec S.C. suppressed larval stages of:
  - face flies
  - horn flies
  - stable flies
  - house flies for periods up to 28 days.
- Similar larval suppression of dung beetles
  - *Avoidance of suppression could be achieved by timing*

Pretoria South Africa, 1998

Approximate annual rainfall 17 inches/year

- Ivermectin injection affected the dung insect community for 3 months after Rx.
  - Most avermectin residue is eliminated via the dung
  - Decreased species diversity
  - Decreased evenness of larval occurrence
  - Ivermectin is lethal to larval stages of various beetles for up to 28 days

Effect of pour-on formulations on dung beetle populations

- Eprinomectin capable of reducing beetle activity in next generation by 25-35%
  - Feces from treated cattle had high mortality during the first 1-2 weeks after treatment
- Pour-on formulation of moxidectin had no detectable effects on development or survival
  - *Australia, 2000*

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Panacur SR bolus vs Ivomec SR Bolus, UK, 1995

- Panacur fecal pats were the same as controls in larval counts and breakdown
  - Non-toxic
- Ivomec pats had significantly fewer beetle larvae and retained solid, compact pats
  - Toxic

In summary

- Increase pasture utilization
- Reduce pathogenic and pest insects in pats
- Prevents surface pollution
- Reduces animal disease by eradicating contaminated feces
- Increases soil nutrients
- Increases effective grazing
- Reduces nitrogen loss in feces

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