ENVIRONMENTAL IMPACT ANALYSIS REPORT
NADA 131-675
SWINE PREMIX

A. February 1983

B. American Hoechst Corporation, Animal Health Division

C. U.S. Hwy. 202-206 North, Somerville, New Jersey 08876

D. Environmental Information

D1. Describe the proposed action

a. Purpose of action

American Hoechst Corporation is seeking approval of NADA 131-675 for the use of fenbendazole premix 20% and 4% as an oral dewormer for swine at a dose level of 3 mg fenbendazole/kg body weight per day for three days. Retreatment after 4-6 weeks may be necessary if the treated swine continue to be exposed to worms. The treated swine can be slaughtered after treatment without withdrawal time.

b. Environment to be affected by proposed action

Approval of the proposed action would allow the production of fenbendazole bulk drug substance at the plant of Hoechst AG in Frankfurt, Federal Republic of Germany, shipment to the United States to the Somerville, New Jersey plant of Hoechst-Roussel Pharmaceuticals Inc. and manufacturing and packaging of the premixes in contract feed mills (Dale Alley, Pfizer, Ralston Purina). The drug will be distributed in the United States for use in swine.

Therefore, the environments affected by the proposed action are:

1. The environment adjacent to the plant in Frankfurt, Federal Republic of Germany.

2. Environment adjacent to the Somerville, New Jersey plant.

3. Environment adjacent to the contract feed mills (Dale Alley Company, St. Joseph, MO., Pfizer Agricultural Division, Lee's Summit, MO., Ralston Purina, St. Louis, MO.)

4. Swine environments receiving residues of the drug contained in animal wastes.

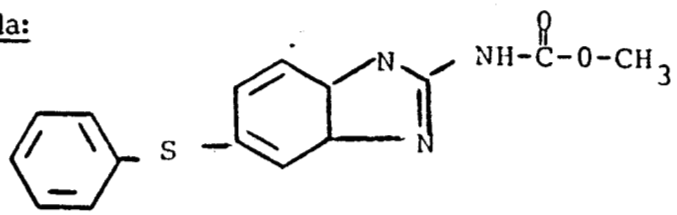
5. Agricultural lands potentially receiving these residue containing wastes.

6. Aquatic systems potentially receiving runoff from feedlots and agricultural lands containing drug residues.

D2a. Probable impact on the environment

Chemical/Physical Properties

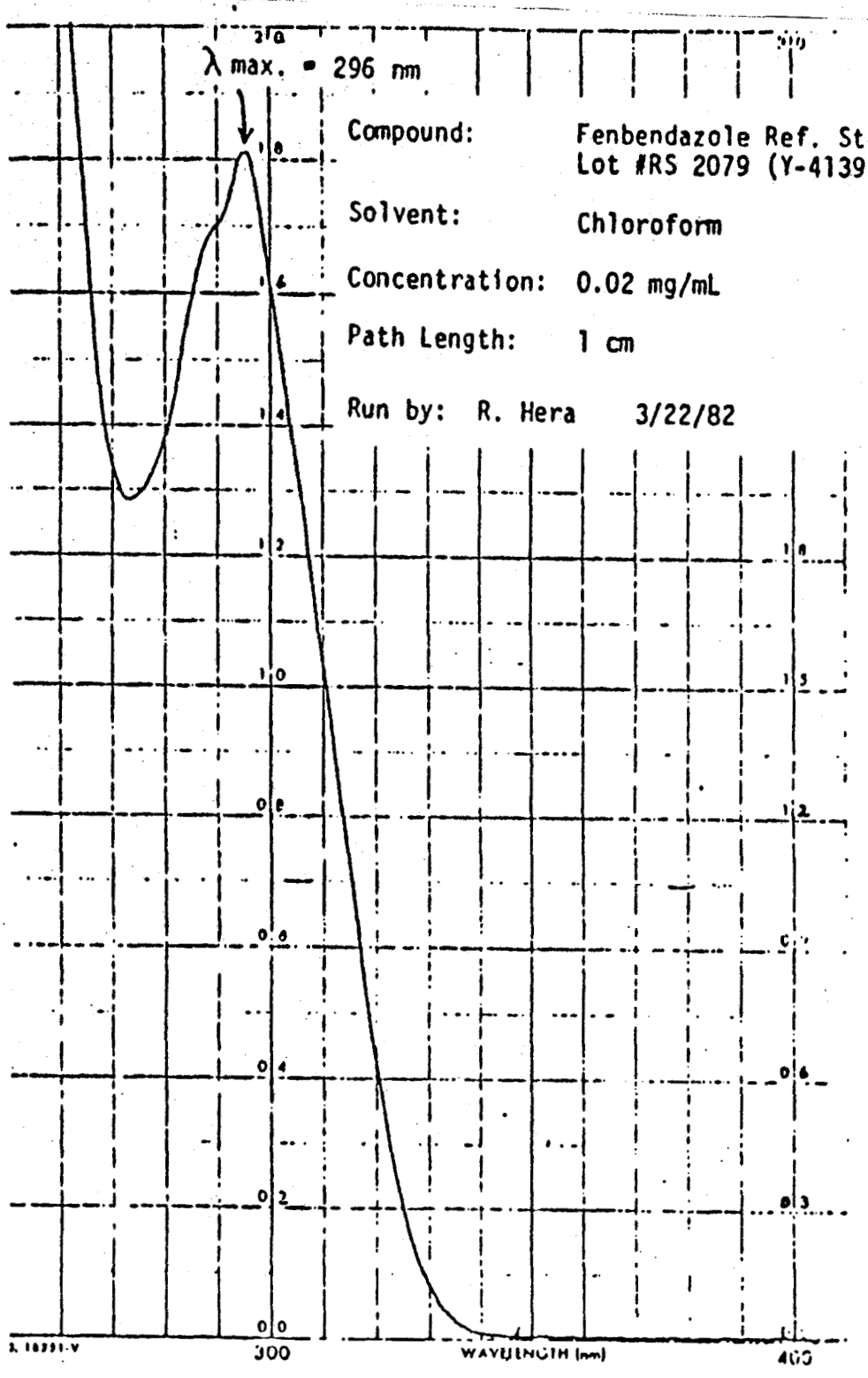
Substance: Fenbendazole (United States Adopted Name)
CAS Registry No: 43210-67-9
CAS Nomenclature: [5-(phenylthio)-1H-benzimidazol-2-yl] -carbamic acid methyl ester.
also: methyl 5-(phenylthio-2-benzimidazol-carbamate.
Structural Formula:



Molecular Formula: $C_{15}H_{13}N_3O_2S$
Molecular Weight: 299.4
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 souble in the usual solvents.
Freely soluble in DMSO.

Octanol: Water partition coefficient - log 3.9

U.V. Absorption Spectrum: Representative spectrum with maximum absorptivity at 296 nm attached.



Toxicological/Pharmacological Properties

Anthelmintic spectrum: fenbendazole is active against gastrointestinal nematodes, lungworms and kidneyworms. Efficacy against the following worms has been demonstrated in the United States:

Lungworms: (Metastrongylus apri, M. pudendotectus)

Stomachworms: (Hyostrongylus rubidus), whipworm (Trichuris suis)

Intestinal worms: nodular worms (Oesophagostomum dentatum, O. quadrispinulatum), roundworm (Ascaris suum)

Kidneyworms: (Stephanurus dentatus - adult & developing 4th and 5th stage larvae)

A total of 8 controlled critical efficacy studies have been conducted by 5 investigators in 5 different geographical locations in the United States. Doses of 3 mg/kg/body weight/day X 3 days were used in these studies.

Fenbendazole was supplied to the investigators as a premix containing 20% or 4% fenbendazole. The drug was administered orally. It was evaluated for efficacy in "controlled critical trials". Groups of swine were treated and postmortem worm counts several days after treatment compared to those of untreated controls. The results (summarized on the attached table) demonstrate that fenbendazole is a highly effective anthelmintic with a wide spectrum of activity when swine are treated at a dose level of 3 mg/kg body weight/day X 3 days.

Metabolism by Target Animals:
Orally administered fenbendazole is excreted as intact parent compound and several metabolites:

TABLE

	Feces	Urine
Parent Compound	44%	1%
NH_2 Metabolite		7%
SO Metabolite	< 7%	
p-OH Metabolite		<13%
Not identified	2 meta-bolites <1%	2 meta-bolites <8%
Total	~52%	~29%

This is a result of studies in which radiolabeled fenbendazole was given to swine.

A finite tolerance of 14.4 ppm in swine liver was established based on extensive safety studies. Residue levels in the liver never reach the tolerance level .

SUMMARY OF CONTROLLED CRITICAL

EFFICACY STUDIES IN SWINE

WITH FENBENDAZOLE AT A DOSE LEVEL OF

3 MG/KG/DAY FOR 3 DAYS ADMINISTERED IN FEED

Investigator/ Location	Study	No. of Animals Control/Treated	% Efficacy									
			Ascaris	Trichuris	Stephanurus		Metastrongylus		Oesophagostomum		Hyostrongylus	Physocephal
					Adult	Immature	M. apri	M. pudendotectus	O. dentatum	O. quadrispinulatum		
Dr. R. Bradley Univ. of FL.	1-E	10/10	100	N P	100	100	N P	N P	100	N P	N P	100
Dr. A. C. Todd Univ. of WI.	4-O	10/10	100	99.4	N P	N P	N P	N P	100	N P	N P	N P
Dr. E. Batte Univ. of N.C.	7-B	6/12	98.9	99.8	N P	N P	N P	N P	N P	N P	N P	N P
Dr. E. Batte Univ. of N.C.	7-D	10/10	N P	N P	100	100	N P	N P	N P	N P	N P	N P
Dr. N. Ferguson Univ. of NB.	14-B	10/10	N P	N P	N P	N P	99.3	N P	N P	N P	N P	N P
Dr. T. B. Stewart Univ. of GA.	34-A	11/11	92.3	65.9	N P	N P	N P	N P	99.8	100	99.9	N P
Dr. T. B. Stewart Univ. of GA.	34-D	11/11	100	93.6	N P	N P	96.9	96.9	99.9	N P	100	N P
Dr. T. B. Stewart Univ. of GA.	34-E	15/15	N P	N P	100	100	N P	N P	N P	N P	N P	N P

N P = Parasite not present.

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 and swine) 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 Dose 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
Swine	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.

Market penetration

We estimate that in the 4th year after introduction, approximately 30 million hogs weighing a maximum of 150 lbs. may be treated with fenbendazole premix 20% or 4%. At a dose of 3 mg fenbendazole/kg body weight/day for 3 days, this would amount to approximately 614 mg per animal or a total of 18,420 kg. 18,420 kg equals 20.3 tons of fenbendazole plus metabolites entering the environment in the United States per year.

30 million hogs at 150 lbs. or 60 million hogs at 75 lbs. body weight can be considered to be approximately 30% of the number of pigs treated in the United States annually. We estimate that approximately 100 million pigs are treated per year. They are usually treated at a weight of less than 150 lbs which allows for an additional margin.

Energy and natural resource use

Fenbendazole bulk drug, acquired from Hoechst AG, Frankfurt, W. Germany, is formulated into a premix using common inert feed grade excipients. Energy requirements for manufacturing are similar to those which would be used in any conventional pharmaceutical operation and feed mill involved in the production and packaging of feed additives.

Introduction into Environment

Manufacture:

The manufacturing facilities in Frankfurt, West Germany comply with local regulations. A statement by Hoechst AG, Frankfurt, West Germany is attached.

Hoechst-Roussel Pharmaceuticals Inc., operates under the following permits:

Air Pollution Control Permits

Permit No.'s	Equipment or Facility Operation
C-2810, C-2811	Dust Collection Equipment
C-2812, C-2813	"
C-2814, C-2815	"
C-2816, C-2817	"
#39327	"
5515	Fluid Bed Dryer Exhaust
3184	Solvent Recovery System
34352	Solvent Recovery System Tank (underground)
C-2818	Table Coating Pan (Polishing) Exhaust
45899 (Temporary)*	Tablet Coating Pan Exhaust

*New Installation

The premix itself will be manufactured by 3 feed manufacturers serving as toll manufacturers: Dale Alley Company, St. Louis, MO., Pfizer Agricultural Division, Lee's Summit, MO., Ralston Purina Company, St. Louis, MO.

Environmental statements by each toll manufacturer are attached.

These represent all the environmental permits currently required for the facilities which will be used for the manufacturing of fenbendazole PREMIX 20%/4%. Other than the temporary permit for exhausts on new coating pans in the New Jersey facility of Hoechst-Roussel Pharmaceuticals Inc., there are no conditions attached to the above permits. The addition of fenbendazole premix 20%/4% manufacturing will not impact or affect the status of these permits.

The manufacturing process of fenbendazole premixes consists of carefully controlled weighing, mixing, and filling operations conducted in a feed mill. These processes are controlled to arrive at a full material balance, and no effluents or pollutants are formed.

Introduction through the Target Animals

For practical purposes, the product will only be introduced into the environment when it is excreted by treated animals. Handling, distribution and storage of the finished product should not cause environmental exposure since the drug is marketed in closed bags.

As discussed above, target animals excrete quantities of the drug as parent compound and metabolites. The excretion of fenbendazole plus metabolites was measured in studies with swine treated with radiolabeled fenbendazole. The studies showed that practically the entire dose, as measured by radioactivity, is excreted again within a few days. For the purpose of this evaluation, we assume that 100% of the administered dose is excreted within 7 days. We assume, that 150 lb. hogs will be treated at a dose level of 3 mg fenbendazole/kg body weight/day X 3 days resulting in a total dose of 614 mg per animal.

A total of 1,000 lbs. live weight of hogs voids 16 tons fresh manure per year (M. E. Ensminger: The Stockman's Handbook, 5th Edition, Interstate Printers & Publishers, Inc., Danville, IL., 1978). 30 million hogs at 150 lbs. each equal 4,500,000,000 lbs. At 16 tons per thousand lbs., these animals would produce 72 million tons of manure annually.

We assume that the weight of pig manure spread on agricultural land approximates that of the excreta. A maximum of 10 tons of pig excreta is spread on 1 acre of agricultural land.

We assume that the 10 tons of excreta spread on one acre mix with approximately 2 million pounds (1000 tons) of soil.

The excreta of 30 million hogs contain 20.3 tons of fenbendazole plus metabolites. They would mix with 1,000 tons of soil per 10 tons excreta or 7,200,000,000 tons of topsoil. Added up:

Soil	7,200,000,000
Excreta	72,000,000
Total	7,272,000,000

A total of 7.272 billion tons topsoil + excreta would contain 20.3 tons fenbendazole + metabolites or 2.8 ppb.

Fenbendazole and its metabolites are excreted in the feces over a period of several days after treatment. The highest levels of fenbendazole in feces are found shortly after treatment. Feces from swine given the recommended treatment were found to contain a range of fenbendazole (and metabolite) concentrations of from 45-86 ppm. A worst case concentration in soil could occur when feces from treated pigs containing the highest level of fenbendazole (and metabolites) are mixed with soil.

In this worst case situation we could assume that ten tons of feces (containing fenbendazole at the concentration of 86 ppm) would be mixed with 1,000 tons of topsoil (10 tons of manure incorporated into the top six inches in one acre of soil).

This represents a dilution factor of 100 resulting in

$\frac{86}{100}$ = 0.86 ppm or 860 ppb fenbendazole + metabolites in topsoil + excreta

This concentration in soil is unlikely to ever be reached because feces excreted with the highest levels of fenbendazole will always be mixed with feces containing much less or no fenbendazole before manure is spread on agricultural land.

Environmental Fate

Since the primary route of introduction of fenbendazole into the environment is through excretion by the target animal, AHC/AHD conducted several studies of the fate of this drug in the environment.

Water Solubility of Fenbendazole:
Fenbendazole was determined to be 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.

Migration of Fenbendazole in Soil:
A migration study using soil thin layer chromatography was done to determine if fenbendazole migrates from the site of introduction into the environment. Radiolabeled fenbendazole was studied in a silt loam soil sample. Fenbendazole adsorbed tightly to particles of this soil type and is expected not to migrate from application sites into runoff or leachate water.

Adsorption of Fenbendazole to Particulate Matter:
An adsorption study was done to determine how tightly fenbendazole is bound to particulate matter in the soil. Radiolabeled fenbendazole was used and 3 soils 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.

Laboratory Runoff Studies with Feces from Animals Treated with Fenbendazole:

Studies have shown that the same metabolites are found in the feces of swine and cattle treated with fenbendazole. Feces from pigs treated with C14 fenbendazole were mixed with soil to a final concentration equivalent to 11.07 mcg 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 mcg/mL which represented 3.19% of the initial 14C activity. The result of this study shows that fenbendazole metabolites just as fenbendazole parent substance are bound tightly to particulate matter and do not migrate into surface waters. (Bio/dynamics, Bound Brook, NJ.)

Biodegradation of Fenbendazole:

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.

It can be concluded from this study that fenbendazole biodegrades very slowly under the test conditions.

Hydrolytic Behavior of Fenbendazole:

A 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.

We concluded from these studies that fenbendazole is not hydrolyzed in the tested range of conditions.

Bioaccumulation:

Octanol/water partitioning coefficient is a chemical measure often indicative of the potential for a chemical to accumulate in lipid-containing tissues of animals and plants. The octanol/water partitioning coefficient (EPA Method, FEDERAL REGISTER, March 16, 1979) for fenbendazole was found to be approximately log 3.9, an intermediate partition coefficient compatible with other test results concerning bioaccumulation of fenbendazole.

Bioaccumulation was determined in additional studies as follows:

Residue studies with radiolabeled fenbendazole in various mammals (cattle, sheep, pigs, rats) showed that the majority of the administered dose of fenbendazole is excreted rapidly with only traces left after 7 days.

Specific studies were done also in fish:

Accumulation and Elimination of 14C Residues by Bluegill Sunfish exposed to 14C Fenbendazole:

Bluegill were continuously exposed to a nominal concentration of .92 nanograms/mL (ng/mL) of 14C labeled fenbendazole in well water for 31 days after which all remaining fish were transferred to flowing, uncontaminated water for a 14 day depuration period.

The concentration of 14C residues measured in the muscle tissue increased during the initial three days of exposure after which a period of equilibrium existed during the remaining 28 days of exposure. The mean equilibrium bioconcentration factor for 14C fenbendazole in muscle tissues (days 3 through 30) was 31 X.

Similarly, an equilibrium was reached in the visceral tissues after 3 days of exposure. The mean equilibrium bioconcentration factor in-viscera was calculated to be 3,500 X.

The 14C residue content measured in the carcass tissue increased during the initial 7 days of exposure after which there was a period of apparent equilibrium for the duration of the exposure period. The mean bioconcentration factor for 14C fenbendazole in bluegill carcass during the equilibrium period (days 7 through 30) was 85 X.

The pattern of accumulation and persistance of 14C residues in the whole body of bluegill exposed to 14C fenbendazole was similar to that observed in the viscera tissue. The mean equilibrium bioconcentration factor for 14C fenbendazole in the whole body of bluegill during the period 3 through 30 days of exposure was 240 X.

Of the 14C residues accumulated in the muscle tissue of bluegill after 31 days of continuous aqueous exposure to 14C fenbendazole, 27% were extractable with hexane, 20% were extractable with methanol, and 53% were nonextractable with either solvent.

The elimination of 14C residues from the selected tissue portions of bluegill exposed for 31 days to 14C fenbendazole was continuous during the 14 day depuration period. Depletion halflife of 14C residues present in the bluegill tissue on day 30 of exposure occurred within the first 24 hours after the transfer to flowing uncontaminated water. By day 14 of depuration, bluegill had eliminated 81%, 99% and 70% of the 14C residues measured in the muscle, viscera and carcass tissue respectively and 93% of the 14C residues calculated for the whole fish on day 30 of exposure.

It should be noted that the results of this study suggest a factor of temporary bioaccumulation that may be higher than under natural circumstances. The water solubility of fenbendazole was determined to be 10-40 ppb. Migration studies showed that fenbendazole and its metabolites are tightly bound to soil particles. Therefore, low concentrations will occur in surface water.

In summary, an intermediate level of accumulation was observed in bluegill continuously exposed to 14C fenbendazole. The calculated mean equilibrium (plateau) bioconcentration factor in the whole body of bluegill was 240X. The factors mitigating concern for the accumulation of fenbendazole in fish consist of 1) the fact that plateau was attained within the first three days of the exposure and continued accumulation did not occur during the remainder of the thirty-day exposure and 2) upon transfer to clean water, the fenbendazole residue accumulated in bluegill (whole body) was rapidly eliminated (half-life

less than 24 hours) and within 14 days had decreased to 7% of the body burden attained at plateau. These data indicate that fenbendazole would not be expected to concentrate or be retained to any great degree by aquatic organisms. From all of the available information we conclude that fenbendazole should not pose a significant problem concerning bioaccumulation.

From all available information, we conclude that fenbendazole should not cause an environmental problem after the treatment of swine as far as bioaccumulation in warm blooded animals or fish is concerned.

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. Metabolism studies have shown that the same metabolites are found in cattle and swine excreta. Therefore, this study can also be used for considerations relative to the use of fenbendazole in swine.

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 3 nanograms/gram (3 ppb). The comparative value for the soil was 490 nanograms/gram.

These values correspond to spreading 10 tons of pig excreta containing 49 ppm fenbendazole + metabolites on 1 acre of agricultural soil. The worst case calculation (refer to "Introduction into the Environment" in this EIAR) considered 86 ppm, a value which is unlikely to be found in reality since excreta containing the highest levels of fenbendazole + metabolites will always be mixed with excreta containing less or no fenbendazole before they are spread on agricultural soil.

Taking into account the low mobility of the drug in soil and the low solubility in water, worst case fenbendazole concentrations in surface waters receiving runoff from agricultural soils where these residues are present can be assumed to be 10-40 ppb. This compares well with the results of a runoff study with manure from treated pigs: the aqueous phase was found to contain maximum concentrations of 39-47 ppb.

Environmental Effects of Fenbendazole

Acute toxicity of fenbendazole to swine and other animals is reported earlier under "Pharmacological/Toxicological Properties".

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 14.4 ppm fenbendazole residues in swine liver was established.

Tests evaluating the antimicrobial activity of fenbendazole
A number of microorganisms were exposed to fenbendazole and no activity of fenbendazole was found.

The microorganisms 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.

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 anaerobius

Variabilis

Peptostreptococcus anaerobius and variabilis

Propionibacterium acnes as well as several clostridia strains including Clostridium perfringens and 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 Entamaoeba 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 mcg/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.

In addition, an earthworm study was conducted with Eisenia foetida.
The effect of fenbendazole on Eisenia foetida (earthworm) in an artificial soil test:

A preliminary range-finding test using earthworms (Eisenia foetida) tested the toxicity of fenbendazole doses of 1000, 500 and 100 mg drug/kg soil. Worm mortality was not observed until 14 days and then only in the 1000 and 500 mg/kg groups. The 14 day LC_{50} was calculated to be 1068 mg/kg with the 95% confidence interval being from about 900-1600 mg/kg. The worms at 100 mg/kg suffered no mortalities, however, by 14 days they had lost almost as much weight (35%) as had the worms at the two higher doses. In comparison to control worms, all treatment with fenbendazole resulted in significant weight losses.

The control worms were able to reproduce (produce cocoons). The only other test group able to reproduce was the 100 mg/kg worms, however, they did so to a smaller degree than did the control worms.

By 7 days at both the 1000 and 500 mg/kg dose levels there was a considerable reduction in the ability of the worms to burrow.

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 and this dose also caused significant sublethal effects. It is difficult to compare and contrast these results with the potential effects at the levels expected to occur in the environment.

The following studies were done to determine the toxicity of fenbendazole to aquatic organisms:

1. Acute Toxicity of Fenbendazole to the Water Flea (Daphnia magna): Nominal concentrations of fenbendazole in water were prepared at 16, 10, 6.4, 3.8, 2.6, 1.6 mcg/L and the appropriate controls added. Three replicates of each concentration were prepared and 5 water fleas were added to each container. The 48 hour LC50 (and 95% confidence interval) for the water flea exposed to fenbendazole was estimated to be 12 mcg/L (11-14 mcg/L).

2. Acute Toxicity of Fenbendazole to Rainbow Trout (Salmo gairdneri): The acute toxicity as expressed by a 96 hr. LC50 could not be determined in rainbow trout. Based on results of the studies, it was estimated to be greater than 7.5 mg/L. The reason for the difficulties may be the low solubility of fenbendazole in water; undissolved fenbendazole was visibly present in all concentrations higher than 1.6 mg/L. The water solubility of fenbendazole was determined to be 0.01-0.04 mg/L. Concentrations tested ranged from 0.58-7.5 mg/L in one and 7.8-100 mg/L in another study. Only the results of the study with concentrations of 0.58-7.5 mg/L could be used because those at higher concentrations were inconsistent. Signs such as

darkened pigmentation, lethargy, rapid respiration were observed at the lowest level tested.

3. Acute Toxicity of 14C Fenbendazole to Bluegill (Lepomis macrochirus) During 21 Days Continuous Exposure:

The study was undertaken to estimate the toxicity, uptake, and elimination of 14C fenbendazole with bluegill during 21 days exposure and 7 days depuration under flowthrough conditions. Measured concentrations of 14C fenbendazole in water were prepared at .061, 0.029, 0.014, 0.0074 and 0.0041 mcg/mL and the appropriate controls added. Ten bluegill were randomly distributed into duplicate test aquaria for a total of 20 fish per concentration. Survival and general appearance were assessed daily. The exposure of bluegill to 14C fenbendazole was continuous for 20 days. After 21 days exposure, all the remaining fish from the lowest test concentration which partially affected the survival of the test population (0.0074 mcg/ml) were transferred to a clean aquarium and held for a depuration period of 7 days. During the initial 10 days of the exposure, 14C fenbendazole did not elicit any effects on the survival of bluegill at any concentration tested. A sharp increase in toxicity occurred between day 10 and 11. From days 11 through 21, a steady increase in the cumulative toxicity of 14C fenbendazole was observed:

LC50, in mcg/mL (95% confidence interval)

Day	4	7	14	21
	$>0.061^a$	$>0.061^a$	0.035^b (0.030-0.041)	0.019^b (0.015-0.024)

[a]empirically estimated

[b]estimated by moving average method

Residue concentrations in muscle, viscera and remaining carcass of bluegill after 21 days of continuous aqueous exposure to 0.0074 mcg/mL 14C fenbendaozle indicate that the concentration of 14C residues in muscle and carcass were similar with bioconcentration factors of 43X and 92X, respectively. The greatest uptake of 14C residues occurred in the viscera which had a bioconcentration factor of 6600X. The whole body bioconcentration factor for bluegill exposed to 0.0074 mcg/mL fenbendazole for 21 days was 580X. After 7 days of depuration, 99% of the 14C residues concentrated in the viscera had been eliminated. The average concentration of 14C residue present in the muscle throughout depuration appears to have been approximately 0.28 mg/kg (average residue measured on days 0, 1 and 7). Based on whole body residues, the halflife for 14C fenbendazole in bluegill tissues was>1<3 days.

4. The Acute Toxicity of Fenbendazole to Bluegill (Lepomis macrochirus) During 21 Days Continuous Exposure:

The study was undertaken to determine if radioactivity was responsible for deaths of bluegills observed in a study with C14 fenbendazole.

The same procedures were used as in the above study. The results in this study were very similar to those observed with C14 fenbendazole. During the initial 7 days of the exposure, fenbendazole did not elicit any effects on the survival of bluegill at any concentration tested. By day 8 of the exposure, 30% and 20% mortality had occurred from exposure to 0.040 and 0.080 mcg/mL fenbendazole respectively. The highest mortality of bluegill exposed to 0.040 and 0.080 mcg/mL fenbendazole occurred between days 8 and 12. From days 12 through 21 of the exposure, relatively few fish died. Estimated LC50 in mcg/ml (95% confidence interval) was:

	LC50 in mcg/ml (95% confidence interval)			
Day /	4	7	14	21
	0.080[a]	0.080[a]	0.033[b] (0.028-0.040)	0.028[b] (0.022-0.037)

[a] Empirically estimated.

[b] Estimated by moving average method.

Water samples from the study were analyzed by a validated analytical method (>98% recovery, standard deviation about 5%) at Hoechst-Roussel Pharmaceuticals Inc. The total concentrations (i.e. fenbendazole in solution plus fenbendazole in suspension) of fenbendazole claimed to have been in the fish tanks were, essentially, correct. They agreed with the fenbendazole concentrations found by C14 measurements in the previous radioactive C14 study.

Many of the concentrations in the tanks were above the saturation point of fenbendazole in water (0.01 mg/L); in these there is strong evidence that it was present as a mixture of:

a. Soluble fenbendazole.

b. Fine particulate - i.e. less than 0.45 micron

c. Course particulate -i.e. greater than 0.45 micron.

However, even the course particulates could not be observed with the naked eye.

The tanks at 0.01 mg/L and 0.005 mg/L (i.e. the saturation concentration, and 1/2 saturation) where the fish did not die, were confirmed as having fenbendazole present. The actual results were about 0.007 mg/L (70% of 0.01) and 0.0033 mg/L (66%), respectively. In the C-14 fenbendazole study, this level could not be measured by the radio carbon C-14 assay.

In summary, fenbendazole was tested for toxicity to water flea, bluegill, and rainbow trout. The 48 hour LC_{50} for the water flea exposed to fenbendazole was estimated to be 12 mcg/L (11-14 mcg/L). Toxicity was found when bluegill were exposed for more than 10 days to concentrations of more than 12-19 mcg fenbendazole/L.

Some signs of toxicity (darkened pigmentation, lethargy, rapid respiration, etc.) were found in rainbow trout but no fish died at concentrations of 0.58-7.5 mg fenbendazole/liter water. We conclude that such concentrations of fenbendazole would not be reached in surface water under natural circumstances because fenbendazole adheres tightly to soil particles, it will be present at very low levels in the soil, and it is soluble in water only at a maximum level of 10-40 mcg/L.

Summary:

American Hoechst Corporation has shown that 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.

D2b. Mitigation of Potential Adverse Environmental Effects.

In light of the data presented above, no such considerations are necessary.

D2c. Environmental Impact of Manufacturing Process.

No measurable effluents will result from the manufacturing process and no pollutants are expected.

The manufacturing facilities in Frankfurt, Federal Republic of Germany, comply with local regulations. A statement by Hoechst AG to that effect is attached.

D2d. Data and Reference:
Summaries of the studies supporting the statements are included in this report.

D3. Probable Adverse Affects which cannot be Avoided:
No adverse effects are expected from the use of fenbendazole.

D4. Alternative to the Proposed Action:
None

D5. Relationship between Local short term uses of the Environment and the Maintenance and Enhancement of Long Term Productivity:
There is no conceivable effect on the environment from either short or long term production.

D6. Irreversible and Irretrievable Commitments resulting from the Proposed Action:

Substances which constitute fenbendazole suspension are taken from natural resources which are either replaceable or are derived from the most commonly existing substances and are logically viewed as insignificant.

Objections raised by other agencies, organizations, or individuals:
American Hoechst Corporation knows of no objections raised regarding the proposed action.

D8. If the Proposed Action should be Taken Prior to 90 Days from the Circulation of a Draft E.I.S. or 30 Days from the Filing of a final E.I.A.S., Explain Why:

American Hoechst Corporation believes that an E.I.S. is not required for the proposed action. If, however, it is determined by the Food & Drug Administration that an E.I.S. is required, American Hoechst Corporation will not request implementation of the proposed action prior to the finalization of such an E.I.S. It would be requested, however, that the FDA expeditiously prepare such a document, if required.

D9. Risk Benefit Analysis

The manufacture and distribution of the new drug demonstrates no risk or potential for risk to the environment.

E. Certification:

The undersigned applicant certifies the information furnished in this Environmental Impact Analysis Report is true, accurate, and complete to the best of his knowledge.

Signature Date

Raine Muser 2/8/1983

Dr. R. K. Muser, Director
Animal Health R&D Dept.
American Hoechst Corporation

Hoechst

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				15.10.1976

Statement

Herewith we state that the production of Panacur ^{R} (generic name: Fenbendazole) at our plant in Frankfurt/Main is carried out in accordance with the pertaining regulations regarding environment control of the Federal Republic of Germany.

HOECHST AG

ppa. Wagner

(Dr. Jürgens) (ppa. Dr. Wagner)