ENVIRONMENTAL ASSESSMENT

NADA 128-620

FENBENDAZOLE SUSPENSION 10% IN DAIRY CATTLE OF BREEDING AGE

1. **DATE:**
May 1995

2. **NAME OF APPLICANT/PETITIONER:**

Hoechst-Roussel Agri-Vet Company

In the United States, Hoechst-Roussel Agri-Vet Company will be the distributor of the product and will control the suspension manufacture.

3. **ADDRESS:**

P.O. Box 2500
Route 202-206
Somerville, New Jersey 08876-1258

4. **DESCRIPTION OF THE PROPOSED ACTION:**

Hoechst-Roussel Agri-Vet Co. is requesting approval to expand the use of fenbendazole (supplemental filing to NADA 128-620) suspension 10% as an oral dewormer to lactating dairy cattle (dairy cattle of breeding age) at a dose level of 5 mg fenbendazole/kg body weight. The recommended dose is given once. Retreatment after 4-6 weeks may be necessary if the treated dairy cattle continue to be exposed to worms. There is no milk withdrawal period following treatment. However, the treated dairy cattle can not be slaughtered for human consumption for a period of 8 days after treatment.

Fenbendazole (Safe-Guard® Suspension 10% and Panacur® Suspension 10%) will be used in lactating dairy cattle at any time during the lactation period and dry period. Safe-Guard® Suspension 10% and Panacur® Suspension 10% will be used as partial replacement for exisitng agents, morantel tartrate and thiabendazole, intended for the same purpose. Safe-Guard® Suspension 10% and Panacur® Suspension 10% will provide an alternative means for deworming lactating dairy cattle.

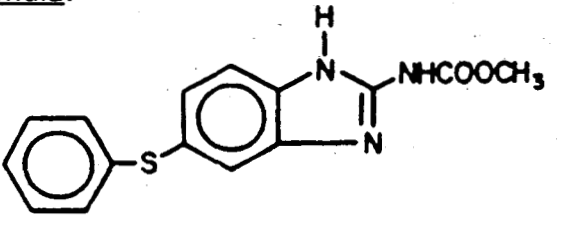
Populations

The number of lactating dairy cattle is projected to be 9 million. Of that number, 5% or about 0.5 million are projected to receive fenbendazole as Safe-Guard® Suspension 10% and Panacur® Suspension 10%.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE SUBJECTS OF THE PROPOSED ACTION:

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. Other related compounds available on the international market include febantel and triclabendazole. 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: [5-(phenylthio)-1H-benzimidazol-2-y1]-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 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 is presented in Appendix 1.
Mode of Administration:	Oral

PRODUCT DESCRIPTION

Fenbendazole is sold as Safe-Guard® Suspension 10% and Panacur® Suspension 10%. Both products have an active ingredient concentration of 100 mg fenbendazole per ml.

MODE OF ACTION

Anthelmintic spectrum: fenbendazole is active against gastrointestinal nematodes and lungworms. Efficacy against the following worms has been demonstrated in the United States:
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 Worm *(Cooperia oncophora, Cooperia punctata)*, Bankrupt Worm *(Trichostrongylus colubriformis)*, Nodular Worm *(Oesophagostomum radiatum)*.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

Approval of the proposed action would allow for the increased production of fenbendazole bulk drug substance at the plant of Hoechst AG in Frankfurt, Germany. The environmental and occupational safety regulations of Germany are presented in Appendix 2. Fenbendazole bulk drug substance will be shipped to the United States to the Somerville, New Jersey plant of Hoechst-Roussel Pharmaceuticals Inc. for manufacturing and packaging of a 10% suspension in the New Jersey facilities. The drug will be distributed in the United States for use in lactating dairy cattle.

Introduction of Substances Through the Manufacturing Process

1. The environment adjacent to the plant in Frankfurt, Germany.
2. The environment adjacent to the plant in Somerville, New Jersey.
3. Dairy facilities and other cattle environments receiving residues of the drug contained in animal wastes.
4. Agricultural lands potentially receiving residue containing wastes.
5. Aquatic systems potentially receiving runoff from dairy facilities and agricultural lands containing drug residues.

The manufacturing facilities in Frankfurt, Germany comply with local regulations. A statement by Hoechst AG, Frankfurt, Germany is included in the original NADA 128-620 (Fenbendazole for Cattle, 48 FR 42809, September 20, 1983). A current manufacturing Environmental Assessment is attached as Appendix 3.

A current manufacturing Environmental Assessment is attached as Appendix 4 for Hoechst-Roussel Pharmaceuticals Inc., manufacturer of the finished dosage form at the facility in Somerville, NJ (USA).

The manufacturing process of fenbendazole suspension consists of carefully controlled weighing, 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.

Introduction of Substances from the Use Site

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 plastic containers.

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 as presented in Appendix 5[1]. For the purpose of this evaluation, we assume that 100% of the administered dose is excreted within 7 days. We assume, that a 1,500 lb. dairy cow will 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. Van Vleck, M. F. Hutjens, 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.

Concentration in Water Run-Off from Dairy Farm

During the year there will be 2 inches of rainfall over an acre of land. Two inches of rainfall on an acre of land weighs approximately 205,500 kilograms. Assume 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 we assume that it is possible to have all of the residue in the run-off, the maximum concentration of fenbendazole in the run-off assuming no degradation equals:

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

It would be expected that the amount of fenbendazole released into water run-off 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).

Concentration in Soil with Waste from Treated Dairy Cattle

The following assumptions can be made:

- o No degradation in the manure before applying to the soil.

- 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.
  (3.4 g fenbendazole/380.8 kg manure per week) X 40,000 kg per acre = 0.356 kg fenbendazole in 40 metric tons manure or 8.9 mg/kg (ppm) manure.

- 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:

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

$$\text{Drug conc. in soil} = 8.9 \text{ mg/kg} \times \frac{40{,}000 \text{ kg manure}}{1 \text{ acre}} \times \frac{\text{acre}}{9.09 \times 10^5 \text{ kg}} = 0.39 \text{ mg/kg (390 ppb) FBZ in soil}$$

As indicated by the above calculations, the amount of fenbendazole (assuming no degradation) that would be released into the soil would be very low.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT:

Since 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 original application NADA 128-620 (48 FR 42809, September 20, 1983).

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. It is clear from these data that fenbendazole is water-insoluble.

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

Photolytic Decomposition of Fenbendazole in Aqueous Solution

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 photodegradation 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 para-nitroacetophenone (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 (T½, days) of fenbendazole at pH 5, 7 and 9 are presented below.

pH	T½ (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.

A summary is presented in Appendix 6.

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 not expected 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 ^{14}C fenbendazole were mixed with soil to a final concentration equivalent to 11.07 micrograms of ^{14}C 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 ^{14}C fenbendazole in the aqueous phase was .045 micrograms/mL which represented 3.19% of the initial ^{14}C 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 may biodegrade very slowly under the test conditions.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

Human Food Safety 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 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
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.

Metabolism by Target Animals

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 as presented in Appendix 7[2].

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.

Environmental Effect Studies

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. 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 anaerobius and *variabilis*
Peptostreptococcus anaerobius and *variabilis*
Propionibacterium acnes as well as several clostridia strains including *Clostridium erfringens* 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 *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).

Earthworm Toxicity (*Eisenia foetida*)

An earthworm study was conducted with *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, 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 1,000 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.

Earthworm Toxicity (*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.

A summary is presented in Appendix 8.

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

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 micrograms/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 LC_{50} (and 95% confidence interval) for the water flea exposed to fenbendazole was estimated to be 12 micrograms/L (11-14 micrograms/L).

Acute Toxicity of Fenbendazole to Rainbow Trout (*Salmo gairdneri*)

The acute toxicity as expressed by a 96 hr. LC_{50} 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 estimated limits of water solubility of fenbendazole.

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 ^{14}C fenbendazole with bluegill during 21 days exposure and 7 days depuration under flowthrough conditions. Measured concentrations of ^{14}C fenbendazole in water were prepared at .061, 0.029, 0.014, 0.0074 and 0.0041 micrograms/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 ^{14}C 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 micrograms/ml) were transferred to a clean aquarium and held for a depuration period of 7 days. During the initial 10 days of the exposure, ^{14}C 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 ^{14}C fenbendazole was observed:

LC_{50} in micrograms/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 micrograms/mL ^{14}C fenbendazole indicate that the concentration of ^{14}C residues in muscle and carcass were similar with bioconcentration factors of 43X and 92X, respectively. The greatest uptake of ^{14}C residues occurred in the viscera which had a bioconcentration factor of 6600X. The whole body bioconcentration factor for bluegill exposed to 0.0074 micrograms/mL fenbendazole for 21 days was 580X. After 7 days of depuration, 99% of the ^{14}C residues concentrated in the viscera had been eliminated. The average concentration of ^{14}C 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 ^{14}C fenbendazole in bluegill tissues was >1 <3 days.

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 ^{14}C fenbendazole.

The same procedures were used as in the above study. The results in this study were very similar to those observed with ^{14}C 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 micrograms/mL fenbendazole respectively. The highest mortality of bluegill exposed to 0.040 and 0.080 micrograms/mL fenbendazole occurred between days 8 and 12. From days 12 through 21 of the exposure, relatively few fish died. Estimated LC_{50} in micrograms/ml (95% confidence interval) was:

LC_{50} in micrograms/ml (95% confidence interval)

Day	4	7	14	21
	0.080[a]	0.0801[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 ^{14}C measurements in the previous radioactive ^{14}C 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 ^{14}C fenbendazole study, this level could not be measured by the radio carbon ^{14}C 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 micrograms/L (11-14 micrograms/L). Toxicity was found when bluegill were exposed for more than 10 days to concentrations of more than 12-19 micrograms fenbendazole/L.

Some signs of toxicity (darkened pigmentation, lethargy, rapid respiration, etc.) were found in rainbow trout but no fish died at concentrations representing the limits of fenbendazole solubility in water. Rainbow trout were not as sensitive as bluegill sunfish and daphnia.

It would be expected that the amount of fenbendazole released into water run-off would be very much lower than 496 ppb because fenbendazole is very insoluble in water, absorbs tightly to soil particles and is rapidly photodegraded. Therefore, fenbendazole is not expected to migrate from application sites into runoff or leachate water; and hence, is not expected to be toxic to aquatic species. Also, fenbendazole will be present at very low levels in the soil, and it is soluble in water only at a maximum level of 10-40 micrograms/L.

Seed Germination and Root Elongation

A study was undertaken to define the effect of fenbendazole on corn(*Zea mays*), cucumber (*Cucumis sativus*), perennial ryegrass (*Lolium 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

A summary of this study is presented in Appendix 9.

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 (controll) 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.

A summary of this study is presented in Appendix 10.

Studies in Plants

Another study was conducted to determine if fenbendazole is accumulated in plants. Feces from a cow which had been treated with ^{14}C 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 3 nanograms/gram (3 ppb). The comparative value for the soil was 490 nanograms/gram.

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 K_{ow} 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 in fish.

Accumulation and Elimination of ^{14}C Residues by Bluegill Sunfish exposed to ^{14}C Fenbendazole.

Bluegill were continuously exposed to a nominal concentration of .92 nanograms/mL (ng/mL) of ^{14}C 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 ^{14}C 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 ^{14}C fenbendazole in muscle tissues (days 3 through 30) was 31X.

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 3,500X.

The ^{14}C 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 ^{14}C fenbendazole in bluegill carcass during the equilibrium period (days 7 through 30) was 85X.

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

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

The elimination of ^{14}C residues from the selected tissue portions of bluegill exposed for 31 days to ^{14}C fenbendazole was continuous during the 14 day depuration period. Depletion halflife of ^{14}C 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 ^{14}C residues measured in the muscle, viscera and carcass tissue respectively and 93% of the ^{14}C 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 ^{14}C 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 cattle as far as bioaccumulation in warm blooded animals or fish is concerned.

Acute Toxicity of Fenbendazole to *Onthophagus gazella*

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 gazella)* 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 16 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 LD_{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.

A summary is presented in Appendix 11.

Environmental Hazard Assessment

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 stream, 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 photodegradation. 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.

Aquatic Levels

- Daphnia Toxicity >> LC_{50} (48 hr.) = 12 ppb
- Trout Toxicity >> LC_{50} (96 hr.) = Limit of H_2O solubility (40 ppb)
- Bluegill Toxicity >> LC_{50} (21 d. continuous exposure) > 19 ppb

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 fenbedazole 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.

Summary

Hoechst-Roussel Agri-Vet Company has shown that fenbendazole used at the proposed levels will not significantly adversely affect microorganisms, 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) and five studies are included in this supplemental application.

9. USE OF RESOURCES AND ENERGY:

Fenbendazole bulk drug, acquired from Hoechst ERG, Frankfurt, Germany, 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 will be involved if the proposed action should be implemented.

This action will not require any significant use of the environment. There are no expectations or evidence to expect short-term or long-term effects. Therefore, there is expected to be no effect upon the depletion of natural resources due to manufacture of the drug.

Environmental impact of manufacturing process.

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

The manufacturing facilities in Frankfurt, Germany, comply with local regulations. A statement by Hoechst AG to that effect is in Section A of Appendix 3.

10. MITIGATION MEASURES:

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

Probable adverse affects which cannot be avoided.
No adverse effects are expected from the use of fenbendazole.

Relationship between local short-term uses of the environment and the maintenance and enhancement of long-term productivity.
There is no conceivable effect of the environment from either short- or long-term production.

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

11. ALTERNATIVES TO THE PROPOSED ACTION:

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.

The only alternative to approval of the New Animal Drug Application is non-approval. This would mean that the dairy cattle industry would not have the choice of the use of this drug. The drug will have the effect of providing an alternative means for deworming dairy cattle.

Objections raised by other agencies, organizations, or individuals: Hoechst-Roussel Agri-Vet Company knows of no objections raised regarding the proposed action.

Hoechst-Roussel Agri-Vet Company believes that an environmental impact statement (E.I.S.) is not required for the proposed action.

12. LIST OF PREPARERS:

Lawrence E. Deetz, Ph.D.
Research Nutritionist
Product Development & Registration
Hoechst-Roussel Agri-Vet Company

Springborn Laboratories , Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571-1075

13. CERTIFICATION:

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

Date 5-15-95

Lawrence E. Deetz, Ph.D.
Research Nutritionist

14. REFERENCES:

Summaries of studies are included in the original NADA 128-620 (48 FR 42809, September 20, 1983) and the supplemental NADA 128-620 (53 FR 40058, October 13, 1988). The following references are attached as part of the Environmental Assessment.

1. Fenbendazole Suspension 10% NADA 128-620 submitted June 4, 1981, Volume IX-B of IX, pages 332-358.

2. Fenbendazole Suspension 10% NADA 128-620 submitted June 4, 1981, Volume VIIII-A of IX, pages 782-799.

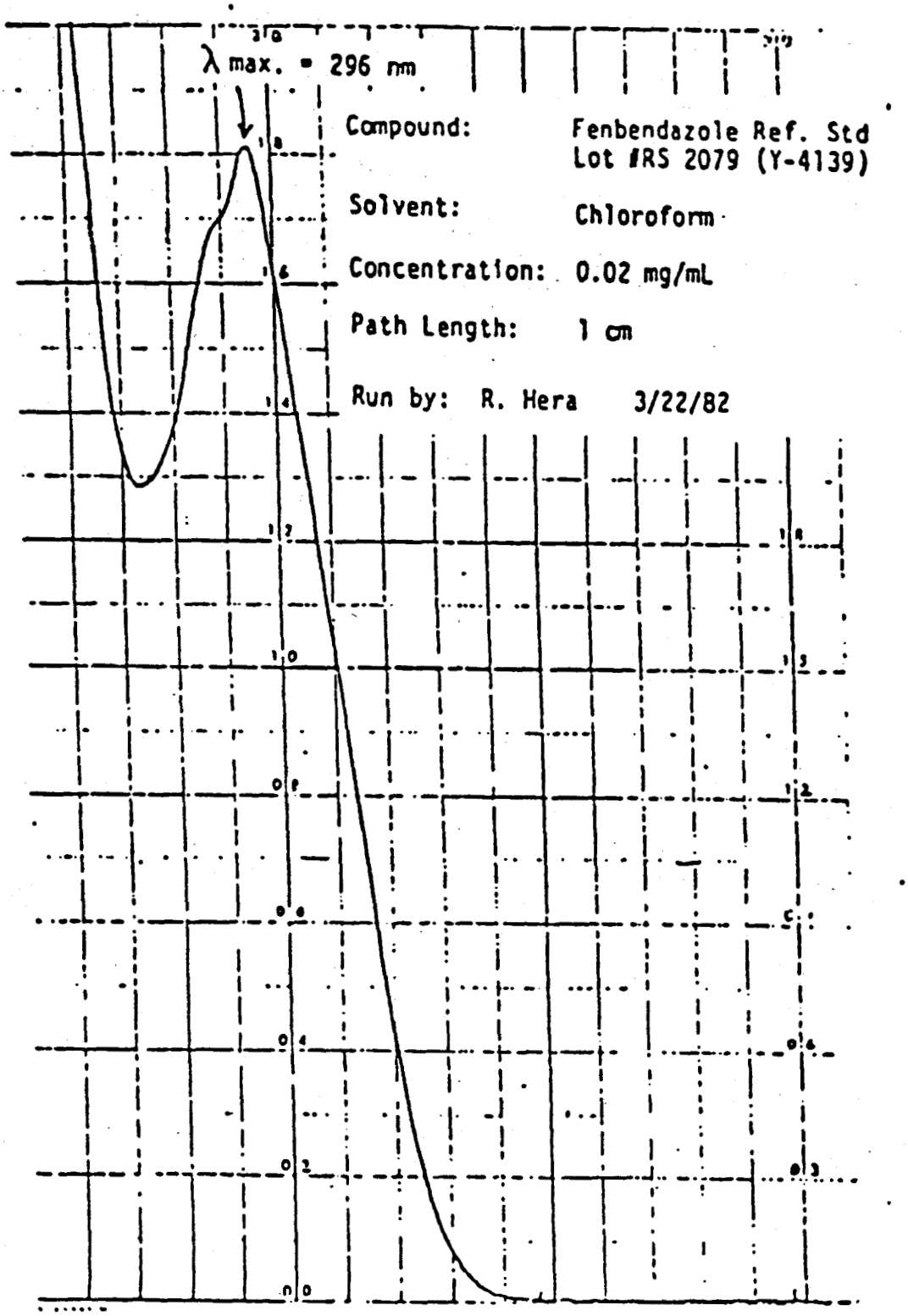
3. Pesticide Registration Rejection Rate Analysis Ecological Effects, EPA 738-R-94-035, December 1994.

15. APPENDICES:

Attached

APPENDIX 1

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



APPENDIX 2

Hoechst

Hoechst AG · Postfach 80 03 20 · D-6230 Frankfurt am Main 80

Hoechst Aktiengesellschaft
Pharma-[redacted]
Qualitätssicherung/GMP, D 610

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Telefax: (0 69) 30 36 65/56

Dresdner Bank AG, Frankfurt am Main 80
(BLZ 500 800 00) Kto. Nr. 735 555 500
Commerzbank AG, Frankfurt am Main 80
(BLZ 500 400 00) Kto. Nr. 2570729
Deutsche Bank AG, Frankfurt am Main 1
(BLZ 500 700 10) Kto. Nr. 926006
Hessische Landesbank – Girozentrale –
Frankfurt am Main 1
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Landeszentralbank in Hessen, Frankfurt am Main 1
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Postgiroamt Frankfurt am Main 1
(BLZ 500 100 60) Kto. Nr. 1442-605

TO WHOM IT MAY CONCERN

Ihre Zeichen	Ihre Nachricht vom	Unsere Zeichen	Telefon Durchwahl	Frankfurt am Main
		Dr. Sch/CT	(0 69) 3 05- 6831	Aug. 17, 1994

Production of Fenbendazole Drug Substance

HOECHST AKTIENGESELLSCHAFT as the producer of Fenbendazole drug substance herewith declares that the estimated increase of production amounts of Fenbendazole is in full compliance with environmental and occupation safety regulations of Germany.

Yours faithfully
HOECHST AKTIENGESELLSCHAFT

ppa. Lehnert ppa. Schwalbe-Fell

(ppa. Dr. Lehnert) (ppa. Dr. Schwalbe-Fehl)

APPENDIX 3

FENBENDAZOLE SYNTHESIS - ENVIRONMENTAL COMPLIANCE

The purpose of this attachment is to provide a statement that Hoechst AG is manufacturing the bulk drug compound in compliance with the Environmental Regulations of Germany.

Included are:

Section A - English translation of
- o Environmental statement
- o Listing of various German laws/regulations applicable to this submission.

Section B - Signed and Sealed Certification in English and German from the Government of Darmstadt for manufacture of Fenbendazole by Hoechst AG

Section C - Material (DIN) Safety Data Sheet (MSDS) - Bulk Product
MSDS - Panacur® Suspension 10%
MSDS - Safe-Guard® Suspension 10%

SECTION A

Statement from Hoechst AG that Production of Fenbendazole
is in Compliance
with the Environmental Regulations of Germany
[Regulations Included]

May 25, 1993

Hoechst

Hoechst AG · Postfach 80 03 20 · D-6230 Frankfurt am Main 80

Hoechst Aktiengesellschaft
Pharma-Technik
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Commerzbank AG, Frankfurt am Main 80
(BLZ 500 400 00) Kto. Nr. 2570729
Deutsche Bank AG, Frankfurt am Main 1
(BLZ 500 700 10) Kto. Nr. 926006
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Frankfurt am Main 1
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Landeszentralbank in Hessen, Frankfurt am Main 1
(BLZ 500 000 00) Kto. Nr. 500 08190
Postgiroamt Frankfurt am Main 1
(BLZ 500 100 60) Kto. Nr. 1442-605

TO WHOM IT MAY CONCERN

Ihre Zeichen	Ihre Nachricht vom	Unsere Zeichen	Telefon Durchwahl	Frankfurt am Main
		Dr. Bdt./CT	(0 69) 3 05- 6831	May 25, 1993

Environmental Assessment

HOECHST AKTIENGESELLSCHAFT, as the producer of drug substances and finished drug products at its factory:

HOECHST AKTIENGESELLSCHAFT
Hoechst Works
Brüningstrasse 50
Postfach 80 03 20
D-6230 Frankfurt/M.-Höchst 80
Federal Republic of Germany

herewith certifies that the above mentioned plant is run in compliance with the existing environmental control laws and regulations of the Federal Republic of Germany.
Environmental protection in the Federal Republic of Germany is subject to a number of laws and regulations which are strictly enforced.
The most important ones are listed below:

- Immissions (Air etc.):

"Gesetz zum Schutz vor schädlichen Umwelteinwirkungen durch Luftverunreinigungen, Geräusche, Erschütterungen und ähnliche Vorgänge ("Bundesimmissionsschutzgesetz"),
(Federal Law for Protection of the Environment against the Adverse Influences Caused by Contamination of the Air, by Noise, Vibration, and Similar Events).
March 15, 1974,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 721,
corrected 1193,
amended May 14, 1990/Federal Law Gazette ("Bundesgesetzblatt") I, 880.

- Water protection:

"Gesetz zum Schutze des Wasserhaushaltes"
("Wasserhaushaltsgesetz"),
(Federal Law for Protection of Water Resources).
October 16, 1976,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 3017,
amended March 28, 1980/Federal Law Gazette ("Bundesgesetzblatt") I, 373.

- Solid Waste:

"Gesetz zur Vermeidung und Entsorgung von Abfällen" ("Abfallgesetz"), (Federal Law for Avoidance and Disposal of Waste).
August 27, 1986,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 1718.

Hoechst

- Technical Directions for Handling of Waste

  "Technische Anleitung zur Lagerung, chemisch-physikalischen und biologischen Behandlung und Verbrennung von besonders überwachungsbedürftigen Abfällen"
  (Technical Directions for Storage, Treatment and Burning of Waste).
  April 10, 1990,
  published in Joint Ministerial Gazette ("Gemeinsames Ministerialblatt") no. 11, 170.

- Technical Directions for Maintaining Clean Air:

  "Technische Anleitung zur Reinhaltung der Luft" ("TA Luft"),
  (Technical Directions for Maintaining Clean Air).
  published in Joint Ministerial Gazette ("Gemeinsames Ministerialblatt") 95; February 27, 1986,
  amended in Joint Ministerial Gazette ("Gemeinsames Ministerialblatt"), 202; April 4, 1986.

- Technical Directions for Noise Protection:

  "Technische Anleitung zum Schutz gegen Lärm" ("TA Lärm"),
  (Technical Directions for Protection Against Noise).
  July 16, 1968,
  published in Enclosure to Federal Register ("Beilage zum Bundesanzeiger") no. 137,
  July 26, 1968.

- Chemicals:

  "Gesetz zum Schutz vor gefährlichen Stoffen"
  ("Chemikaliengesetz"),
  (Federal Law for Protection Against Dangerous Chemicals).
  March 14, 1990,
  published in Federal Law Gazette ("Bundesgesetzblatt") I, 521.

Hoechst

- Regulations for Dangerous Goods:

"Gefahrstoffverordnung",
(Regulations for Dangerous Goods).
April 23, 1990,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 790.

- Regulations for Pressurized Systems

"Druckbehälterverordnung",
(Regulations for pressurized systems, e.g. pressurized containers etc.).
April 21, 1989,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 830.

- Regulations for Notifications of Immissions:

"Zwölfte Verordnung zur Durchführung des Bundesimmissionsschutzgesetzes" ("Störfallverordnung"),
(12th Regulation for the Implementation of the Federal Law for Protection of the Environment Against the Adverse Influences Caused by Contamination of the Air, by Noise, Vibration, and Similar Events).
May 19, 1988,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 626.

- Regulations for Storage of Inflammable Liquids:

"Verordnung über Anlagen zur Lagerung, Abfüllung und Beförderung brennbarer Flüssigkeiten zu Lande",
(Regulations for Facilities for Storage, Filling, and Transportation of Inflammable Liquids on Land).
February 27, 1980,

published in Federal Law Gazette ("Bundesgesetzblatt") I, 229, amended May 3, 1982 in Federal Law Gazette ("Bundesgesetzblatt") I, 569.

Hoechst

- Regulations for Transportation of Dangerous Goods (road, rail, sea, river, air):

  -- For Transportation on the Road:
  "Gefahrgutverordnung Straße",
  (Regulations for Transportation of Dangerous Goods on the Road).
  July 22, 1985,
  published in Federal Law Gazette ("Bundesgesetzblatt") I, 1550,
  amended December 21, 1987,
  published in Federal Law Gazette ("Bundesgesetzblatt") I, 2858.

  -- For Transportation by Rail:
  "Gefahrgutverordnung Eisenbahn",
  (Regulations for Transportation of Dangerous Goods by Rail).
  July 22, 1985,
  published in Federal Law Gazette ("Bundesgesetzblatt") I, 1560,
  amended December 21, 1987,
  published in Federal Law Gazette ("Bundesgesetzblatt") I, 2862.

  -- For Transportation by Sea:
  "Gefahrgutverordnung See",
  (Regulations for Transportation of Dangerous Goods by Sea).
  July 5, 1978,
  published in Federal Law Gazette ("Bundesgesetzblatt") I, 1917,
  amended December 21, 1987,
  published in Federal Law Gazette ("Bundesgesetzblatt") I, 2863.

-- For Transportation on Waterways within Germany:
"Gefahrgutverordnung Binnenschiffahrt",
(Regulations for Transportation of Dangerous Goods on Waterways within the Federal Republic of Germany).
March 24, 1983,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 1977,
amended March 16, 1989,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 489.

-- Dangerous Products Regulations of the IATA (International Air Transport Association):
"IATA - DGR" (Dangerous Goods Regulations),
28th edition.

- Regulations for Drinking Water:

"Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe",
(Regulations for Drinking Water and for Water to be Used in Food Industries).
May 22, 1986,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 760.

- Feedstuffs (Animal Nutrition):

"Futtermittelgesetz",
(Federal Law on Feedstuffs).
July 2, 1975,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 1745,
and:
"Futtermittelverordnung",
(Regulations on Feedstuffs).
April 8, 1981,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 352.

Hoechst

Empfänger: TO WHOM IT MAY CONCERN | Unsere Zeichen: Dr. Bdt./CT | Datum: May 25, 1993 | Blatt: 7

- Regulations for Work Places:

"Verordnung über Arbeitsstätten" ("Arbeitsstättenverordnung"), (Regulations for Work Places).
May 20, 1975,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 729.

- Drug Law:

"Gesetz über den Verkehr mit Arzneimitteln" ("Arzneimittelgesetz"), (Federal Law for Handling of Drugs).
August 24, 1976,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 2445, 2448,
last amendment April 11, 1990,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 717;
and:

"Betriebsverordnung für pharmazeutische Unternehmer",
(Operations Ordinance for Pharmaceutical Entrepreneurs).
March 8, 1985,
published in Federal Law Gazette ("Bundesgesetzblatt") I, 546,
(and amendments).

Yours faithfully
HOECHST AKTIENGESELLSCHAFT

(Dr. Schütte) (ppa. Dr. Bauerschmidt)

900 2 012

SECTION B

Certification from the Government of Darmstadt (Federated State of Hesse)
Specific for the Manufacture of Fenbendazole by Hoechst AG
at Frankfurt, Germany

Dated August 8, 1994

Regierungspräsidium
Darmstadt

II 16 e - 18 l C2/03 (8) - FA 49

Luisenplatz 2
D-64278 Darmstadt, den 08. August 1994

Telephon: (06151) 12 62 42
12 53 13
Telefax: (06151) 12 57 89

1. Confirmation

In the Federal Republic of Germany the supreme Health Authority of each individual federal state is responsible for issuing and confirming documents, for signatures to confirm for the production and distribution of medicines, pharmaceutical raw materials, food stuffs and cosmetics.
For the

Hoechst Aktiengesellschaft
Brüngingstr. 50
D-65926 Frankfurt/Main

my office is the responsible authority. A second confirmation of a certificate issued or certified by my office by a superior federal authority is not necessary and will not be carried out for fundamental reasons.

Recipient of this document:

To whom it may concern.

For registration and all official purposes in

United Staates of America.

1. Bestätigung

Für die Ausstellung und Bestätigung von Urkunden und Unterschriften über die Herstellung und den Vertrieb von Arzneimitteln, pharmazeutischen Rohstoffen, Lebensmitteln und Kosmetika sind in der Bundesrepublik Deutschland die Bundesländer zuständig.

Für die Firma

Hoechst Aktiengesellschaft
Brüningstr. 50
D-65926 Frankfurt/Main

ist mein Haus die zuständige Behörde. Eine Überbeglaubigung eines von mir ausgestellten oder beglaubigten Zertifikates scheidet im übrigen aus grundsätzlichen Erwägungen aus.

Zu Registrierungs- und allen amtlichen Zwecken in:

Vereinigte Staaten von Amerika.

2. Statement of Manufacturing/Processing Sales Activities

The Hoechst AG is in the Federal Republic of Germany an acknowledged pharmaceutical and chemical factory. They have permission to produce pharmaceutical products (§ 13 Drug Law).

They are producing chemical and pharmaceutical raw materials for the manufacture of finished pharmaceutical products, cosmetics and food-additives.

They are marketing and selling these products at home and abroad.

3. Certificate

This is to certify that the Hoechst AG holds a manufacturing licence as a pharmaceutical manufacturer according to § 13 of the Drug Law from August 24 th in 1976 (BGBl. I, page 2445).

It is certified, that

a) the manufacturing plant, in which the product is produced is subject to inspections at suitable intervals,

- 2 -

2. Herstell-/Verkaufsbescheinigung Verkaufstätigkeit

Die Firma Hoechst AG ist in der Bundesrepublik Deutschland eine anerkannte pharmazeutische und chemische Fabrik. Sie hat die Erlaubnis zur Herstellung von Arzneimitteln (§ 13 Arzneimittelgesetz).

Sie stellt chemisch-pharmazeutsiche Rohmaterialien zur Fabrikation von Arzneimitteln und Kosmetika sowie Lebensmittelzusatzstoffe her.

Sie verkauft und vertreibt diese Produkte im Inland und im Ausland.

3. Bescheinigung

Hiermit wird der Firma Hoechst AG bestätigt, daß sie als pharmazeutischer Herstellerbetrieb im Besitz einer Herstellungserlaubnis gemäß § 13 des Gesetzes über den Verkehr mit Arzneimitteln vom 24.08.1976 (BGBl. I, S. 2445) ist.

Es wird bestätigt, daß

a) der Herstellerbetrieb, in dem das Produkt hergestellt wird, in angemessenen Abständen überwacht wird,

b) the manufacturer conforms to requirements for good practices in manufacture and quality control, as recommended by the World Health Organization, in respect of products to be sold or distributed within the country of origin or to be exported.

4. Product list

This certificate refers to the following product:

Fenbendazole.

- 3 -

b) der Hersteller hinsichtlich der Produkte, die im Herkunftsland verkauft und vertrieben werden oder für die Ausfuhr vorgesehen sind, den von der Weltgesundheitsorganisation empfohlenen Grundregeln für die Herstellung von Arzneimitteln und die Sicherung ihrer Qualität entspricht.

4. Aufzählung der Produkte

Diese Bescheinigung gilt für folgendes Produkt:

Fenbendazol.

Im Auftrage

REGIERUNGSPRÄSIDIUM DARMSTADT

(Völler)

SECTION C

Material (DIN) Safety Data Sheet (MSDS)

Summary of Physicochemical Properties of Fenbendazole

Procedures for Processing Waste
(incineration, landfill, microbial/chemical treatment)

Environmental Safety Data
(toxicological, ecological)

Dated October 28, 1991

MSDS - Panacur® Suspension 10% Dated October 12, 1994

MSDS - Safe-Guard® Suspension 10% Dated October 12, 1994

Product Name: FENBENDAZOLE
Product Code: 101870
MSDS Number : 00601
Version Date: 05/16/1995

Material Safety Data Sheet

Print date -- May 17th, 1995 2:05 p.m. PS PSA PSFHV - 1.1 (1/6)

1. CHEMICAL PRODUCT and COMPANY IDENTIFICATION

Product Name: FENBENDAZOLE
Product Code: 101870
MSDS Number : 00601

SYNONYMS: FENBENDAZOLE
METHYL-5-PHENYLTHIO-2-BENZIMIDAZOLE-CARBAMATE

HOECHST-ROUSSEL AGRI-VET COMPANY
ROUTE 202-206
P.O. BOX 2500
SOMERVILLE, NJ 08876-1258
UNITED STATES

PRODUCT USE:
Fenbendazole is the active ingredient in Panacur(R) and Safeguard(R) products, which are animal dewormers.

2. COMPOSITION / INFORMATION on INGREDIENTS

COMPONENT	CAS NUMBER
FENBENDAZOLE	43210-67-9

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW:
Fenbendazole is a solid which is non-reactive, relativley non-toxic, and insoluble in water.

POTENTIAL HEALTH EFFECTS
There are no known adverse health effects associated with this product.

DELAYED/LONG TERM EFFECTS

3. HAZARDS IDENTIFICATION (Continued)

CARCINOGENIC:

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

4. FIRST AID MEASURES

SKIN:

Wash with soap and water. If irritation develops, get medical attention.

EYES:

Flush with water for 15 minutes. If irritation develops, get medical attention.

INHALATION:

In cases of difficult breathing, remove to fresh air. If not breathing, give artificial respiration and get medical attention immediately.

INGESTION:

If conscious, give water to drink and induce vomiting. Never give anything by mouth to an unconscious person. Contact medical personnel for observation or treatment as needed.

NOTE TO PHYSICIANS:

Fenbendazole is a broad spectrum anthelmintic approved for use in animals. It is non-toxic.

5. FIRE FIGHTING MEASURES

FLAMMABLE PROPERTIES

Spontaneous ignition point: 230°C.

EXTINGUISHING MEDIA:

Water, Water mist, alcohol foam, of dry chemical.

5. FIRE FIGHTING MEASURES (Continued)

FIRE FIGHTING INSTRUCTIONS:

Wear full bunker gear, including SCBA, for fighting fires involving this material. Keep upwind.

6. ACCIDENTAL RELEASE MEASURES

PROCEDURES IN CASE OF SPILL OR LEAK:

Sweep and shovel up spilled material. Place in a secure container for disposal.

7. HANDLING and STORAGE

HANDLING:

Flow of material may generate static electricity. Do not pour contents into vessels containing flammable liquids or vapors.

STORAGE:

Store at room temperature. Keep material dry. Protect containers from damage.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

PROTECTIVE EQUIPMENT

EYES:

Prevent eye contact by wearing appropriate eye protection for handling tasks (safety glasses, goggles, or face shield) and by using good work practices.

INHALATION:

Avoid breathing dust. Wear dust respirator if local exhaust ventilation is not available.

9. PHYSICAL and CHEMICAL PROPERTIES

Melting Point: 200 degrees C
Odor: Odorless
Physical Form: Solid
Solubility: Insoluble in water

10. STABILITY and REACTIVITY

CHEMICAL STABILITY:
Stable

HAZARDOUS POLYMERIZATION:
Will not occur.

11. TOXICOLOGICAL INFORMATION

Oral LD50 : rat greater than 10,000 mg/kg
Oral LD50 : mouse greater than 10,000 mg/kg
Skin irritation: negative
Eye irritation: negative

12. ECOLOGICAL INFORMATION

ECOTOXICITY:

Fish Toxicity (LC50): >500 mg/l (Zebrafish) 48 & 96 hrs.
Daphnia Toxicity (LC50): 12 micrograms/l 48 hrs.
Trout Toxicity (LC50): 40 micrograms/l 96 hrs.
Bluegill Sunfish Toxicity (LC50): >19 micrograms/l 21 days.
Earthworm Toxicity (LC50): 180 mg/kg 28 days.
Dung Beetle Toxicity (LD50): >770 mg/kg 7 days.

Note: Fenbendazole can be eliminated in water treatment plants.

13. DISPOSAL CONSIDERATIONS

Waste should be incinerated.

14. TRANSPORT INFORMATION

DOT proper shipping name :Not regulated by DOT

15. REGULATORY INFORMATION

STATE REGULATIONS

The following chemicals associated with the product are subject to the right-to-know regulations in these states:
No components regulated

U.S. FEDERAL REGULATIONS

SARA 313 : No components listed

16. OTHER INFORMATION

REVISION INDICATORS:

The following sections have been revised:

SECTION 12: ECOLOGICAL INFORMATION
ECOTOXICITY

DISCLAIMER:

These data are based on today's state of the art. They are intended to describe our products with regard to safety requirements and do not therefore have the connotation of guaranteeing certain properties.

The information contained herein is offered only as a guide to the handling of this specific material. Since such information does not relate to use of the material with any other material or in any process, any person using this information must determine for him self its suitability for any particular application. The buyer and user assumes all risk and liability of use, storage and/or handling of this product not in accordance with the

DISCLAIMER: (Continued)

terms of the product label.

Hoechst-Roussel Agri-Vet Company

Hoechst Roussel

Product Name: PANACUR® (FENBENDAZOLE) SUSPENSION 10%
Product Code: 20103510
MSDS Number : 00757
Version Date: 10/12/1994

Material Safety Data Sheet

Print date -- November 29th, 1994 1:11 a.m. 3820 PG1A XCH21001 -- 4.1 (16/130)

1. CHEMICAL PRODUCT and COMPANY IDENTIFICATION

Product Name: PANACUR® (FENBENDAZOLE) SUSPENSION 10%
Product Code: 20103510
MSDS Number : 00757

SYNONYMS: FENBENDAZOLE
METHYL-5-PHENYLTHIO-2-BENZIMIDAZOLE-CARBAMATE

HOECHST-ROUSSEL AGRI-VET COMPANY
ROUTE 202-206
P.O. BOX 2500
SOMERVILLE, NJ 08876-1258
UNITED STATES OF AMERICA

PRODUCT USE:
This product is a dewormer (anthelmintic) for horses and cattle.

2. COMPOSITION / INFORMATION on INGREDIENTS

COMPONENT	CAS NUMBER
FENBENDAZOLE	43210-67-9

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW:
Fenbendazole is non-reactive and relatively non-toxic.

POTENTIAL HEALTH EFFECTS
There are no known adverse health effects associated with this product.

DELAYED/LONG TERM EFFECTS

CARCINOGENIC:

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

4. FIRST AID MEASURES

SKIN:
Wash with soap and water. If irritation develops, get medical attention.

EYES:
Flush with water for 15 minutes. If irritation develops, get medical attention.

INHALATION:
In cases of difficult breathing, remove to fresh air. If not breathing, give artificial respiration and get medical attention immediately.

INGESTION:
If conscious, give water to drink and induce vomiting. Never give anything by mouth to an unconscious person. Contact medical personnel for observation or treatment as needed.

NOTE TO PHYSICIANS:
Fenbendazole is a broad spectrum anthelmintic approved for use in animals. It is non-toxic.

5. FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA:
Water, Water mist, alcohol foam, or dry chemical.

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.

Hoechst-Roussel Agri-Vet Company

Hoechst Roussel

Product Name: PANACUR® (FENBENDAZOLE) SUSPENSION 10%
Product Code: 20103510
MSDS Number : 00757
Version Date: 10/12/1994

Print date – November 29th, 1994 1:11 a.m. 3820 PG1A X0H-21001 – 4.3 (18/130)

7. HANDLING and STORAGE

STORAGE:

Store at room temperature. Keep material dry. Protect containers from damage. Keep out of reach of children.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

PROTECTIVE EQUIPMENT

For Bulk Use:

EYES:

Prevent eye contact by wearing appropriate eye protection for handling tasks (safety glasses, goggles, or face shield) and by using good work practices.

9. PHYSICAL and CHEMICAL PROPERTIES

APPEARANCE	: White to off-white
PH	: 5.0 TO 7.0

10. STABILITY and REACTIVITY

CHEMICAL STABILITY:

Stable

HAZARDOUS POLYMERIZATION:

Will not occur.

11. TOXICOLOGICAL INFORMATION

Oral LD50 : rat greater than 10,000 mg/kg
Oral LD50 : mouse greater than 10,000 mg/kg
Skin irritation: negative
Eye irritation: negative

12. ECOLOGICAL INFORMATION

ECOTOXICITY:

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

13. DISPOSAL CONSIDERATIONS

Waste should be incinerated.

14. TRANSPORT INFORMATION

DOT proper shipping name :Not regulated by DOT

15. REGULATORY INFORMATION

STATE REGULATIONS

The following chemicals associated with the product are subject to the right-to-know regulations in these states:
No components regulated

U.S. FEDERAL REGULATIONS

SARA 313 : No components listed

16. OTHER INFORMATION

DISCLAIMER:

The information contained herein is offered only as a guide to the handling of this specific material. Since such information does not relate to use of the material with any other material or in any process, any person using this information must determine for himself its suitability for any particular application. The buyer and user assumes all risk and liability of use, storage and/or handling of this product not in accordance with the terms of the product label.

Hoechst-Roussel Agri-Vet Company

Hoechst Roussel

Product Name: SAFE-GUARD® (FENBENDAZOLE) SUSPENSION 10%
Product Code: 20303536
MSDS Number : 00757
Version Date: 10/12/1994

Material Safety Data Sheet

Print date -- November 29th, 1994 1:11 a.m. 3820 PG1A X0H21001 -- 7.1 (28/130)

1. CHEMICAL PRODUCT and COMPANY IDENTIFICATION

Product Name: SAFE-GUARD® (FENBENDAZOLE) SUSPENSION 10%
Product Code: 20303536
MSDS Number : 00757

SYNONYMS: FENBENDAZOLE
METHYL-5-PHENYLTHIO-2-BENZIMIDAZOLE-CARBAMATE

HOECHST-ROUSSEL AGRI-VET COMPANY
ROUTE 202-206
P.O. BOX 2500
SOMERVILLE, NJ 08876-1258
UNITED STATES OF AMERICA

PRODUCT USE:
This product is a dewormer (anthelmintic) for horses and cattle.

2. COMPOSITION / INFORMATION on INGREDIENTS

COMPONENT	CAS NUMBER
FENBENDAZOLE	43210-67-9

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW:
Fenbendazole is non-reactive and relatively non-toxic.

POTENTIAL HEALTH EFFECTS
There are no known adverse health effects associated with this product.

DELAYED/LONG TERM EFFECTS

CARCINOGENIC:

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

Hoechst-Roussel Agri-Vet Company

Hoechst Roussel

4. FIRST AID MEASURES

SKIN:
Wash with soap and water. If irritation develops, get medical attention.

EYES:
Flush with water for 15 minutes. If irritation develops, get medical attention.

INHALATION:
In cases of difficult breathing, remove to fresh air. If not breathing, give artificial respiration and get medical attention immediately.

INGESTION:
If conscious, give water to drink and induce vomiting. Never give anything by mouth to an unconscious person. Contact medical personnel for observation or treatment as needed.

NOTE TO PHYSICIANS:
Fenbendazole is a broad spectrum anthelmintic approved for use in animals. It is non-toxic.

5. FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA:
Water, Water mist, alcohol foam, or dry chemical.

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.

Hoechst-Roussel Agri-Vet Company

Hoechst Roussel

Product Name: SAFE-GUARD® (FENBENDAZOLE) SUSPENSION 10%
Product Code: 20303536
MSDS Number : 00757
Version Date: 10/12/1994

Print date -- November 29th, 1994 1:11 a.m. 3820 PG1A X0H21001 -- 7.3 (30/130)

7. HANDLING and STORAGE

STORAGE:

Store at room temperature. Keep material dry. Protect containers from damage. Keep out of reach of children.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

PROTECTIVE EQUIPMENT

For Bulk Use:

EYES:

Prevent eye contact by wearing appropriate eye protection for handling tasks (safety glasses, goggles, or face shield) and by using good work practices.

9. PHYSICAL and CHEMICAL PROPERTIES

APPEARANCE	: White to off-white
PH	: 5.0 TO 7.0

10. STABILITY and REACTIVITY

CHEMICAL STABILITY:

Stable

HAZARDOUS POLYMERIZATION:

Will not occur.

11. TOXICOLOGICAL INFORMATION

Oral LD50 : rat greater than 10,000 mg/kg
Oral LD50 : mouse greater than 10,000 mg/kg
Skin irritation: negative
Eye irritation: negative

Hoechst-Roussel Agri-Vet Company

Hoechst Roussel

Product Name: SAFE-GUARD® (FENBENDAZOLE) SUSPENSION 10%
Product Code: 20303536
MSDS Number : 00757
Version Date: 10/12/1994

Print date -- November 29th, 1994 1:11 a.m. 3820 PG1A X0H21001 -- 7.4 (31/130)

12. ECOLOGICAL INFORMATION

ECOTOXICITY:

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

13. DISPOSAL CONSIDERATIONS

Waste should be incinerated.

14. TRANSPORT INFORMATION

DOT proper shipping name :Not regulated by DOT

15. REGULATORY INFORMATION

STATE REGULATIONS

The following chemicals associated with the product are subject to the right-to-know regulations in these states:
No components regulated

U.S. FEDERAL REGULATIONS

SARA 313 : No components listed

16. OTHER INFORMATION

DISCLAIMER:

The information contained herein is offered only as a guide to the handling of this specific material. Since such information does not relate to use of the material with any other material or in any process, any person using this information must determine for himself its suitability for any particular application. The buyer and user assumes all risk and liability of use, storage and/or handling of this product not in accordance with the terms of the product label.

APPENDIX 4

PANACUR® (fenbendazole) Suspension 10%

NADA 128-620

Form FDA 356V
Chemistry, Manufacturing, and Controls
Supplement

PANACUR® (fenbendazole) Suspension 10%

NADA 128-620

TABLE OF CONTENTS

PANACUR® (fenbendazole) Suspension 10%

NADA 128-620

SUMMARY

This present submission is a Supplement to the NADA 128-620 for PANACUR® (fenbendazole) Suspension 10%. This supplemental submission is for the use of PANACUR® (fenbendazole) Suspension 10% in dairy cattle of breeding age. Information is submitted in this Supplement as it pertains to the environmental assessment for manufacturing PANACUR® (fenbendazole) Suspension 10% (drug product) at Hoechst-Roussel Pharmaceuticals Inc. (HRPI), Somerville (Bridgewater), New Jersey.

Supporting information for the Supplement is being submitted in Item 10 of the NADA. All other information in the NADA remains unchanged. For convenience in FDA review, the information has been organized in accordance with the original FDA Form 356V.

PANACUR® (fenbendazole) Suspension 10%

NADA 128-620

Form FDA 356V
Chemistry, Manufacturing, and Controls
Supplement

Item 10

PANACUR Suspension 10%
NADA 128-620
Form FDA 356V

(10) ENVIRONMENTAL ASSESSMENT (EA)

The bulk formulation, filling, packaging, and quality control testing of the drug product, PANACUR® (fenbendazole) Suspension 10%, are performed by Hoechst-Roussel Pharmaceuticals Inc. (HRPI) at their facility in Somerville (Bridgewater), New Jersey.

Revised Environmental Assessment information regarding the operations performed at HRPI, including disposal operations, is submitted in the attached EA Report, dated August 24, 1993.

Abbreviated Environmental Assessment (EA) Information

PANACUR® (fenbendazole) Suspension 10%

NADA 128-620

Items 1-5: 21 CFR Section 25.31 a (a)

1. **DATE:** August 24, 1993

2. **NAME OF APPLICANT/PETITIONER:**

   Hoechst-Roussel Pharmaceuticals Inc.

3. **ADDRESS:**

   Route 202-206
   P.O. Box 2500
   Somerville (Bridgewater), NJ 08876-1258

4. **DESCRIPTION OF THE PROPOSED ACTION:**

   Briefly describe the requested approval; need for the action; the locations where the products will be produced; to the extent possible, the locations where the products will be used and disposed of; and the types of environments present at and adjacent to those locations.

   The purpose of this Supplemental NADA application is to provide a claim for the use of PANACUR® (fenbendazole) Suspension 10% in dairy cattle of breeding age. PANACUR® (fenbendazole) Suspension 10% will be used as an oral anthelmintic to treat dairy cattle against gastrointestinal and lung worms. It has already been used for 16 years in horses (NADA 104-494) and 10 years in cattle, excluding dairy cattle of breeding age (NADA 128-620).

   The proposed action consists of manufacturing and control of the drug product at Hoechst-Roussel Pharmaceuticals Inc. (HRPI). All manufacturing and control operations for the drug product (bulk formulation, filling, packaging, quality control testing) are performed at the facilities of HRPI at the above address.

   Federal, State, and local environmental regulations determine the appropriate system for disposal of waste at HRPI. Waste resulting from manufacturing, packaging, quality-control testing, and distribution of the drug product, including waste resulting

from rejected, returned, or outdated drug product, is incinerated. The incineration facilities are government-permitted. HRPI does not use landfills.

HRPI, manufacturer of the drug product, is located in a suburban region of New Jersey. The land is hilly and the temperature is moderate.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION:

Provide complete nomenclature, CAS Reg. No. (if available), molecular weight, structural formulae, physical description, additives, and impurities. This information is required to be adequate to allow accurate location of data about chemicals in the scientific literature and to allow identification of closely related chemicals.

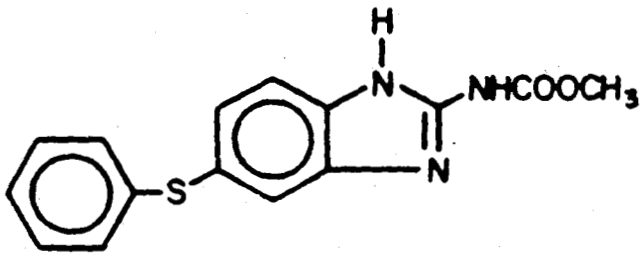
Substance: Fenbendazole (United States Adopted Name)

CAS Registry No.: 43210-67-9

CAS Nomenclature: [5-(phenylthio)-1*H*-benzimidazol-2-yl]-carbamic acid methyl ester

also: Methyl 5-(phenylthio)-2-benzimidazolecarbamate

Structural Formula:



PANACUR® (fenbendazole) Suspension 10%
Environmental Assessment Information

Molecular Formula: $C_{15}H_{13}N_3O_2S$

Molecular Weight: 299.35

Description: White to light brownish or grayish powder; essentially odorless.

Melting Point: Approximately 233° (with decomposition).

pKa: 3.75 ± 0.07

Solubility: Practically insoluble in water (approximately 10-40 ppb.)
Sparingly soluble in dimethylformamide.
Very slightly soluble in methanol.
Freely soluble in DMSO.
Soluble in anhydrous acetic acid

Octanol:Water Partition Coefficient: 3.9

U.V. Absorption Spectrum: Maximum absorptivity at 296 nm.

Impurities in the drug substance and drug product are limited to structurally-related compounds that are derived from synthesis and/or from storage.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

Abbreviated Item 6: 21 CFR Section 25.31a(b)(4)(i)

For the site(s) of production: list the substances expected to be emitted; state the controls exercised; include a citation of, and statement of compliance with, applicable emissions requirements (including occupational) at the Federal, State, and local level; and discuss the effect the approval will have upon compliance with current emissions requirements at the production site(s). Estimate the maximum yearly market volume of the drug product to aid in determining whether approval of the application could result in potentially significant environmental introductions from use of the product.

As a result of the following emission controls exercised at HRPI, substances from manufacturing, packaging, quality-control testing, distribution, and disposal of drug product, will be emitted within established limits of existing discharge permits:

Air-Emission Controls - The physical transfer of product components is controlled to prevent their airborne escape. The air in the manufacturing and control facilities is

filtered, and the used filters are incinerated. Differential-pressure systems control the movement of airborne substances into the manufacturing areas.

There is no fenbendazole discharged into the outside air during the manufacturing of PANACUR® (fenbendazole) 10% Suspension, as the fenbendazole is trapped by dust collectors. Discarded dust collectors and filters are transported by HRPI to licensed incineration facilities.

Water-Emission Controls - Small amounts of fenbendazole are discharged into the sewerage system as a result of cleaning of exposed surfaces and equipment used in manufacturing PANACUR® (fenbendazole) Suspension. The liquid sewage waste stream is treated by chemical and microbial action in the local sewage-waste treatment plant (Somerset Raritan Valley Sewerage Authority). The Authority has granted HRPI discharge Permit No. 10A.

PANACUR® (fenbendazole) 10% Suspension is manufactured in tanks and then fed directly into the filling line to be packed in 500-mL, 1000-mL and 1 gallon containers. Under this process, there is no manufacturing loss of bulk suspension. The waste generated from filling of each container size was calculated using random batches from 1991 production. Average loss during filling of 500-mL containers was 2.893-kg of drug product which is equivalent to 289-g of fenbendazole (0.06% of starting amount of drug product). Average loss during filling of 1000-mL containers was 11.131-kg of drug product which is equivalent to 1.131-kg of fenbendazole (0.25% of starting amount of drug product). Average loss during filling of 1 gallon containers was 3.400-kg of drug product equivalent to 340-g fenbendazole (0.08% of starting amount of drug product). All waste was lost into the sewer system.

Soil-Emission Controls - Waste resulting from manufacturing and filling (spillage or breakage of containers), quality-control testing, and distribution of the drug product, including waste resulting from rejected, returned or outdated drug product, is disposed by incineration. HRPI does not use landfills for disposal of waste. As a result of these practices, terrestrial ecosystems are not exposed.

The applicable emission-(air, water, soil) and occupational-control regulations of the State of New Jersey Department of Environmental Protection (NJDEP) are provided in *Attachment 10-1*. The Federal EPA recognizes NJDEP as agent in administering these regulations. The local Somerville (Bridgewater) government depends on the state government for emission- and occupational-control regulations.

HRPI certifies that the operations involved in manufacturing, packaging, quality-control testing, and distribution of the drug product, and disposal of waste, are in compliance with these emission- and occupational-control regulations.

PANACUR® (fenbendazole) Suspension 10%
Environmental Assessment Information

Once market saturation (1998) for the use of PANACUR® (fenbendazole) Suspension in dairy cattle of breeding age is achieved, production of an additional 11,000 liters of product is anticipated. This is approximately a < 8% increase over the current production volume of this product for use in cattle and horses.

The estimated increase in production of drug product from approval of the proposed action will have no adverse effect upon compliance with emission- and occupational-control regulations of Federal, State, and local governments.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT
8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES
9. USE OF RESOURCES AND ENERGY
10. MITIGATION MEASURES
11. ALTERNATIVES TO THE PROPOSED ACTION

Items 7-11 are intentionally omitted as permitted by 21 CFR Section 25.31a(b)(4)(ii).

12. LIST OF PREPARERS

Those persons preparing the assessment together with their qualifications (expertise, experience, professional disciplines) shall be listed. Persons and agencies consulted shall also be listed.

Janice P. Muller

Master of Arts (Organic Chemistry) 1981
University of California, Santa Barbara

Hoechst-Roussel Agri-Vet Company
Project Manager, Quality Assurance/Manufacturing
Product Development and Registration

Over 10 years experience in the areas of research (medical diagnostics, immunomodulators), technical services (animal health product support), and regulatory affairs (preparation of registration information and project management).

PANACUR® (fenbendazole) Suspension 10%
Environmental Assessment Information

13. CERTIFICATION

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

SIGNATURE: _____ **DATE:** 23 AUGUST 1993

Louis G. Pavloff
Vice President, Operations
HOECHST-ROUSSEL PHARMACEUTICALS INC.

14. REFERENCES

List complete citations for all referenced material. Copies of referenced articles not generally available should be attached.

Not applicable.

15. APPENDICES:

(a) Data summary charts (eg, structural formula, vapor pressure, water solubility, n-octanol/water partition coefficient, bio-degradation half-life, LC_{50} for each species tested, etc.).

(b) Test reports (for each experiment: research objective, experimental design and procedure, all data relevant to interpretation of the test result given in Item 15(a), sample calculations and statistical analyses).

This item is intentionally omitted as permitted by 21 CFR Section 25.31a(b)(4)(ii).

ATTACHMENT 10-1

Environmental and Occupational Regulations
Applicable to the HRPI Somervillle (Bridgewater), NJ Site

Hoechst-Roussel Pharmaceuticals Inc.

Route 202-206 • PO Box 2500 • Somerville, NJ 08876-1258
Telephone (908) 231-2000 • FAX (908) 231-3225

Hoechst

Direct dial number:

Environmental Compliance Summary

Hoechst Celanese Corporation
Hoechst-Roussel Pharmaceuticals, Inc.
(HRPI)

Somerville (Bridgewater), NJ

Air Emissions

The Hoechst Celanese Corporation Bridgewater Site is required to comply with the air emission regulations administered by the New Jersey Department of Environmental Protection and Energy Subchapters 3, 4, 5, 6, 8, 13, 16, and 18, et seq. (NJAC 7:27-et seq.)

The Bridgewater Site is also subject to Federal air emission regulations, which are codified at 40 CFR Parts 60, 61, 70, and 82, as well as the new Clean Air Act Amendments of 1990.

Currently, the Bridgewater Site has 33 active air permits, issued by the NJDEPE, which regulate active sources. Annual inspections are performed by the Regional Enforcement Bureau of the NJDEPE. (See attached list of Permits.)

Wastewater

Wastewater discharges from the Bridgewater Site are subject to both State and Federal regulations. State regulations are found at NJAC 7: 1E, 8, 9, 14, and 14A, Federal Regulations are found at 40 CFR Parts 112, 116, 117, and 122. Additional requirements are enforced by the Somerset Raritan Valley Sewerage Authority (SRVSA).

The Bridgewater Site discharges process and sanitary wastewater to the SRVSA under a permit issued by the SRVSA. Semi-annual sampling and analysis is conducted and reported. Annual inspections are conducted by the SRVSA.

Non-hazardous aqueous wastes from reactor washes and rinses, and non-hazardous aqueous chemical wastes are collected and transported to DuPont Environmental in Deepwater, NJ for biological treatment.

Stormwater from the site discharges into Peters Brook. The site has obtained a permit under the NJDEPE General Stormwater Permit program.

Solid Waste Disposal

The site is required to comply with the United States Environmental Protection Agency (USEPA) Resources Conservation and Recovery Act (RCRA) regulations, codified at 40 CFR Parts 259, 260, 261, 262, and 268. Additional requirements are set forth by NJDEPE at NJAC 7:26-3, 3A, 4A, 7, 8, and 9, as well as 7:26B.

Currently, the site is designated as a "90-day generator"; that is, hazardous waste that is generated on the site must be removed and transported off site to a licensed Treatment, Storage or Disposal Facility (TSDF), within 90 days of the date the waste was generated.

Disposal of medical wastes are regulated by the NJDEPE at NJAC 7:26-3A. All medical wastes generated at the site are collected and transported routinely to a licensed TSDF for incineration.

It is the policy of Hoechst Celanese Corporation, that all wastes be destroyed to the extent that technology exists for the destruction of particular materials. Under no circumstances are any of these wastes placed into a landfill. (See attached list of TSDF's).

Superfund Amendments and Reauthorization Act (SARA)/CERCLA

Sections 312 and 313 of SARA Title III, are complied with. Each year, a SARA 312/NJ Right-To-Know survey (Chemical substances inventory) is completed and submitted to NJDEPE and various local agencies and authorities. In addition, each year the site submits a "negative declaration of non-applicability" pursuant to the requirements for annual reporting of releases under Section 313.

In addition, any accidental release of a hazardous substance is subject to the requirements of the USEPA Comprehensive Environmental Response, Compensation and Liability Act (CERCLA).

The above requirements are codified at NJAC 7:1G and 40 CFR Parts 302, 355, 370, and 372.

Safety

In addition to the environmental regulations cited above, the Bridgewater Site also complies with the requirements for worker safety, set forth in the various areas of the Occupation Safety and Health Act. Most of the applicable regulations are set forth in 29 CFR Part 1910 et seq. Federal OSHA regulates worker safety in the state of New Jersey. Federal OSHA does not issue an occupational safety permit. A separate state agency for occupational safety does not exist in New Jersey.

Hoechst-Roussel Pharmaceuticals Inc.

Route 202-206 • PO Box 2500 • Somerville, NJ 08876-1258
Telephone (908) 231-2000 • FAX (908) 231-3225

Hoechst

Direct dial number:

Hoechst Celanese Corporation
Hoechst-Roussel Pharmaceuticals, Inc.
(HRPI)
Somerville (Bridgewater), NJ

Compliance Certification

Hoechst Celanese Corporation, HRPI certifies that it is in compliance with applicable Environmental, Health and Occupational Safety regulations. Hoechst Celanese Corporation, HRPI certifies that the information contained herein is true, accurate and complete to the best of knowledge. (See attached list of Regulations.)

S/ F. Olp

Steven F. Olp
Manager, EH&S
Bridgewater Site

9/21/94

Date

Hoechst Celanese
Regulatory Operating Permits
Bridgewater Site

Permit Type	Permit #	Source	Expires	Agency
Air	(See attached list of Air Permits.)		Various	NJDEPE
Wastewater	10A	Sanitary/Process	10/95	SRVSA
Hazardous Waste	NJD045787991	Waste Generation	N/A	NJDEPE
Medical Waste	57468	Waste Generation	Annual	NJDEPE
Stormwater	NJ0088315	Stormwater	11/97	NJDEPE

BRIDGEWATER SITE AIR PERMIT INVENTORY LIST

Bldg.	Cert. #	Stack #	Description	Permit Fee	Approval	Effective	Expires	Plant ID #
F	1910416	N/A	DIESEL GENERATOR #2		4/11/91	4/11/91	4/11/96	35080
F	195088	N/A	CUMMINS GENERATOR N855-F N1IRS61F		3/27/95	3/27/95	3/27/00	35080
J	119867	1	J-122 DC-164 DUST COLLECTOR	$250	4/24/77	12/8/94	12/8/99	35080
J	119868	2	J-1028 DC-166 DUST COLLECTOR	$250	4/24/77	12/8/95	12/8/99	35080
J	119869	3	J-122 DC-163 DUST COLLECTOR	$250	4/24/77	12/8/94	12/8/99	35080
J	119870	4	J-1028 DC-167 DUST COLLECTOR	$250	4/24/77	12/8/94	12/8/99	35080
J	119871	5	J-1053 DC-162 DUST COLLECTOR	$250	4/24/77	12/8/95	12/8/95	35080
J	119872	6	J-1053 DC-161 DUST COLLECTOR	$250	4/24/77	12/8/95	12/8/99	35080
J	119873	7	J-1028 DC-165 DUST COLLECTOR	$250	4/24/77	12/8/94	12/8/99	35080
J	119874	8	J-1052 VAC-3 CENTRAL HOUSE VACUUM	$250	2/26/90	12/8/94	12/8/95	35080
J	5155	10	J-1064 GLATT FLUID BED DRYER	$1,000	12/8/94	1/25/77	1/25/97	35080
G	1951337	16	G-DUST COLLECTOR		3/24/95	3/24/95	3/24/99	35080
F	190038	18	F-BOILER #2		10/22/80	10/22/80	3/17/99	35080
F	190037	19	F-BOILER #1		10/21/80	10/21/80	3/17/99	35080
Q	36235	20	Q-VENTURI SCRUBBER		9/25/79	9/25/79	2/23/99	35080
F	190039	22	F-BOILER #3		3/17/94	3/17/94	3/17/99	35080
J	119875	23	J-122 VAC-2 CENTRAL HOUSE VACUUM	$250	11/21/78	12/8/94	12/8/99	35080
G	45899	24	G-RM 305D ACELLA COTA		7/21/80	10/10/81	1/2/98	35080
J	119876	25	J-1036 PRTE-1 ROTO-CLONE	$1,000	8/30/85	12/8/94	3/7/00	35080
J	119877	26	J-1036 PRTE-2 ROTO-CLONE	$1,000	4/25/85	12/9/94	3/8/00	35080
J	119878	31	J-1036 PRTE-5 ROTO-CLONE	$1,000	6/14/85	12/8/94	6/5/95	35080
J	119879	32	J-1036 PRTE-6 ROTO-CLONE	$1,000	6/14/85	12/8/94	6/5/95	35080
J	119880	33	J-1036 PRTE-7 ROTO-CLONE	$1,000	6/14/85	12/8/94	9/6/96	35080
J	119881	34	J-1036 PRTE-8 ROTO-CLONE	$1,000	6/14/85	12/8/94	6/5/95	35080
J	119882	35	J-RM 1096 AZO PNEUMATIC CONVEYOR	$250	8/17/89	12/8/94	12/8/99	35080
J	119883	36	J-1064 MICHIGAN OVEN #1 & #2	$1,000	10/5/90	12/8/94	12/8/99	35080
F	116309	38	DIESEL GENERATOR #1		7/9/90	7/9/90	7/9/99	35080
T	103304	41	STORAGE TANK T-1		10/2/91	10/2/91	10/2/96	35080
T	103305	42	STORAGE TANK T-2		10/2/91	10/2/91	10/2/96	35080
T	1117191	43	STORAGE TANK T-3		10/2/91	10/2/91	10/2/96	35080
T	103625	44	STORAGE TANK T-4		10/2/91	10/2/91	10/2/96	35080
T	103626	45	STORAGE TANK T-5		10/2/91	10/2/91	10/2/96	35080
J	119884	46	J-122 VAC-1 CENTRAL HOUSE VACUUM	$250	2/20/92	12/8/94	12/18/99	35080
J	119885	47	J-1043 AEROMATIC FLUID BED DRYER	$1,000	12/21/90	12/8/94	12/8/99	35080
F	111235	48	COGENERATION SYSTEM		1/29/93	1/29/93	7/17/95	35080
K	1931198	49	BL-3		3/26/93	3/26/93	3/26/98	35080
Q	118049	50	DIESEL GENERATOR #3		6/15/94	6/15/94	6/15/99	35080
Number of active permits:		37		$11,750				

Off-Site Treatment, Storage and Disposal Facilities

Name/Location	Permit#	Exp. Date	Materials Handled	Facility Type
E.I. DuPont Nemours Chambers Works - Rte 130 Deepwater, NJ 08023	NJD002385730	N/A	Nonhazardous Aqueous Wastewater	Biological Treatment
Marisol, Inc. 125 Factory Lane Middlesex, NJ 08846	NJD002454544	N/A	Hazardous Wastes	Fuel Blending
Safety Kleen 515 E. Main Street Bound Brook, NJ 08805	NJD000768077	N/A	Hazardous Wastes	Solvent Recovery
CWM Resources 4301 Infirmary Road West Carrolton, OH	OHD093945293	N/A	Hazardous Wastes	Fuel Blending
ThermalKem, Inc. P.O. Box 2664 Rock Hill, SC	SCD044442333	N/A	Hazardous Wastes	Incineration
Advanced Environmental Technology Corp. One Eden Lane Flanders, NJ 07836	NJD980536593	N/A	Hazardous Wastes	Transfer Facility
Perma-Fix 1940 NW 67th Place Gainsville, FL 32006	FLD980711071	N/A	Low Level Radioactive Liquid Wastes	Fuel Bending
Metro NY Health Waste Processing 910 E. 138th Street Bronx, NY 10454	2-6007-00023/00001-0	N/A	Medical Wastes	Incineration
CMTI 100 Nix Street Hampton, SC 29924	SCN000000002	N/A	Medical Wastes	Incineration
Ogden Martin of Babylon 125 Gleam Street West Babylon, NY 11704	1-4720-00777/00002-0	N/A	Medical Wastes	Incineration
Advanced Environmental Recycling Corp 2591 Mitchell Avenue Allentown, PA 18103	PAD987367216	N/A	Hazardous Wastes (Mercury)	Recycling

N/A - Not Available. The permit numbers listed for Off-Site Disposal Facilities are Federal and/or State EPA ID numbers. These permits are not subject to expiration.

Environmental Regulations

Air

- New Jersey Administrative Code (NJAC) Title 7 Chapter 27 - Air Pollution Control

  - Subchapter 3 - Control and Prohibition of Smoke from Combustion of Fuel (NJAC 7:27-3)
  - Subchapter 4 - Control and Prohibition of Particles from Combustion of Fuel (NJAC 7:27-4)
  - Subchapter 5 - Prohibition of Air Pollution (NJAC 7:27-5)
  - Subchapter 6 - Control and Prohibition of Particles from Manufacturing Processes (NJAC 7:27-6)
  - Subchapter 8 - Permits and Certificates (NJAC 7:27-8)
  - Subchapter 13 - Ambient Air Quality Standards (NJAC 7:27-13)
  - Subchapter 16 - Control and Prohibition of Air Pollution by Volatile Organic Compounds (NJAC 7:27-16)
  - Subchapter 18 - Control and Prohibition of Air Pollution from New or Altered Sources Affecting Ambient Air Quality (Emission Offset Rules) (NJAC 7:27-18)

- Code of Federal Regulations (CFR)

  40 CFR Part 60 - Standards of Performance for New Stationary Sources
  40 CFR Part 61 - National Emission Standards for Hazardous Air Pollutants
  40 CFR Part 70 - State Operating Permit Programs
  40 CFR Part 82 - Protection of the Stratospheric Ozone
  Clean Air Act Amendments - November 1990

Water

- NJAC Title 7 Chapter 1E - Discharges of Petroleum and Other Hazardous Substances (NJAC 7:1E)

- NJAC Title 7 Chapter 8 - Stormwater Management (NJAC 7:8)

- NJAC Title 7 Chapter 9 - Water Pollution Control (NJAC 7:9)

- NJAC Title 7 Chapter 14 - Water Pollution Control Act (NJAC 7:14)

- NJAC Title 7 Chapter 14A - The New Jersey Pollutant Discharge Elimination System (NJAC 7:14A)

- Code of Federal Regulations (CFR)

  40 CFR Part 112 - Oil Pollution Prevention
  40 CFR Part 116 - Designation of Hazardous Substances
  40 CFR Part 117 - Determination of Reportable Quantities of Hazardous Substances
  40 CFR Part 122 - National Pollutant Discharge Elimination System

Solid And Hazardous Waste

- NJAC Title 7 Chapter 26 - Division of Waste Management (NJAC 7:26)

  - Subchapter 3 - Transportation (NJAC 7:26-3)
  - Subchapter 3A - Regulated Medical Waste (NJAC 7:26-3A)
  - Subchapter 4A - Hazardous Waste Fees (NJAC 7:26-4A)
  - Subchapter 7 - Labeling, Records and Transportation Requirements (NJAC 7:26-7)
  - Subchapter 8 - Hazardous Waste Criteria, Identification and Listing (NJAC 7:26-8)
  - Subchapter 9 - Requirements for Hazardous Waste Facilities (NJAC 7:26-9)

- NJAC Title 7 Chapter 26B - Environmental Cleanup Responsibility Act Rules (NJAC 7:26B)

- Code Of Federal Regulations (CFR)

  40 CFR Part 259 - Standards for the Tracking and Management of Medical Waste
  40 CFR Part 260 - Hazardous Waste Management System - General
  40 CFR Part 261 - Identification and Listing of Hazardous Waste
  40 CFR Part 262 - Standards Applicable to Generators of Hazardous Waste
  40 CFR Part 268 - Land Disposal Restrictions

SARA Title III/CERCLA

- NJAC Title 7 Chapter 1G - Worker and Community Right to Know Regulations (NJAC 7:1G)

- Code of Federal Regulations (CFR)

  40 CFR Part 302 - Designation, Reportable Quantities and Notification
  40 CFR Part 355 - Emergency Planning and Notification
  40 CFR Part 370 - Hazardous Chemical Reporting: Community Right-To-Know
  40 CFR Part 372 - Toxic Chemical Release Reporting: Community Right-To-Know

Safety

- Code of Federal Regulations (CFR)

  29 CFR 1910 - Occupational Safety and Health Standards

APPENDIX 5

#266

TRANSLATION

Please note Hoe 881 is the code number for Fenbendazole (generic name), the active ingredient of Panacur®.

Hoechst AG
Pharma Forschung (Research)
Radiochemistry

Dr. Kellner, Dr. Christ

April 25, 1975
Dr. Kn/Tr - 5776 -

Re: Pharmacokinetic Studies on Oral Administration of HOE 881 - ^{14}C to a Cow.

Summary:

The oral administration of 2.25 g HOE 881-^{14}C (≙ about 5.3 mg/kg) as a prepared 2% aqueous suspension to a lactating, black and white lowland cow (schwarzbunte niederungskuh) resulted in slow absorption with a peak in blood levels of 0.52 µg/ml and 0.71 µg/ml in serum occurring 28-30 hours postadministration. After this time, the levels were lower in the milk than in serum. The predominant half-life in blood, serum, and milk was 15 h.

Almost 77% of the applied dose was recovered with the feces, 14% with urine, and only 0.3% in the milk. Excretion was as rapid as elimination from blood and serum. Fifteen days after dosing the overall concentration was very low with the liver showing the highest level of radioactivity at 1.4 µg/g (≙ 0.30% of the applied dose).

In all other organs and tissues the concentrations remained under 0.1 µg/g and for the most part even under 0.01 µg/g.

1. Purpose

The pharmacokinetics of the broad-spectrum anthelmintic HOE 881 has been thoroughly studied in small ruminants (sheep) among other species (1,2). Since the compound is also intended for the treatment of worm infestation in cattle, pharmacokinetic studies in large ruminants were also necessary. A lactating cow was projected as test animal.

2. Material and Methods

2.1. Compound and Formulation

All data involving the labeling of HOE 881 - ^{14}C is contained in the first report (1). In this case, however, the specific activity of batch 4072 IIa was 10.1 mCi/g (3.7×10^8 sec^{-1} g^{-1} ; for purity test see Fig. 1). The compound had a specific surface of 20 m^2/g (measured on 12-6-74 acording to Brunauer, Emmett, and Teller (BET), gas absorption with argon in the Dept. for Applied Physics).

The Galenik Laboratory (Pharmaceutical Development Lab) prepared an aqueous suspension with an active ingredient content of 2.6% (see attached release form from Pharma Galenik (Pharmaceutical Devel.). This suspension is comparable in formulation to the commercial 2.5% aqueous suspension for oral use.

#266

2.2. Test Animal

2.2.1. Housing and Feed

The test animal was a 3-year-old black and white cow (schwarzbunte Niederungskuh) (right ear tag No. H35) with a 6 liter average daily milk production. The cow was acclimated to the housing by holding her in a medium length metabolism stall for one week before the study. The official weight of the cow was 449 kg on the day of stalling. Feed intake dropped in the first few days but increased again on the fourth day and stabilized to a constant daily consumption rate. In this initial preconditioning phase in the metabolism stall, a slight weight reduction estimated at about 25-30 kg (see 2.2.2. dose), that is, below 10%, was observed.

Feed consisted of hay and water (automatic drinking bowls) ad libitum and about 2 kg dairy feed and 3 kg oats in 2 daily rations.

Room temperature was between 22° and 24° C; relative humidity between 55 and 60%.

2.2.2. Application and Dose

Administration of drug was instituted after feed consumption and milk yield had stabilized. Examination by a veterinarian before the test did not yield pathological findings. The drug was given in the morning by stomach tube. In an 87-ml suspension a dose of 2250 mg HOE 881- ^{14}C (- 22.7 m Ci) was administered. At an estimated weight of 420 kg the cow received a dose equal to 5.3 mg HOE 881 - ^{14}C per kg body weight. To eliminate suspension residues in the relatively long tube, it was flushed with about 500 ml tap water. Roughage was withdrawn from the cow on the evening before dosing. Immediately after the dose, hay was again made available.

2.2.3. Obtaining Test Samples

A balloon catheter was inserted into the urinary bladder under extradural anesthesia to allow separate collection of urine and fecal samples. Subsequently, the balloon was filled with fluid preventing expulsion of catheter. The catheter was anchored to the animal's tail in such a way that constant tension was put on the bag to seal the internal orifice of the bladder. In this way it was possible to collect the urine without any leakage. The catheter was attached to connecting tubing leading to a glass receptacle. It was necessary to add a few thymol crystals to the collecting vessel each day in order to prevent immediate bacterial contamination that could lead to an ascending urinary bladder infection.

The cow was killed by exsanguination after tranquilizing with 0.8 ml Rompun (Bayer) and sodium pentobarbital (Nembutal, Abbott) anesthesia.

A so-called feces "catcher" was attached directly under the anus to collect fecal samples.

Blood samples were taken from the jugular vein.

Milk was collected at the intervals shown in the attached table.

2.3. Processing of Samples and Counting Method

Radioactivity was measured by liquid scintillation counting using a suitable colloid-forming mixture of xylene, a polyethoxyethanol, and ethyl alcohol with 1.1% PPO and 0.1% dimethyl - POPOP. This scintillator is comparable to the commercial product Instagel (Packard Instrument Co., USA) or Unisolve (Kochlight GB). The ratio of liquid samples to scintillator was maintained at a level that provided clear, homogeneous test samples.

Processing of blood, urine, feces, and tissues was described in detail in the first study (1).

One ml milk was mixed with 1 ml Digestin (Merck, Darmstadt) and then added to the scintillator.

Subtraction of background radioactivity was made by processing and measuring comparable biological material from untreated animals.

3. Comments on mathematical evaluation

Data in µg were calculated from radioactivity measurements made in the samples. Possible metabolism of radioactivity was not taken into account. When necessary, the complex curves were separated into the individual components by plotting on graph paper. Half-life values were determined by calculating fitted curves.

4. Results

4.1. Blood Level

Data on kinetics in blood and serum are contained in Table 1 and the graph in Figure 2.

Drug absorption was steady and slow with blood levels detectable only after 3 hours post dosing. The blood level reached a peak of 0.52 µg/ml 28 to 30 hours after the drug.

Serum levels were measured parallel to the blood and were higher in all instances (Tab. 1). The difference between these two concentrations was greater initially but decreased with time. Commencing from the time of peak levels, serum concentration was still 40% above blood levels of drug.

Concentrations in both serum and blood declined at an equally rapid rate. The concentration in blood could only be measured for five days (0.1 ml - test sample). Half-life for the time period between maximum and 4 days postdose was 15 hours (segment B). This is also the half-life in the same time period in serum (segment S). Since 2-ml samples were measured in the serum, the levels could be followed for a considerably longer time making it possible to plot the total curve.

A double-phase elimination was noted. A half-life of 5 days was recorded for the slow phase III_S. Phase II_S yielded a half-life of 11 hours. The 15-hour value for segment S stemmed from the overlapping of both phases.

4.2. Elimination

4.2.1. Milk

With milk, 0.30% of the deliveredradioactivity was eliminated (Tab. 1). The highest concentration of 0.59 µg/ml was found in the milk between 8 and 22 hours post drug. Comparison of milk and serum levels (Fig.2) showed that concentrations in the milk were markedly higheronly during the first 8 hours after administration At the following collection interval both values were practically equal and subsequent milk concentrations were always below serum levels (Tab.1). The level of radioactivity fell until it reached assay limit after about 6 days (0.002µg/ml) with a half-life of 14 hours. There is no slow phase in contrast to serum (blood) levels.

4.2.2. Urine and Feces

Almost 77% of the orally administered radioactivity was found in the feces and 14% in the urine (Tab. 2). Figure 3 shows the elimination curve in urine and feces. Excretion of radioactivity with the urine begins from the third day post dose; with the feces, from the second day. Excretion follows a 2-phase process as was also established for serum. In addition, half-lives are similar to that found in serum.

After the slower phase only 3% of the dose was eliminated.

4.3. Distribution

The cow was killed 15 days after dosing and the organs and tissues listed in Table 3 were measured for radioactivity.

Out of the very low total concentrations found, only in the liver was a level distinctly above the assay limit detected at 1.4 µg/g $\triangleq$ 0.30% of given dose. Considerably lower concentrations of 0.09 µg/g in the kidneys and 0.05 µg/g in the gallbladder followed in these two systems involved with elimination. Detectable levels of 0.02 µg/g in the lungs and gonads were already just slightly above the assay limit. The same was the case for the adrenals.

Recovery amounted to 91%. The studies were carried out by:

Dr. Herok, RCL: Synthesis of HOE 881 - ^{14}C
Dr. Lötzsch, RCL: Purity test
Mr. Ebel, Pharmacist and Mr. Tillmann, Pharma-Galenik (Pharmaceutical Development Laboratory): Manufact. of Preparation

(signed)

Christ, Kellner

References:

(1) Dr. Kellner; Dr. Christ
Pharmacokinetic studies after oral administration of Hoe 881 to sheep, dogs, rabbits, and rats
(AN 3376, 6-29-73)

(2) Dr. Kellner; Dr. Christ
Pharmacokinetic studies in rats, rabbits, dogs, sheep, and pigs, after i.v. and oral administration of Hoe 881-C^{14}
(AN 4912, 9-2-74)

Concentrations in blood, serum, and milk

Content in -- milk

Ratio of serum to blood as well as milk to serum

after oral administration of ~5.3 mg/kg Hoe 881-C^{14} to a cow.

Time after Administr.	Concentration in Blood µg/ml	Concentration in Serum µg/ml	Time aft. Administr.	Concentr. in Milk µg/ml	% Content of Milk Milch	Serum / Blood	Milk / Serum +)
1 h	< 0.02	< 0.002	0-8 h	0.16	0.011	-	1.9
2	< 0.02	0.009	8-22	0.59	0.096	-	1.1
3	0.02	0.037	22-33	0.54	0.056	1.85	0.78
4	0.05	0.086	33-46	0.41	0.064	1.72	0.66
5	0.09	0.15	46-57	0.27	0.031	1.67	0.63
6	0.13	0.20	57-70	0.14	0.022	1.54	0.56
7	0.16	0.26	70-81	0.060	0.0060	1.62	0.43
8	0.22	0.33	81-94	0.034	0.0049	1.50	0.44
			94-105	0.020	0.0024		0.44
24	0.50	0.67	105-118	0.010	0.0018	1.34	0.29
26	0.49	0.69	118-129	0.006	0.0007	1.41	0.20
28	0.52	0.71	129-142	0.004	0.0006	1.37	0.15
30	0.52	0.71	142-153	0.002	0.0003	1.37	0.08
32	0.50	0.67	153-166	< 0.002	< 0.0003	1.34	
			166-177	< 0.002	< 0.0003		
2 d	0.35	0.49	177-190	< 0.002	< 0.0003	1.40	
2.3	0.26	0.35	0-190 h		0.30	1.35	
3	0.12	0.17				1.42	
3.3	0.08	0.12				(1.50)	
4	0.04	0.049				(1.22)	
5	0.02	0.033				(1.65)	
6	< 0.02	0.024					
6.3	< 0.02	0.023					
7		0.021					
7.3		0.020					
8		0.018					
8.3		0.018					
9		0.014					
9.3		0.016					
10		0.013					
10.3		(0.007)					
15		0.007					

+) Serum concentrations were taken from the curve in Figure 2.

Table 2: Excretion with urine, feces, and milk (milk: see also Table 1) after oral administration of ~5.3 mg Hoe 881-C^{14} per kg body weight to a cow.

Time after Administration	Excretion of Administered Radioactivity in %			
	Urine	Feces	Lavage	Milk
0-2 h	0.004			
2-4	0.027			
4-6	0.11			
6-8	0.17			
8-24	2.8			
0-1 d	3.1	28		
1-2	4.5	29	0.09	
2-3	3.7	11	0.04	
3-4	1.8	2.3		
4-5	0.53	4.7		
5-6	0.19	0.63	0.01	
→ 6-7	0.091	0.38	0.003	
→ 7-8	0.052	0.21		
8-9	0.029	0.075		
9-10	0.020	0.057		
10-11	0.013	0.046		
11-12	0.010	0.033		
12-13	0.008	0.025		
13-14	0.007	0.022		
14-15	0.005	0.014		
0-15 d	14.1	76.5	0.14	0.30
Σ Excretion	91.0			

Table 3: Distribution 15 days after oral administration of ~5.3 mg/kg Hoe 881-C^{14} to a cow.

	Concentration µg/g	%
Pancreas	< 0.01	
Spleen	< 0.05	
Adrenals	0.01	
Stomach	< 0.01	
Small intestine	< 0.01	
Colon	< 0.01	
Kidneys	0.09	
Gonads	0.02	
Liver	1.4	0.30
Gall bladder	0.05	
Heart	< 0.01	
Lung	0.02	
Bladder	< 0.01	
Blood	< 0.02	
Muscles	< 0.01	
Subcutaneous Fat	< 0.01	
Suet	< 0.01	
Brain	< 0.01	
Uterus	< 0.01	
Omasum	< 0.01	
Abomasum	< 0.01	
Udder	< 0.01	

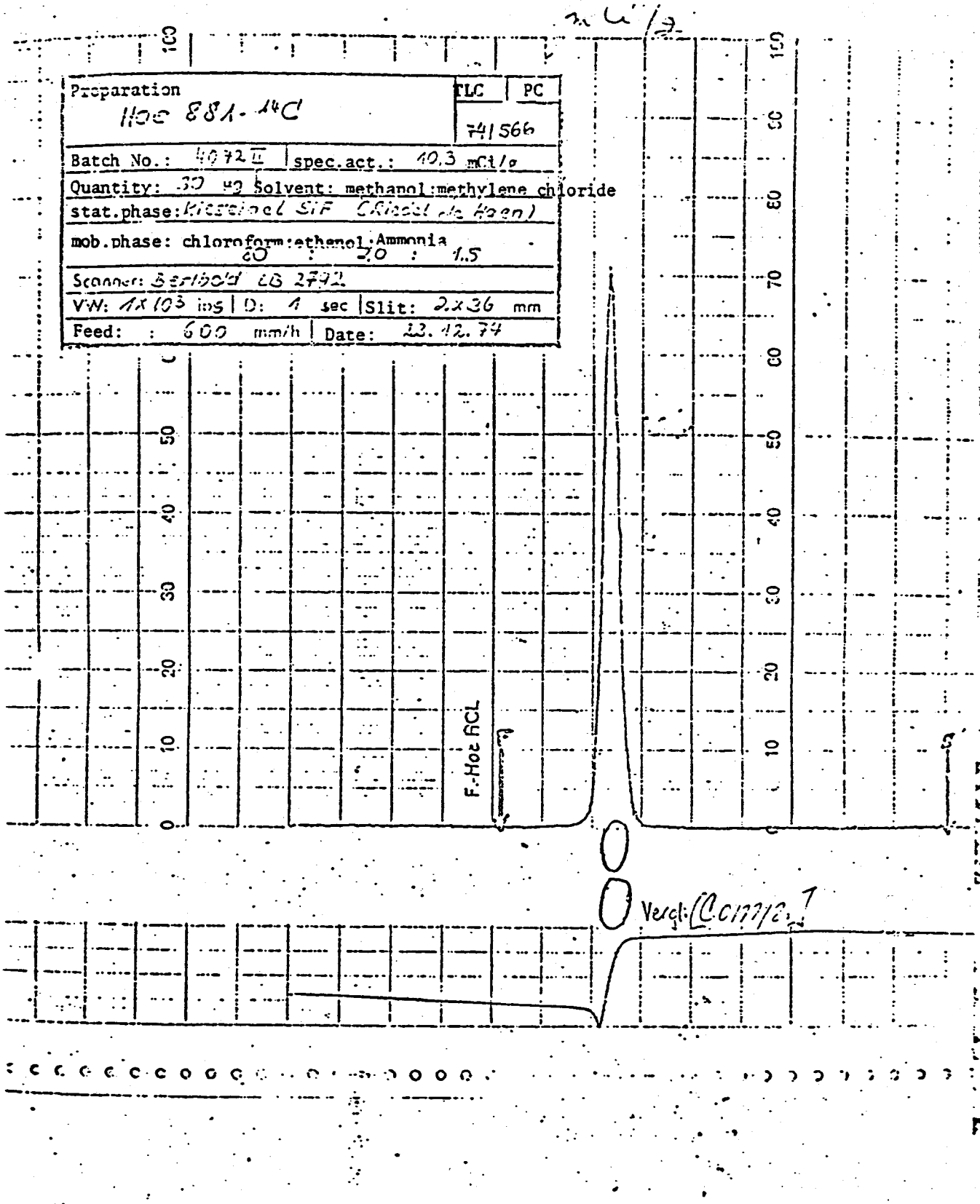


Figure 1: Radio Thin-layer Chromatogram

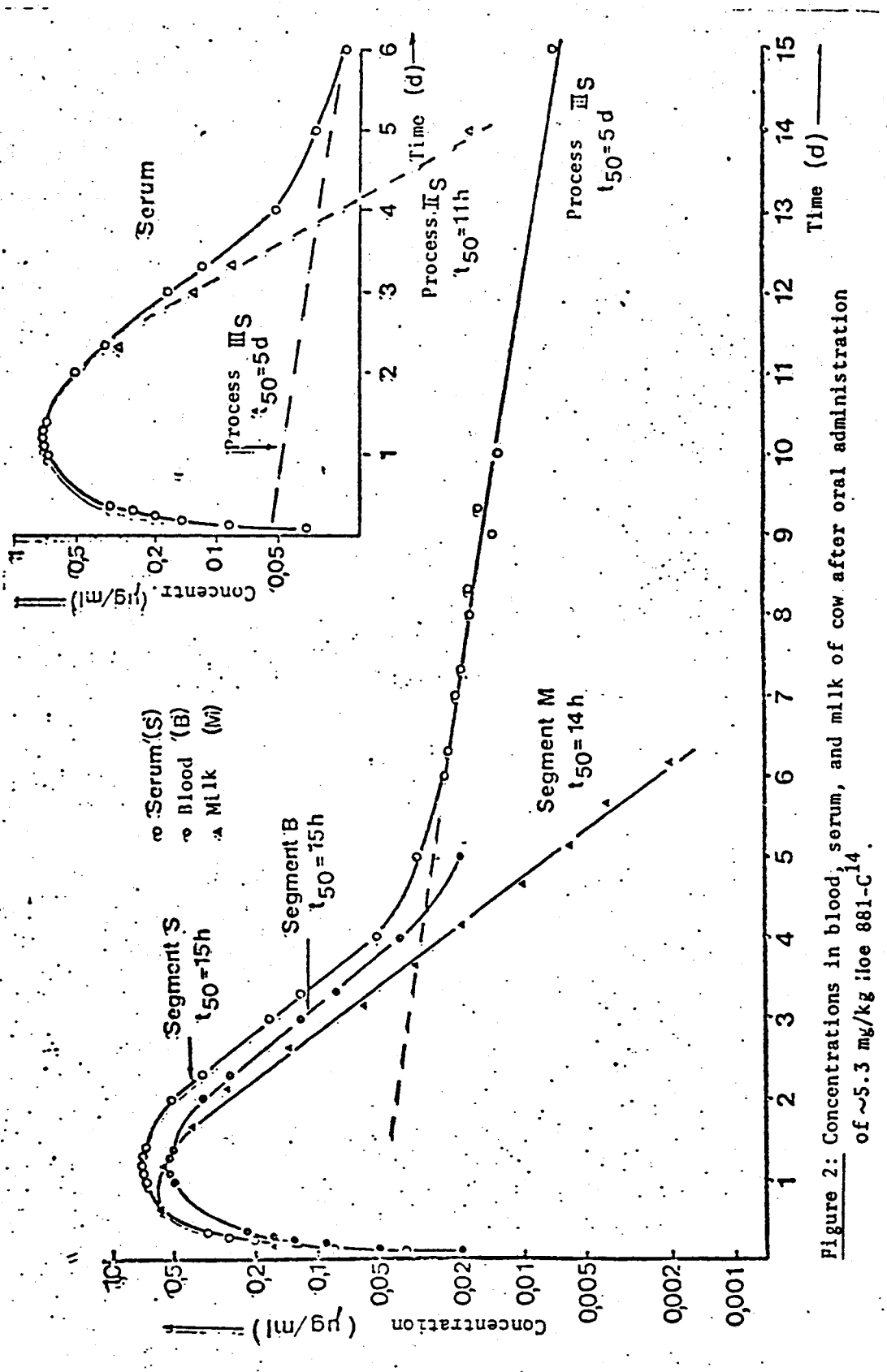


Figure 2: Concentrations in blood, serum, and milk of cow after oral administration of ~5.3 mg/kg iloe 881-C^{14}.

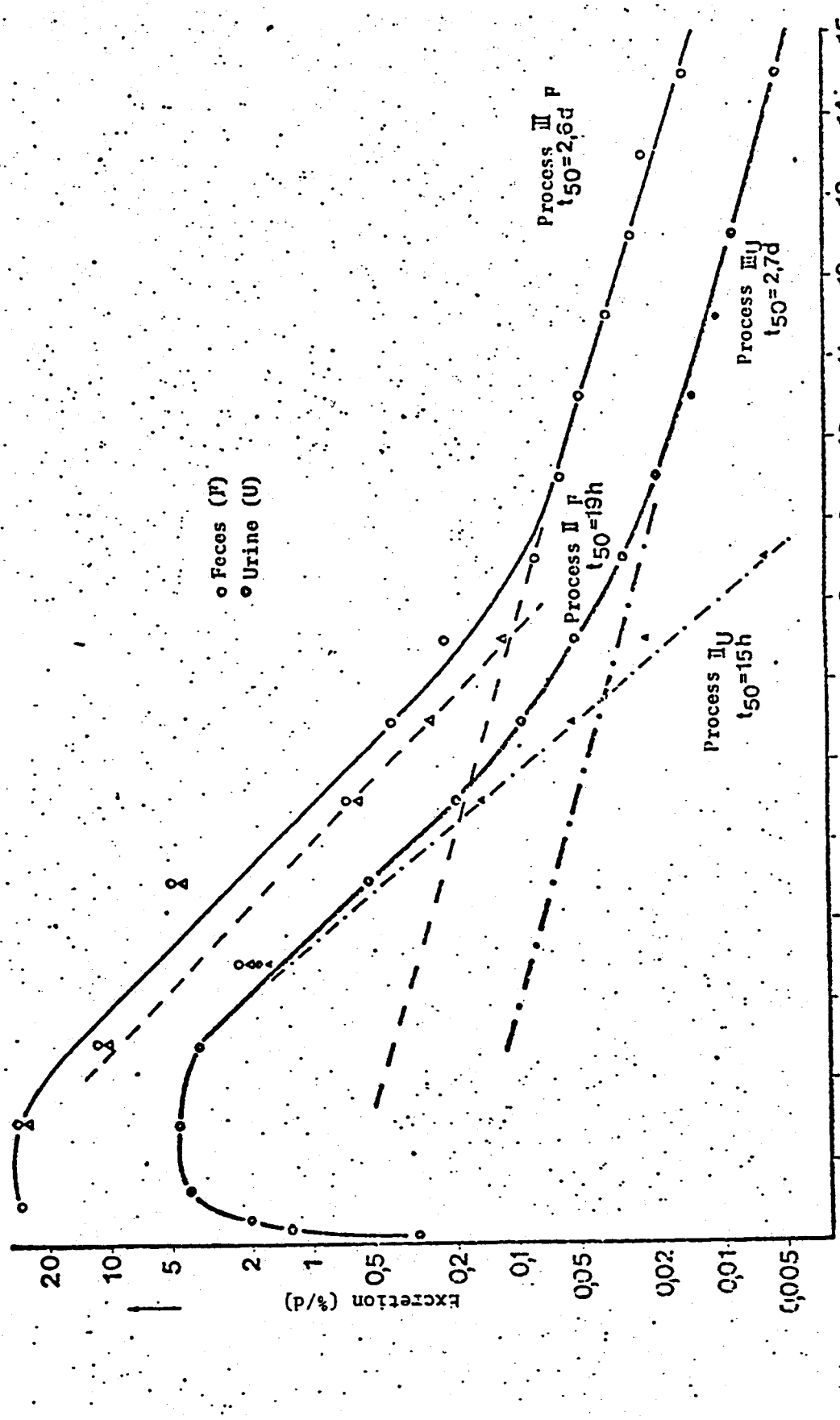


Table 3: Excretion with urine and feces after oral administration of ~5.3 mg/kg Hoe 881-C^{14} to a cow.

HOECHST

Enclosure to AN RCL Dr.Kn/Tr -5776- April 25, 1975

TO: Dr. Christ, RCL, Med.Dept. via Pharmac.Research, G 838
FROM: Pharmaceutical Development

6230 Frankfurt/M-80, January 17, 1975
Ebel (Pharm.) 34/75

In compliance with your order No. 10/75 you receive:

ca. 160 g of a 2.5% Suspension
Preparation Hoe 881-C^{14}
Batch No. 4072 II a

Activity	:	260.9 µCi/ml
Act.substance	:	Batch No. 4072 II
Spec.activity	:	10.1 mCi/g
Contents	:	25.8 mg/ml
Test certificate	:	No.817, date 1-14-75 from Dr. Christ's Laboratory/RCL

(sign. Ebel)

Hoechst

Hoechst Aktiengesellschaft
Pharma Forschung
Radiochemie

Dr. Kellner, Dr. Christ

F-Höchst, 25. April 1975
Dr.Kn/Tr - 5776 -

Report Submitted to FDA
Under ~~NADA~~ # NADA 1684
Date 8/5/75

Betr.: Untersuchungen zur Pharmakokinetik nach oraler Gabe von Hoe 881-^{14}C an eine Kuh

Zusammenfassung: Nach oraler Gabe von 2.25 g Hoe 881-^{14}C (≙ ca. 5.3 mg/kg) als 2.6 %-ige gebrauchsfertige wäßrige Suspension an eine laktierende schwarzbunte Niederungskuh führte die langsame Resorption 28-3o h p.appl. zu maximalen Spiegeln im Blut von o.52 µg/ml und im Serum von o.71 µg/ml. Danach waren die Konzentrationen in der Milch niedriger als im Serum. Die dominierende Halbwertszeit in Blut, Serum und Milch war 15 h.

Im Kot wurden fast 77 % der zugeführten Dosis wiedergefunden, im Urin 14 % und in der Milch nur o.3 %. Die Ausscheidung verlief ähnlich schnell wie die Elimination aus Blut und Serum. Fünfzehn Tage p.appl. wies bei den insgesamt sehr niedrigen Konzentrationen die Leber mit 1.4 µg/g (≙ o.3o % der appl. Dosis) die höchste Radioaktivität auf.

In allen anderen Organen/Geweben lagen die Konzentrationen unter o.1 µg/g, zum großen Teil sogar unter o.o1 µg/g.

1. Fragestellung

Das Breitspektrumanthelminthikum Hoe 881 ist hinsichtlich seines pharmakokinetischen Verhaltens neben anderen Tierarten ausführlich am kleinen Wiederkäuer (Schaf) untersucht worden (1,2). Da das Präparat auch für die Behandlung des Wurmbefalls bei Rindern vorgesehen ist, waren pharmakokinetische Untersuchungen auch am großen Wiederkäuer erforderlich. Als Versuchstier war eine laktierende Kuh vorgesehen.

2. Material und Methoden

2.1 Präparat und Formulierung

Alle Angaben, die die Markierung von Hoe 881-^{14}C betreffen, sind in der ersten Mitteilung enthalten (1). Abweichend davon betrug die spezifische Radicaktivität der verwendeten Charge 4o72 IIa 1o.1 mCi/g ($3.7 \times 1o^{8}$ $sec^{-1}g^{-1}$; Ergebnis der Reinheitsprüfung s. Abb. 1). Das Präparat hatte eine spezifische Oberfläche von 2o m^2/g (gemessen am 6.12.1974 nach BET, Gasadsorption mit Argon in der Abteilung für Angewandte Physik).

Die vom Galenischen Laboratorium angefertigte Zubereitung war eine wäßrige Suspension mit einem Wirkstoffgehalt von 2.6 % (s. Abgabeschein Pharma-Galenik im Anhang). Sie entsprach in ihrer übrigen Zusammensetzung der gebrauchsfertigen 2.5 %-igen wäßrigen Suspension für orale Zwecke.

2.2 Versuchstier

2.2.1 Haltung und Futter

Als Versuchstier diente eine etwa 3-jährige schwarzbunte Niederungskuh (Ohrmarke rechts Nr. H 35) mit einer durchschnittlichen täglichen Milchleistung von ca. 6 l. Sie wurde zur Eingewöhnung bereits eine Woche vor Versuchsbeginn in ein Stoffwechselgestell gebracht, das in seinen Abmessungen einem Mittellangstand entsprach. Am Tag der Aufstallung wog die Kuh nach dem amtlichen Wiegeschein 449 kg. In den ersten Tagen ging die Futteraufnahme etwas zurück, nahm aber vom 4. Tag an wieder zu und stabilisierte sich dann auf eine täglich konstante Futtermenge. In dieser Anfangsphase der Gewöhnung an das Stoffwechselgestell trat eine leichte Gewichtsreduktion ein, die auf ca. 25-3o kg geschätzt wurde (s. 2.2.2 Dosis), also unter 1o % lag.

Die Fütterung bestand aus Heu und Wasser (Selbsttränke) ad libitum und ca. 2 kg Milchviehfutter und 3 kg Hafer in 2 Tagesportionen.

Die Raumtemperatur lag zwischen 22 und 24 °C, die relative Luftfeuchte zwischen 55 und 6o %.

2.2.2 Applikation und Dosis

Die Applikation wurde erst vorgenommen, nachdem Futteraufnahme und Milchleistung konstant blieben. Die vor der Behandlung durchgeführte tierärztliche Untersuchung ergab keinen Hinweis auf eine Erkrankung. Die Applikation erfolgte morgens mit einer Nasenschlundsonde. In 87 ml Suspension wurden 2 25o mg Hoe 881-^{14}C (≙ 22.7 mCi) verabreicht. Diese Menge würde bei einem geschätzten Gewicht der Kuh von 42o kg einer Dosis von 5.3 mg Hoe 881-^{14}C pro kg Körpergewicht entsprechen. Um Suspensionsrückstände in der relativ langen Sonde zu vermeiden, mußte mit ca. 5oo ml Leitungswasser nachgespült werden. Am Abend vor der Applikation war der Kuh das Rauhfutter entzogen worden. Unmittelbar nach der Gabe konnte sie Heu wieder nach Belieben aufnehmen.

2.2.3 Gewinnung von Untersuchungsmaterial

Um den Urin getrennt vom Kot gewinnen zu können, wurde ein Ballonkatheter in die Harnblase unter extraduraler Anaesthesie eingeführt. Anschließend wurde der Ballon mit Flüssigkeit gefüllt und damit ein Herauspressen des Katheters verhindert. Der Katheter war so am Schwanz der Kuh fixiert, daß er ständig unter leichtem Zug stand und so das Orificium internum der Harnblase verschloß. Damit war ein völlig verlustfreier Gewinn des Urins gewährleistet. Der Katheter war mit einer Schlauchleitung verbunden, die in ein Glassammelgefäß führte. Dem Urin im Sammelgefäß mußten täglich einige Thymolkristalle zugesetzt werden, um eine sofortige bakterielle Zersetzung zu verzögern, die zu einer aszendierenden Harnblaseninfektion hätte führen können.

Die Kuh wurde durch Entbluten getötet, nachdem sie vorher mit einem Tranquilizer (o.8 ml Rompun, Bayer) ruhiggestellt und mit Pentobarbital-Na (Nembutal, Abbott) anaesthesiert worden war.

Der Kot wurde über eine sog. Kotschürze, die direkt unter dem After befestigt war, in das Kotsammelgefäß geleitet.

Blutproben stammten aus der Vena jugularis.

Für Milch können die Sammelintervalle den Tabellen im Anhang entnommen werden.

2.3 Aufarbeitung der Proben und Meßtechnik

Für die Radioaktivitätsmessung nach dem Flüssigkeitsszintillationsverfahren diente eine zur Kolloidbildung geeignete Mischung aus Xylol, einem Polyäthoxyäthanol und Äthanol mit 1.1 % PPO und 0.1 % Dimethyl-POPOP. Dieser Szintillator ist mit den Handelsprodukten Instagel (Packard Instrument Comp., USA) bzw. Unisolve (Kochlight, GB) vergleichbar. Das Verhältnis der wäßrigen Proben zur Szintillatorflüssigkeit war stets so gewählt, daß klare, homogene Meßproben vorlagen.

Die Aufarbeitungsverfahren für Blut, Urin, Kot und Gewebe wurden bereits bei den ersten Untersuchungen ausführlich beschrieben (1).

Von der Milch wurde 1 ml mit 1 ml Digestin (Merck, Darmstadt) versetzt und dann der Szintillator zugegeben.

Zur Nullwertsubtraktion wurde entsprechendes biologisches Material von unbehandelten Tieren aufgearbeitet und gemessen.

3. Anmerkungen zur rechnerischen Auswertung

Die Angaben in µg wurden aus dem Radioaktivitätsgehalt der Proben errechnet. Eine mögliche Metabolisierung blieb unberücksichtigt. Die komplexen Kurven wurden - wenn erforderlich - durch graphische Analyse in die einzelnen Komponenten zerlegt. Die Bestimmung der angegebenen Halbwertszeiten erfolgte durch Ausgleichsrechnung.

4. Ergebnisse

4.1 Blutspiegel

Die Daten über die Kinetik in Blut und Serum sind in Tabelle 1 enthalten und in Abbildung 2 graphisch dargestellt.

Die Resorption verlief langsam und stetig, der erste nachweisbare Blutspiegel trat 3 h p.appl. auf. Das Maximum im Blut war 28 bis 3o h nach der Gabe mit o.52 µg/ml erreicht.

Im Serum, das parallel zum Blut gemessen wurde, waren die Konzentrationen zu allen Zeiten höher (Tab. 1), dabei war dieser Unterschied am Anfang größer, verringerte sich aber mit zunehmender Zeit. Etwa vom Maximum an lagen die Serumspiegel noch 4o % über den Blutspiegeln.

Die Konzentrationsabnahme in Serum und Blut war gleich schnell. Im Blut konnte die Konzentration nur bis 5 Tage gemessen werden (o.1 ml-Meßproben). Die Halbwertszeit für den zeitlichen Bereich zwischen Maximum und 4 d p.appl. betrug 15 h (Segment B). Diese Halbwertszeit gilt auch für das Serum im selben zeitlichen Bereich (Segment S). Da beim Serum 2 ml-Proben zu messen waren, konnte der Spiegel hier wesentlich länger verfolgt werden, wodurch eine graphische Analyse des Gesamtverlaufs möglich war.

Es läßt sich ein zweiphasischer Abfall erkennen. Für den langsamen Vorgang III_s ergab sich eine Halbwertszeit von 5 d. Für Vorgang II_s lieferte die graphische Analyse eine Halbwertszeit von 11 h. Der Wert von 15 h im Segment S kommt durch Überlagerung dieser beiden Vorgänge zustande.

4.2 Ausscheidung

4.2.1 Milch

Mit der Milch wurden o.3o % der applizierten Radioaktivität ausgeschieden (Tab. 1). Die höchste Konzentration wurde in dem Gemelk zwischen 8 und 22 h p.appl. mit o.59 µg/ml gefunden. Ein Vergleich mit den Serumspiegeln (Abb. 2) macht deutlich, daß die Konzentrationen in der Milch nur in den ersten 8 h p.appl. deutlich höher als im Serum gelegen haben. Im nächsten Sammelintervall waren sie fast schon gleich und danach in der Milch immer niedriger als im Serum (Tab. 1).

Der Radioaktivitätsspiegel fiel bis zum Erreichen der Nachweisgrenze nach rund 6 Tagen (0.002 µg/ml) mit einer Halbwertszeit von 14 h ab. Im Unterschied zum Serum (Blut) fehlte der langsame Vorgang.

4.2.2 Urin und Kot

Fast 77 % der oral zugeführten Radioaktivität wurden im Kot und 14 % im Urin gefunden (Tab. 2). Abbildung 3 zeigt den Ausscheidungsverlauf für Urin und Kot. Die Exkretion folgte beim Urin vom dritten, beim Kot vom zweiten Tag p.appl. an einem 2-phasischen Prozeß, wie er auch beim Serum festgestellt wurde. Auch die Halbwertszeiten sind den dort gefundenen ähnlich.

Nach dem langsameren Vorgang wurden nur etwa 3 % der Dosis ausgeschieden.

4.3 Verteilung

Die Kuh wurde 15 d nach Applikation getötet und die in Tabelle 3 aufgeführten Organe und Gewebe auf ihren Radioaktivitätsgehalt untersucht.

Von den insgesamt sehr niedrigen Konzentrationen wies nur die Leber mit 1.4 µg/g ≙ 0.3o % der gegebenen Dosis einen deutlich über der Nachweisgrenze liegenden Wert auf. Mit wesentlich niedrigeren Konzentrationen von 0.09 µg/g bzw. 0.05 µg/g folgten mit Niere und Galle 2 weitere Systeme, die mit der Ausscheidung in Zusammenhang stehen. Die in Lunge und Gonaden nachgewiesenen Konzentrationen von 0.02 µg/g lagen schon sehr nahe an der Nachweisgrenze. Dasselbe gilt auch für die Nebennieren.

Die Bilanz betrug 91 %.

An den Untersuchungen waren beteiligt:

Dr. Herok, RCL: Synthese von Hoe 881-^{14}C
Dr. Lötzsch, RCL: Reinheitsprüfung
Ap.Ebel/Herr Tillmann, Pharma-Galenik: Anfertigung der Zubereitung

Z i t a t e

(1) Dr. Kellner, Dr. Christ
Pharmakokinetische Untersuchungen nach oraler Gabe von Hoe 881 an Schafe, Hunde, Kaninchen und Ratten
(AN 3376 vom 29.6.1973)

(2) Dr. Kellner, Dr. Christ
Untersuchungen zur Pharmakokinetik nach intravenöser und oraler Gabe von Hoe 881-^{14}C an Ratten, Kaninchen, Hunde, Schafe und Schweine
(AN 4912 vom 2.9.1974)

Tab. 1: KONZENTRATION IN BLUT, SERUM UND MILCH UND %-GEHALT DER MILCH SOWIE QUOTIENTEN AUS SERUM UND BLUT SOWIE MILCH UND SERUM NACH ORALER GABE VON ~ 5.3 mg Hoe 881-^{14}C PRO kg KÖRPERGEWICHT AN EINE KUH

Zeit p.appl.	Konzentration im Blut µg/ml	im Serum µg/ml	Zeit p.appl.	Konzentr. i.d.Milch µg/ml	%-Gehalt der Milch	Serum/Blut	Milch/Serum +)
1 h	< 0.02	< 0.002	0-8 h	0.16	0.011	-	1.9
2	< 0.02	0.009	8-22	0.59	0.096	-	1.1
3	0.02	0.037	22-33	0.54	0.056	1.85	0.78
4	0.05	0.086	33-46	0.41	0.064	1.72	0.66
5	0.09	0.15	46-57	0.27	0.031	1.67	0.63
6	0.13	0.20	57-70	0.14	0.022	1.54	0.56
7	0.16	0.26	70-81	0.060	0.0060	1.62	0.43
8	0.22	0.33	81-94	0.034	0.0049	1.50	0.44
			94-105	0.020	0.0024		0.44
24	0.50	0.67	105-118	0.010	0.0018	1.34	0.29
26	0.49	0.69	118-129	0.006	0.0007	1.41	0.20
28	0.52	0.71	129-142	0.004	0.0006	1.37	0.15
30	0.52	0.71	142-153	0.002	0.0003	1.37	0.08
32	0.50	0.67	153-166	< 0.002	<0.0003	1.34	
			166-177	< 0.002	<0.0003		
2 d	0.35	0.49	177-190	< 0.002	<0.0003	1.40	
2.3	0.26	0.35	0-190 h		0.30	1.35	
3	0.12	0.17				1.42	
3.3	0.08	0.12				(1.50)	
4	0.04	0.049				(1.22)	
5	0.02	0.033				(1.65)	
6	< 0.02	0.024					
6.3	< 0.02	0.023					
7		0.021					
7.3		0.020					
8		0.018					
8.3		0.018					
9		0.014					
9.3		0.016					
10		0.013					
10.3		(0.007)					
15		0.007					

+) Serumkonzentrationen wurden dem Kurvenverlauf in Abb. 2 entnommen

Tab. 2: AUSSCHEIDUNG MIT URIN, KOT UND MILCH (MILCH s. AUCH Tab. 1) NACH ORALER GABE VON ~5.3 mg Hoe 881-^{14}C PRO kg KÖRPERGEWICHT AN EINE KUH

Zeit p.appl.	Prozent der applizierten Radioaktivität			
	Urin	Kot	Spüle	Milch
o-2 h	o.oo4			
2-4	o.o27			
4-6	o.11			
6-8	o.17			
8-24	2.8			
o-1 d	3.1	28		
1-2	4.5	29	o.o9	
2-3	3.7	11	o.o4	
3-4	1.8	2.3		
4-5	o.53	4.7		
5-6	o.19	o.63	o.o1	
6-7	o.o91	o.38	o.oo3	
7-8	o.o52	o.21		
8-9	o.o29	o.o75		
9-1o	o.o2o	o.o57		
1o-11	o.o13	o.o46		
11-12	o.o1o	o.o33		
12-13	o.oo8	o.o25		
13-14	o.oo7	o.o22		
14-15	o.oo5	o.o14		
o-15 d	14.1	76.5	o.14	o.3o
∑Ausscheidung	91.o			

Tab. 3: VERTEILUNG 15 d NACH ORALER GABE VON ~5.3 mg Hoe 881-^{14}C PRO kg KÖRPERGEWICHT AN EINE KUH

	Konzentration	
	µg/g	%
Pankreas	< 0.01	
Milz	< 0.05	
Nebennieren	0.01	
Magen	< 0.01	
Dünndarm	< 0.01	
Dickdarm	< 0.01	
Nieren	0.09	
Gonaden	0.02	
Leber	1.4	0.3o
Galle	0.05	
Herz	< 0.01	
Lunge	0.02	
Harnblase	< 0.01	
Blut	< 0.02	
Muskulatur	< 0.01	
Unterhautfett	< 0.01	
Nierenfett	< 0.01	
Gehirn	< 0.01	
Uterus	< 0.01	
Blättermagen	< 0.01	
Labmagen	< 0.01	
Euter	< 0.01	

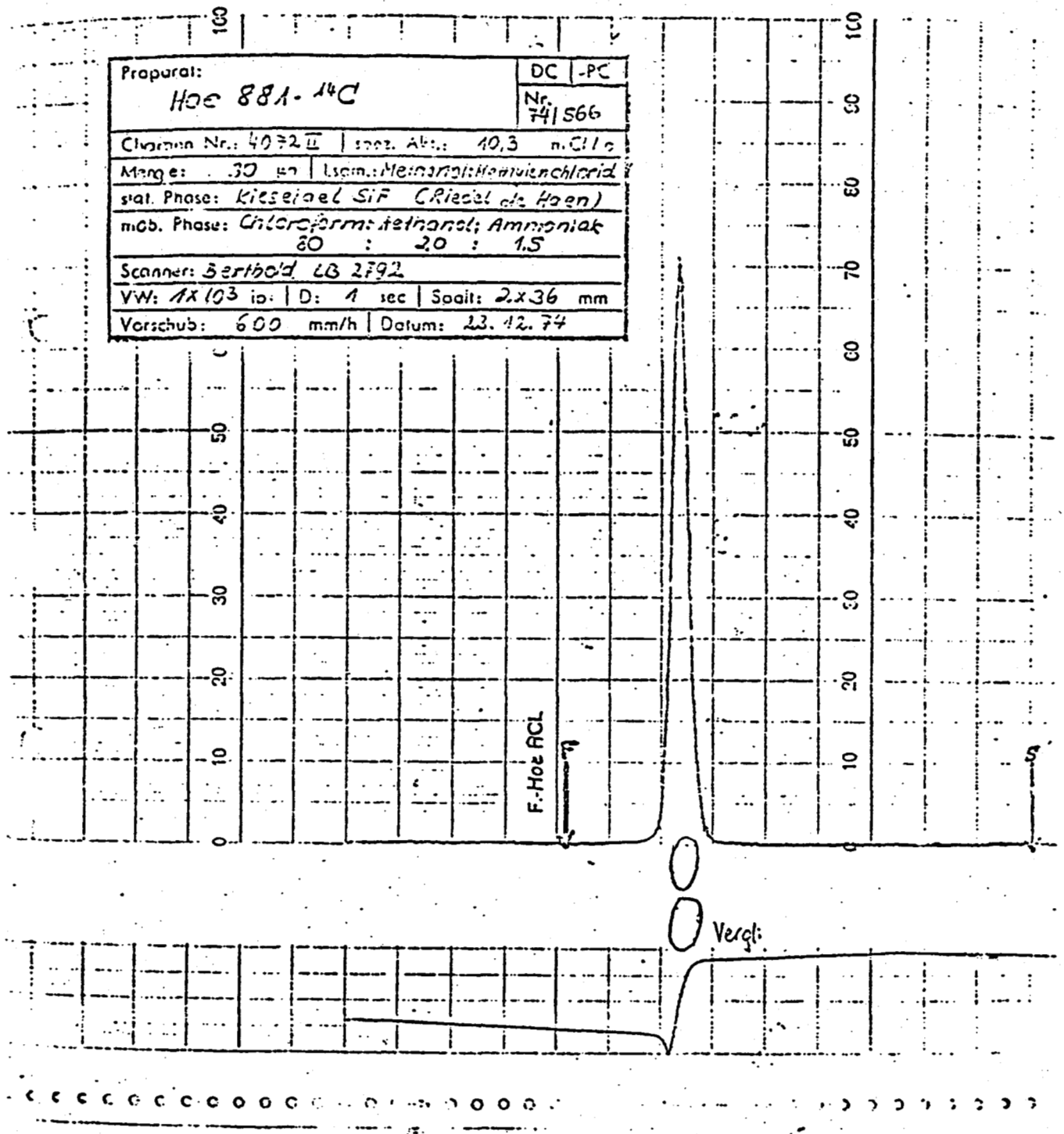


Abb. 1: RADIODÜNNSCHICHTCHROMATOGRAMM

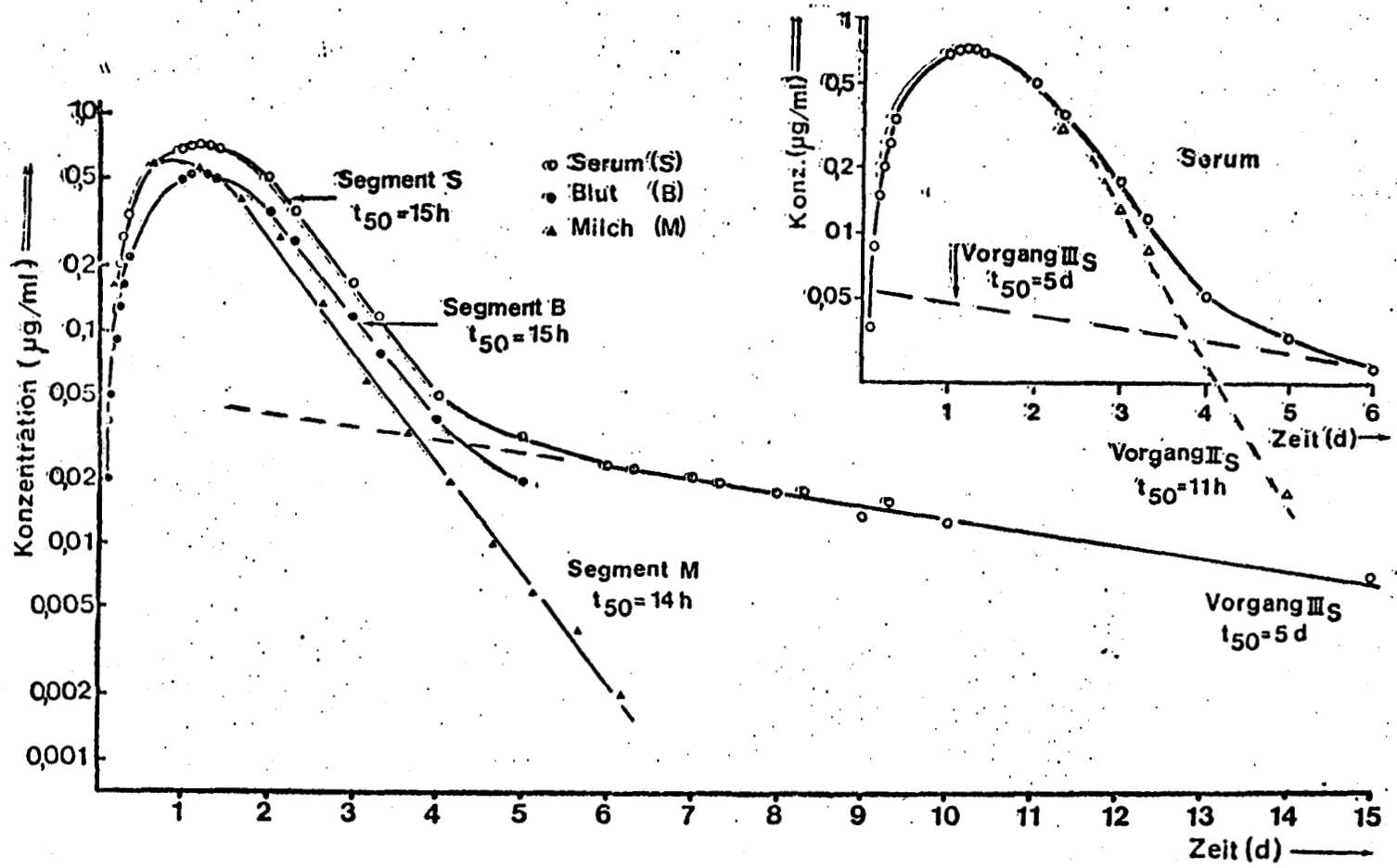


Abb. 2: KONZENTRATIONEN IN BLUT, SERUM UND MILCH NACH ORALER GABE VON ~5.3 mg Ibo 881-^{14}C PRO kg KÖRPERGEWICHT AN RIND Kü

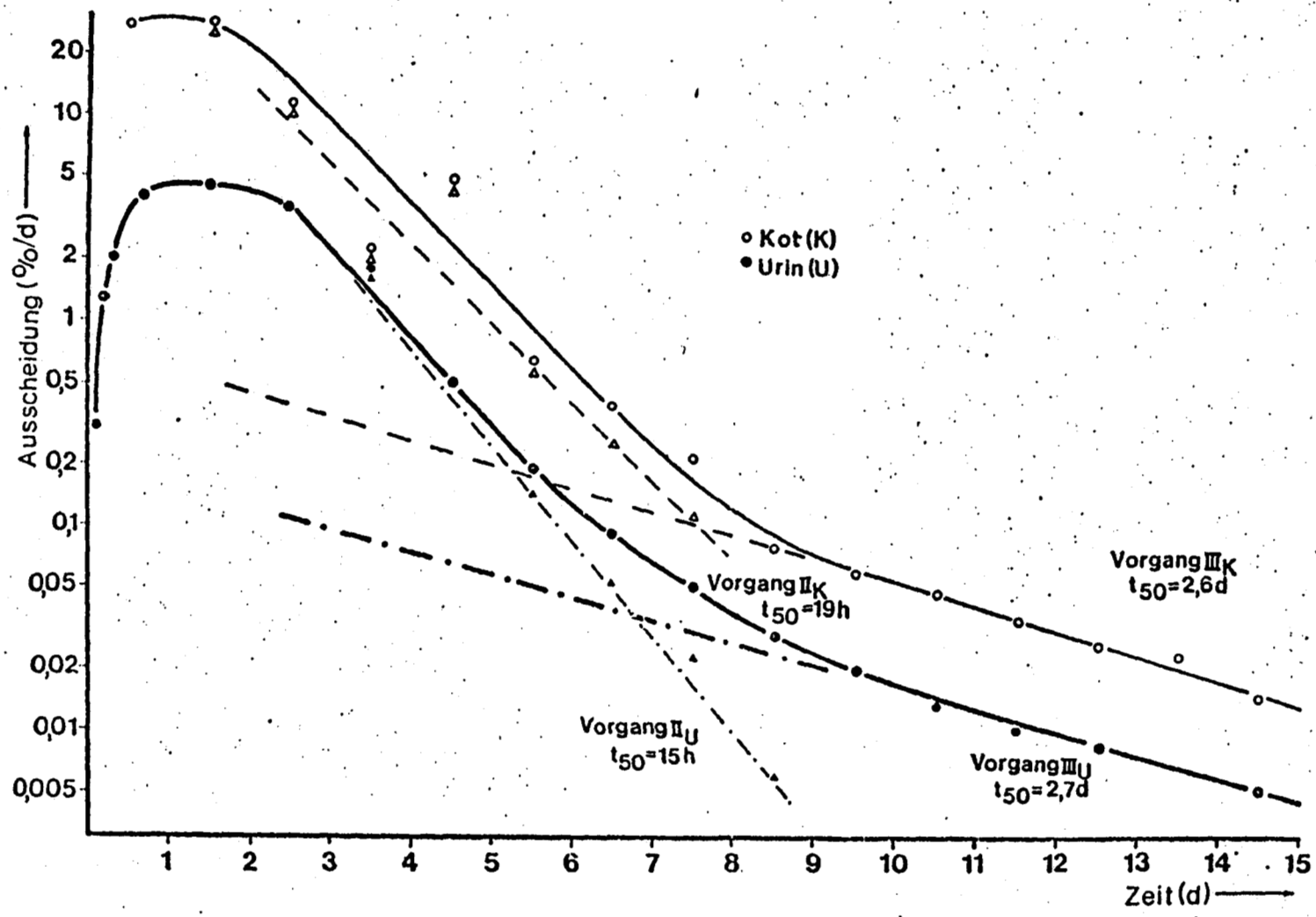


Abb. 3: AUSSCHEIDUNG MIT URIN UND KOT NACH ORALER GABE VON ~ 5,3 mg Ibe 881-^{14}C PRO kg KÖRPERGEWICHT AN EINE KUH

Anlage zur AN RCL Dr.Kn/Tr -5776- vom 25.4.1975

Hoechst

Empfänger Herr Dr.Christ, RCL
Medizinische Abteilung
über Pharma Forschung, G 838

Absender	Bearbeiter / Hausruf Nr.	Bericht Nr.	6230 Frankfurt (M) 80
Pharma Galenik	Apoth.Ebel 5801	34 / 75	17.1.75

Gemäß Auftrag Nr. 10 / 75 erhalten Sie: ca. 160 g
2,5%ige Suspension
Präparat Hoe 881-^{14}C

Zusammensetzung: wie Op.3092 III a v.19.3.74
Op. 4072 II a

Aktivität : 260,9 µCi/ml
Wirkstoff : Op. 4072 II
Spez.Akt. : 10.1 mCi/g
Gehalt : 25,8 mg/ml
Prüfschein : Nr. 817 v. 14.1.75 v.Lab.Dr.Christ/RCL

84487-2104

APPENDIX 6

Fenbendazole - Photodegradation in Water with Simulated Sunlight

SPONSOR:	Hoechst-Roussel Agri-Vet Company
PROTOCOL TITLE:	"Fenbendazole: Photodegradation in Water with Natural Sunlight, Following FDA Technical Assistance, Document 3.10.", Springborn Laboratories, Protocol #031894/HRAV/FDA TAH# 3.10 and Protocol Amendment #1 dated 12 January 1995.
REPORT NUMBER:	95-4-5792
STUDY NUMBER:	1719-0994-6241-720
TEST ARTICLE & REFERENCE STANDARDS:	Fenbendazole, Lot No. Y-10911, CAS Registry No. 43210-67-9, a white powder with a purity of 100.4% reported by the Study Sponsor with an expiration date of 1 August 1995, was received from Hoechst-Roussel Agri-Vet Company, Inc. on 30 September 1994. [^{14}C] Fenbendazole, Batch No. 6119I, with a radiochemical purity of 98.3% and specific activity of 7.86 µCi/mg, was received from Hoechst-Roussel on 4 October 1994. A reference standard of fenbendazole sulfoxide (oxfendazole), Lot No. A 001, with a purity of 95.1% and an expiration date of November 1996 reported by the Study Sponsor, was received from Hoechst-Roussel on 4 October 1994. A reference standard of fenbendazole sulfone, Lot No. MR 12 972, with a purity of 99.1% and an expiration date of July 1996 reported by the Study Sponsor, was received from Hoechst-Roussel on 4 October 1994.
DEFINITIVE TEST DATES:	31 January to 23 March 1995

PROCEDURES FOLLOWED:

The effect of simulated sunlight on the photolytic degradation of aqueous solutions of fenbendazole was tested at pH 5, 7 and 9. Although, testing at each pH is not required for test articles which do not dissociate in water, the study was conducted at pH 5, 7 and 9 to provide data under a wide range of conditions, especially since no degradation pathways have been established in the environment. Actinometer (reference material) solutions of para-nitroacetophenone (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 to 5 separate tubes for the light-exposed and dark control solutions were 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 constructed to quantify the concentration of fenbendazole present. Samples for PNAP were analyzed by high performance liquid chromatographic analysis with UV detection.

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 the Sponsor supplied standards.

RESULTS:

The following table summarizes the photolytic rate constants, half lives and quantum yields of fenbendazole determined during the study.

Aqueous Photolysis of Fenbendazole & Actinometer Solutions Experimental Values

	Rate Constant k_p (day^{-1})	Coefficient of Determination (r^2)	Half-Life $t_{½}$ (day)	Quantum Yield Φ^c_E
		Light-exposed		
pH 5 (FBZ)	1.15	0.816	0.604	0.0326
PNAP	0.242	0.917	2.87	NA[a]
pH 7(FBZ)	1.47	0.358	0.470	0.0441
PNAP	0.203	0.871	3.42	NA[a]
pH 9 (FBZ)	1.65	0.499	0.421	0.0493
PNAP	0.203	0.947	3.41	NA[a]

Note: All linear regressions were forced through zero.

[a] These values are not applicable; quantum yield of actinometer solutions are used as a reference.

Aqueous Photolysis of Fenbendazole Environmental Values

	Direct Photoreaction Rate Constant k_{pE} (day^{-1})	Environmental Half Life $t_{1/2E}$ (days)
pH 5	0.972	0.713
pH 7	1.32	0.527
pH 9	1.47	0.471

CONCLUSIONS: Fenbendazole was rapidly photolyzed in aqueous solutions at pH 5, 7 and 9. Many small polar peaks were formed during exposure, with no single photodegradate greater than 10% of the applied dose. Therefore, identification and characterization was not of concern. The photolytic half-lives were experimentally determined to be 14.5, 11.3 and 10.1 hours in aqueous solutions at pH 5, 7 and 9, respectively. The environmentally relevant half-lives (corrected for surface water geometry) of fenbendazole were 17.1, 12.6 and 11.3 hours at pH 5, 7 and 9, respectively. Therefore, fenbendazole is expected to be rapidly photolyzed in natural bodies of water.

APPENDIX 7

TRANSLATION

Dr. G. Klöpffer
Biochemistry South H 821

November 27, 1975/Bl.

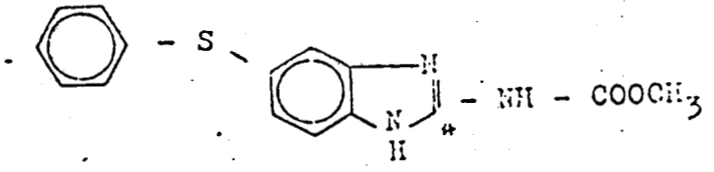
METABOLISM STUDIES WITH S 71 1881 IN SHEEP, CATTLE AND PIGS

Summary

After single oral administration of 5 mg/kg each of Hoe 881-^{14}C, a quantitative metabolite pattern was determined in the urine and feces of sheep, cattle and pigs. The main metabolites were isolated from animals treated with inactive compound and their chemical structure was established (diagram 1). Compound 2-carbomethoxy-amino-5-(4'hydroxythiophenoxy)-benzimidazole was found to be the most important metabolite in sheep and cattle urine. In pig urine, 4 metabolites are present in large concentrations, among these also the hydroxy compound. In the feces of all animals, most of the compound is excreted unchanged, apart from 4 to 5 metabolites in low concentrations. One of these was identified as a sulfoxide derivative of the original compound.

Compound:

S 71 1881



*) The asterisk shows the site of labeling with carbon-14.

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Animal experiments:

The experiments with labeled substance were performed at the Radiochemical laboratory. The specific activity amounted to 10.8 mCi/g, 7.1 mCi/g and 10.1 mCi/g, depending on the batch used. Five sheep, three pigs and one cow were treated orally with 5 mg/kg Hoe 881-^{14}C. One sheep was intravenously injected with 0.51 mg/kg Hoe 881-^{14}C. For the metabolic tests we used urine and feces from pigs collected over 0 - 72 hours, from the cow and sheep over 0 - 120 hours after administration. All samples were stored deep-frozen until analysis. The exact study outline is given in the reports of Drs. Kellner/Christ, dated June 29, 1973, Sept. 2, 1974 and April 25, 1975.

The animal tests without radioactively labeled substance for isolating the metabolites were performed in collaboration with Dr. Düwel (Laboratory for Helminthology). The sheep were treated with 500 mg/kg Hoe 881, the pigs with 100, 500 or 1000 mg/kg, and the cattle with 500 or 2000 mg/kg. Urine and feces were collected up to 120 hours.

Analytic methods:

Before extraction with organic solvent, the samples were incubated with an enzyme mixture of β-glucuronidase/arylsulfatase in acetate buffer (pH = 5.5) for 16 hours at 37° to split any conjugates.

After several extractions with the five-fold volume of chloroform, 85 - 95 % of the radioactivity present in the urine was transferred from the incubated urine solution to the organic solvent. The samples of feces could be extracted almost quantitatively.

The separation of the individual substances was performed by thin-layer chromatography on precoated silica gel plates $PF_{254 + 366}$. As eluents we used:

A: ethanol - acetone - benzene - 25 % ammonia solution = 5-45-45-1

B: chloroform - ethyl acetate - acetic acid = 50-50-4

C: methylene chloride - methanol = 9-1

With the acid eluent variants of different composition were used.

After the chromatograms had developed, the plates were dried and the activity distribution was recorded with a thin-layer radioscanner. The number and position of the peaks showed the qualitative metabolite pattern. The concentration of the individual metabolites was established by extracting the silica gel layer, which had been previously divided into 6-mm strips, with dimethyl sulfoxide, and then the radioactivity was measured in the liquid scintillation counter.

Preparative thin-layer chromatography (eluents A, B and C) of inactive extracts from urine and feces enabled us to isolate and purify the individual metabolites for chemophysical structural determination. The site of the bands in question was determined by comparing the fluorescence quenching in the UV spectrum with the blank value, and by comparing the Rf-values with the radioactive peaks. Identification was made by

- chromatographic separation tests in at least three different eluents with mixtures containing synthetic metabolites produced by Dr. Loewe (Pharma Synthesis);
- group-specific staining reactions on the thin-layer plate, e.g. blue coloration of phenolic OH-groups with Gibbs reagent, or yellow coloration of sulfide-bound sulfur with palladium chloride;
- recording of mass spectra (performed and evaluated by Dr. Fehlhaber, Pharma Synthesis);
- comparison of UV spectra of natural and synthetic metabolites;
- mixing of the active and inactive extracts and comparison of Rf-values obtained by thin-layer chromatography.

Results:

Sheep

The excretion of radioactive substances amounts to 6 - 9 % during the initial 120 hours in the urine of the five sheep treated orally, and 76-87 % in the feces.

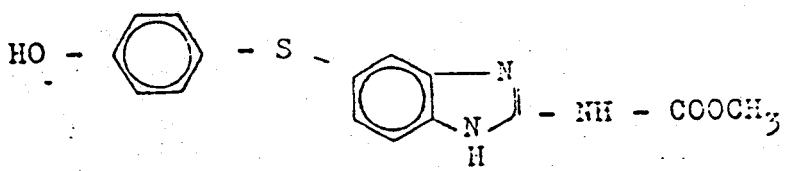
Figs. 1 and 2 show the qualitative metabolite patterns in eluent A determined in sheep No..5. Table 1 contains the quantitative distribution of metabolites found in the urine and feces of all sheep.

Table 1

	Sheep 1	Sheep 2	Sheep 4	Sheep 5	Sheep 6
Urinary metabolite 1	1 %	1 %	1 %	1 %	≤ 1 %
" 2	1 %	1 %	1 %	1 %	2 %
" 3	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %
" 4	3 %	3,5%	4 %	3,5%	5 %
Original compound	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %
Fecal metabolite 1	11 %	11 %	8 %	10 %	9 %
" 2	9 %	16 %	5 %	11 %	9 %
" 3	14 %	4 %	20 %	9 %	13 %
Original compound	58 %	61 %	46 %	46 %	40 %

The urine extract of sheep No. 3, which was treated intravenously, shows a practically identical metabolite pattern when compared with that of the orally treated animals.

The main metabolite in the urine of sheep (urinary metabolite 4) is the hydroxy compound making up an average of 4 %.



The site of the specifically stained OH-group was shown by mass spectrometry. Comparisons of thin-layer chromatograms with synthesized p- and o-hydroxy compounds resulted in a complete agreement between metabolite and p-hydroxy compound Rf-values, but separation from the o-hydroxy compound. The m-position is to be excluded, because of the generally lower reacting ability of this position and its certainly different speed of migration on the silica gel plate. In the urine, the original compound occurs in traces only.

Almost one half of the administered compound is excreted in the feces as original substance. Fecal metabolite No. 3 could be identified as the sulfoxide of the original compound by comparing the thin-layer chromatogram with synthetic substance. This metabolite will be dealt with in more detail in the section on pigs.

Cattle

Excretion during the initial five days amounted to 13.6 % in the urine and 75 % in the feces. The metabolite pattern in both cases consists of 4 metabolites each, whose concentration is shown in % of the administered dose in Table 2. In comparison with the other animal species, it should be noted that like metabolite numbers do not indicate chemical identity.

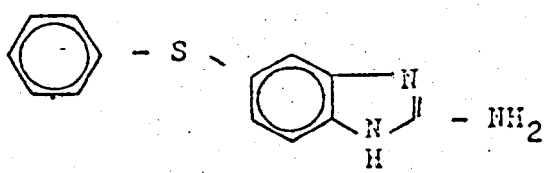
Table 2

METABOLITE PATTERN IN CATTLE

Urine			Feces		
Urinary metabolite	1	3 %	Fecal metabolite	1	5 %
"	2	2,5 %	"	2	2 %
"	3	0,5 %	"	3	8 %
"	4	6,5 %	"	4	10 %
Original compound		0,5 %	Original compound		48 %

Figs. 3 and 4 show the separation of the urinary and fecal extract in eluent A.

Identification showed the following results: the p-hydroxyphenyl derivative is urinary metabolite No. 4 (= urinary metabolite No. 4 in sheep). From a test with inactive substance an additional metabolite was isolated and identified as 2-amino-5-thiophenoxy-benzimidazole. According to its Rf-value, it belongs to urinary metabolite 1 or 2.



Fecal metabolite 3 is the sulfoxide which has already been mentioned in the section on sheep; it was identified by mixing experiments with the radioactive extract. In cattle, too, one half of the administered compound is excreted unchanged in the feces.

Pig

Excretion of radioactive compounds in the urine of the three pigs during the initial three days amounted to 30 - 35 % of the administered quantity, and 50 - 58 % in the feces. The thin-layer radiochromatogram of the urine shows 6 different metabolites; and that of the feces, 5 in addition to the original compound.

Table 3 contains the distribution of the metabolites in urine and feces of the individual animals.

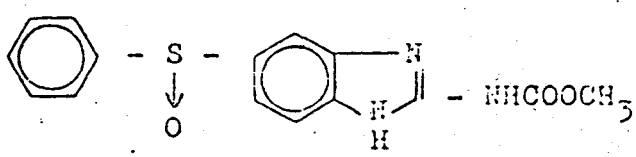
Table 3

	Pig 1	Pig 2	Pig 3
Urinary metabolite 1	6 %	8 %	5 %
" 2	10 %	6 %	4 %
" 3	1 %	1 %	2 %
" 4 + 5 + 6	13 %	10 %	16 %
Original compound	1 %	1 %	1 %
Fecal metabolite 1	1 %	< 1 %	< 1 %
" 2	< 1 %	< 1 %	< 1 %
" 3 + 4 + 5	8 %	4 %	8 %
Original compound	42 %	52 %	37 %

Figs. 5 and 6 show thin-layer radiochromatograms using samples from pig No. 3 and eluent A. Although pecks 4+ 5+ 6 in the urine and peaks 3+4+5 in the feces can be recognized, they lie too closely together to be quantitatively separated and evaluated. Therefore, the total of the three peaks in each case was measured and recorded.

Compared to ruminants, Hoe 881 is quantitatively metabolized in a different way in pigs. While sheep and cattle excrete approx. 10 % in the urine, pigs excrete approx. 30 %. Furthermore, the 4 urinary metabolites are present in approximately equal concentrations. By thin-layer chromatographic comparison with the synthetic substance and ninhydrin staining, urinary metabolite 2 was identified as 2-amino-5-thiophenoxybenzimidazole, which has been described in more detail in the section on cattle. An additional metabolite was isolated from a test with inactive substance and identified as the p-hydroxyphenyl compound. According to its Rf-value, this metabolite belongs to urinary metabolite 4, 5 or 6.

Out of the fecal metabolites 3+ 4+5, the largest portion of activity is shown by the compound identified as the sulfoxide (= fecal metabolite 4).



Pigs also eliminate 40 – 50 % in the feces as original compound.

Tests with unincubated urine resulted in a lower extraction yield (sheep 61 %, cattle 39 %, pig 75 %).

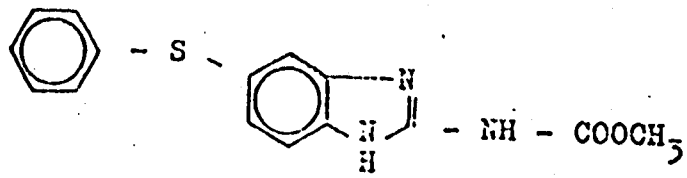
signed: Dr. Klöpffer

7 enclosures

Translated
January 12, 1975
Oe.

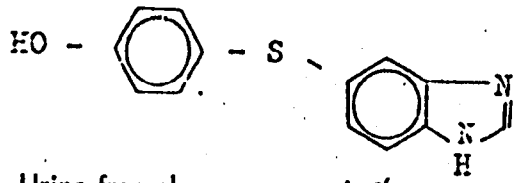
Diagram 1

Metabolism of S 71 1881

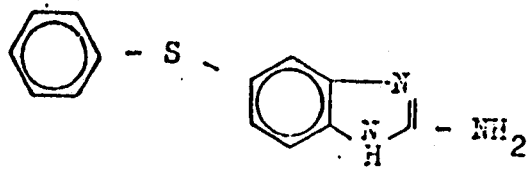


S 71 1881

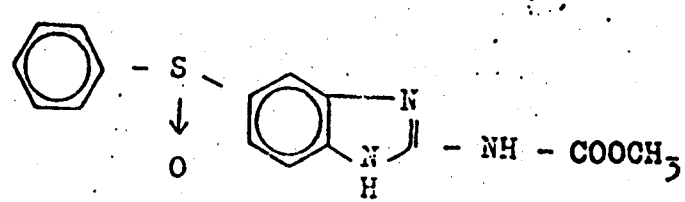
Sheep : urine ≤ 1 %; feces 50 %
Cattle : urine ≤ 1 %; feces 48 %
Pig : urine 1 %; feces 44 %



Urine from sheep : 4 %
Urine from cattle : 6 %
Urine from pig : < 15 %



Urine from cattle : 3 %
Urine from pig : 7 %



Feces from sheep : 12 %
Feces from cattle : 8 %
Feces from pig : < 7 %

Fig. 1

THIN-LAYER RADIOCHROMATOGRAM

SHEEP NO. 5

Urinary extract 0 - 120 h

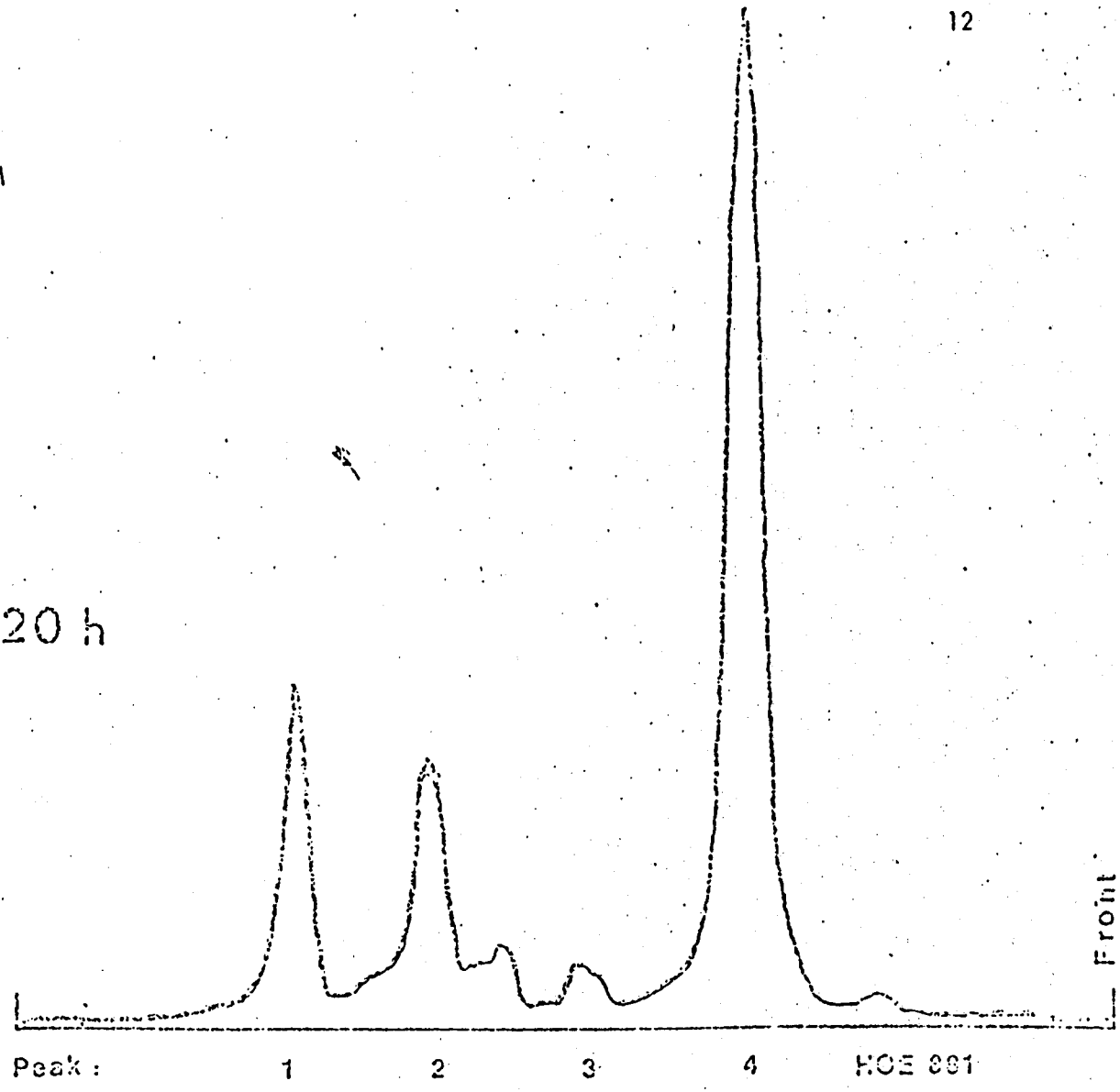


Fig. 2

THIN-LAYER RADIOCHROMATOGRAM

SHEEP No. 5

Fecal extract 0-120 h

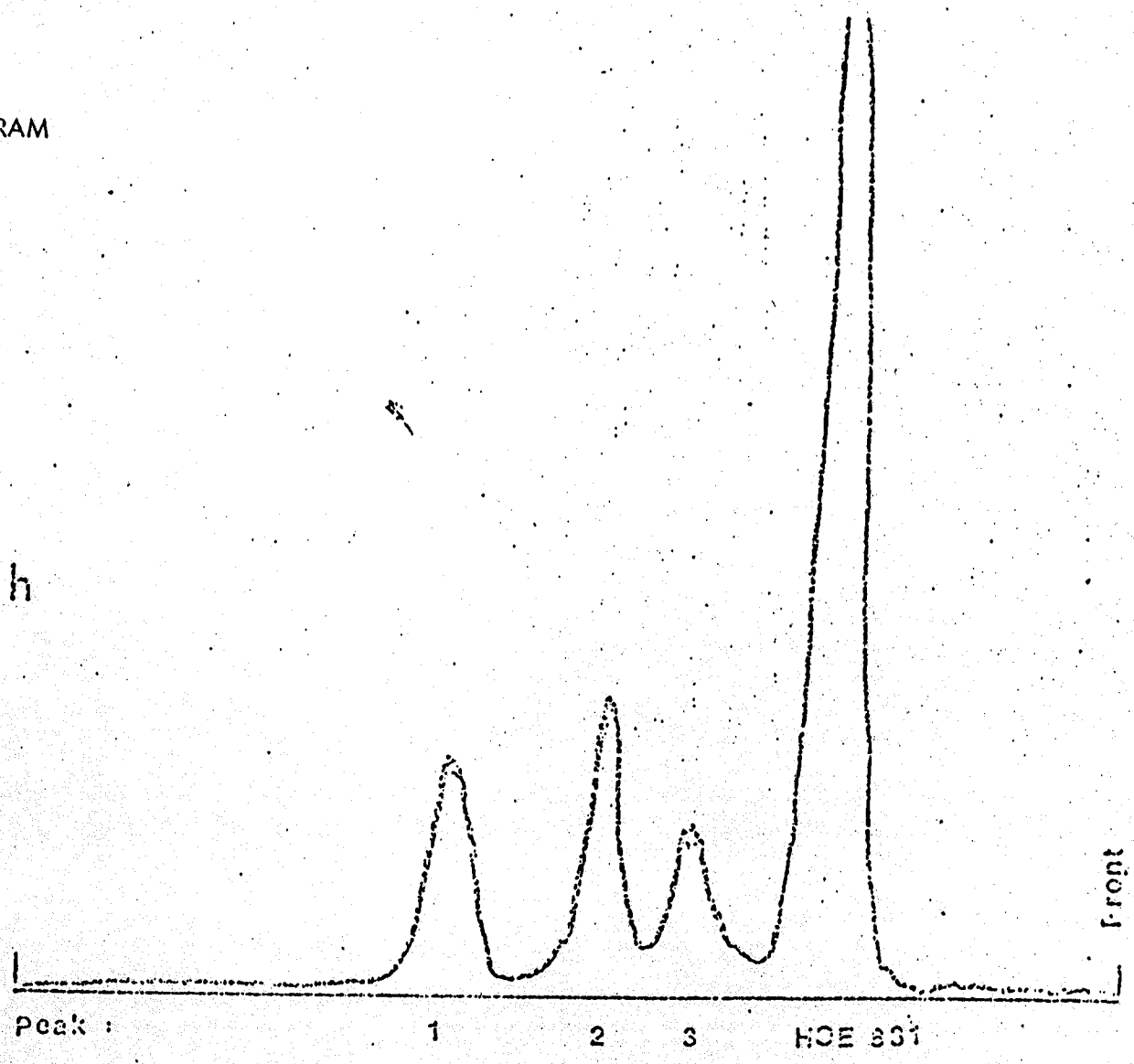


Fig. 3

THIN-LAYER RADIOCHROMATOGRAM

COW No. 1

Urinary extract 0 – 120 h

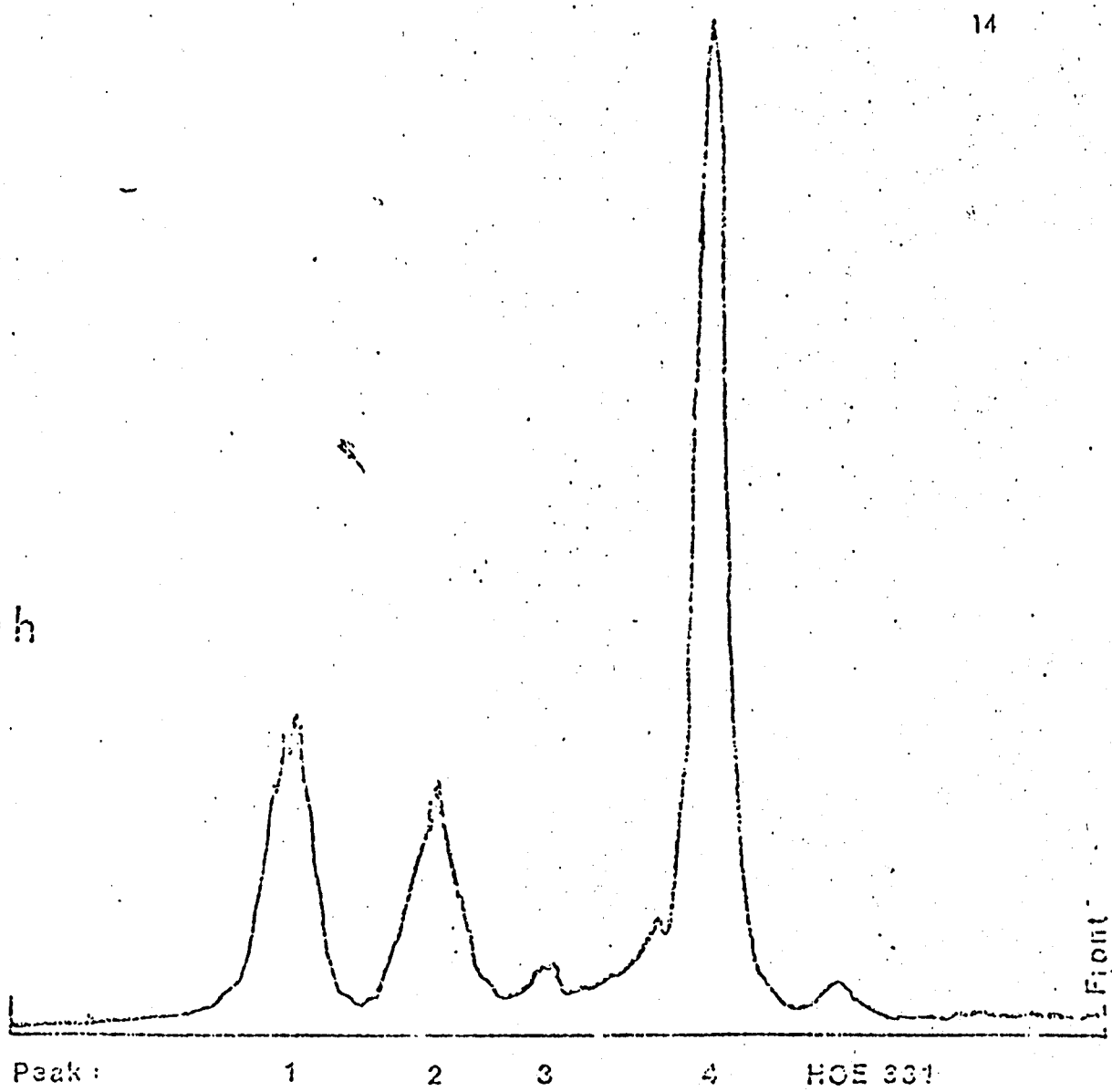
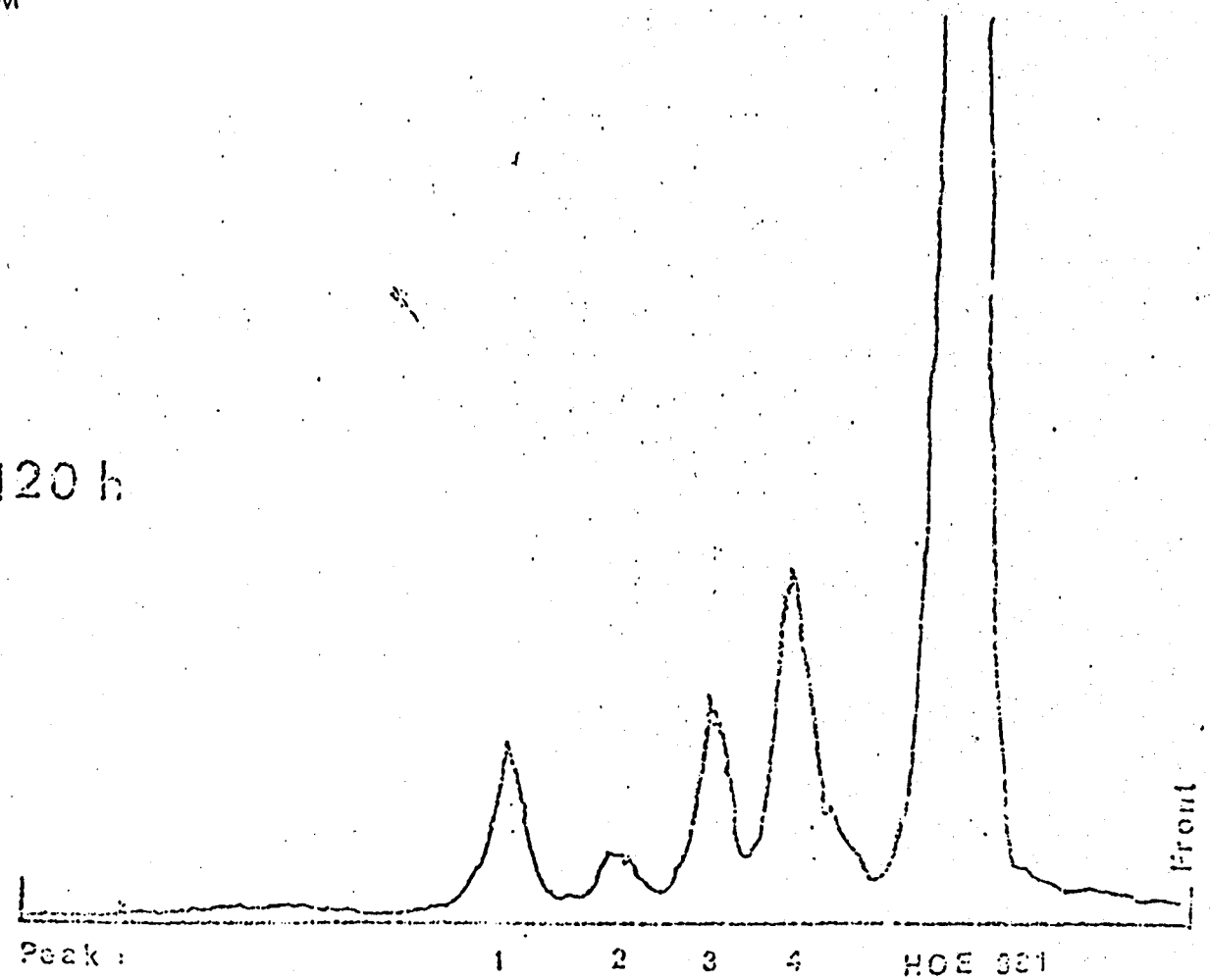


Fig. 4

THIN-LAYER RADIOCHROMATOGRAM

COW No. 1

Fecal extract 0 - 120 h



THIN-LAYER RADIOCHROMATOGRAM

PIG NO. 3

Urinary extract 0 - 72 h

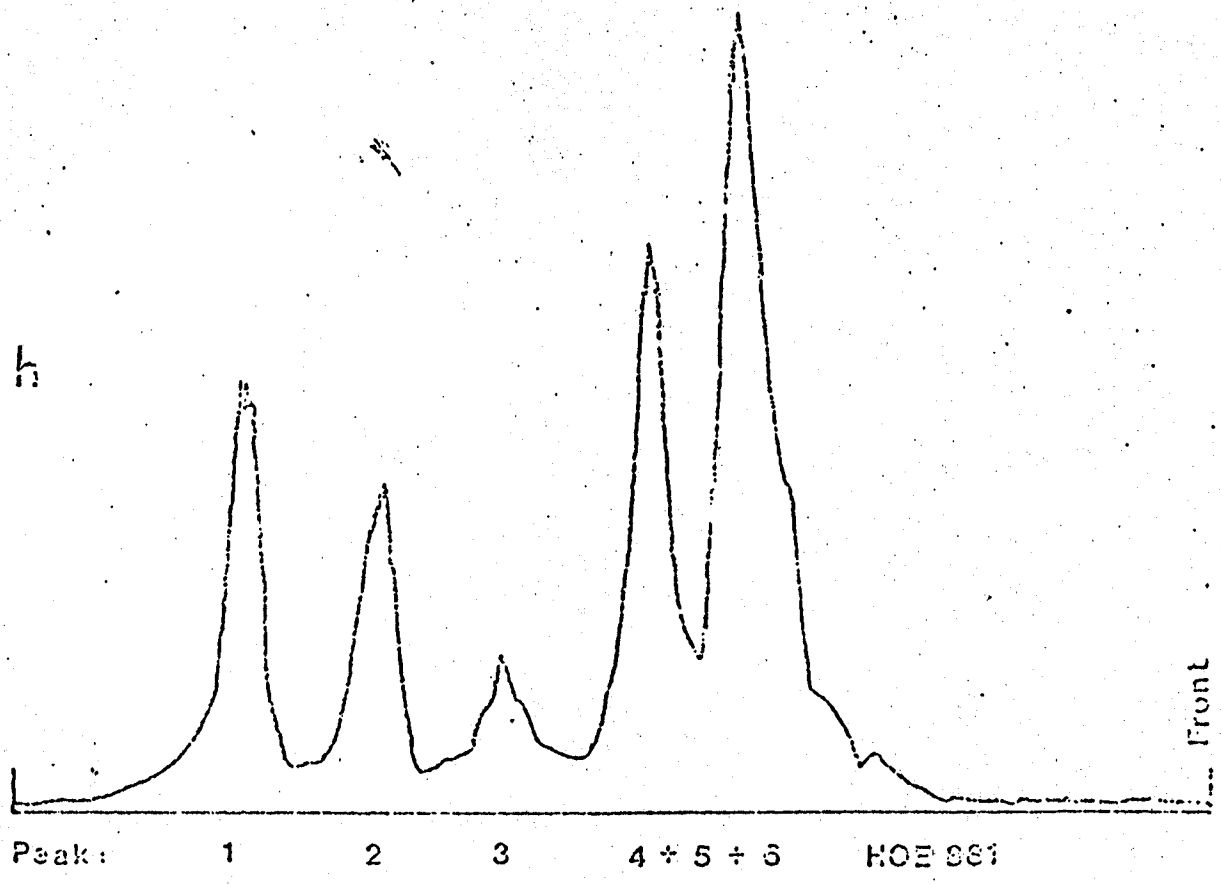
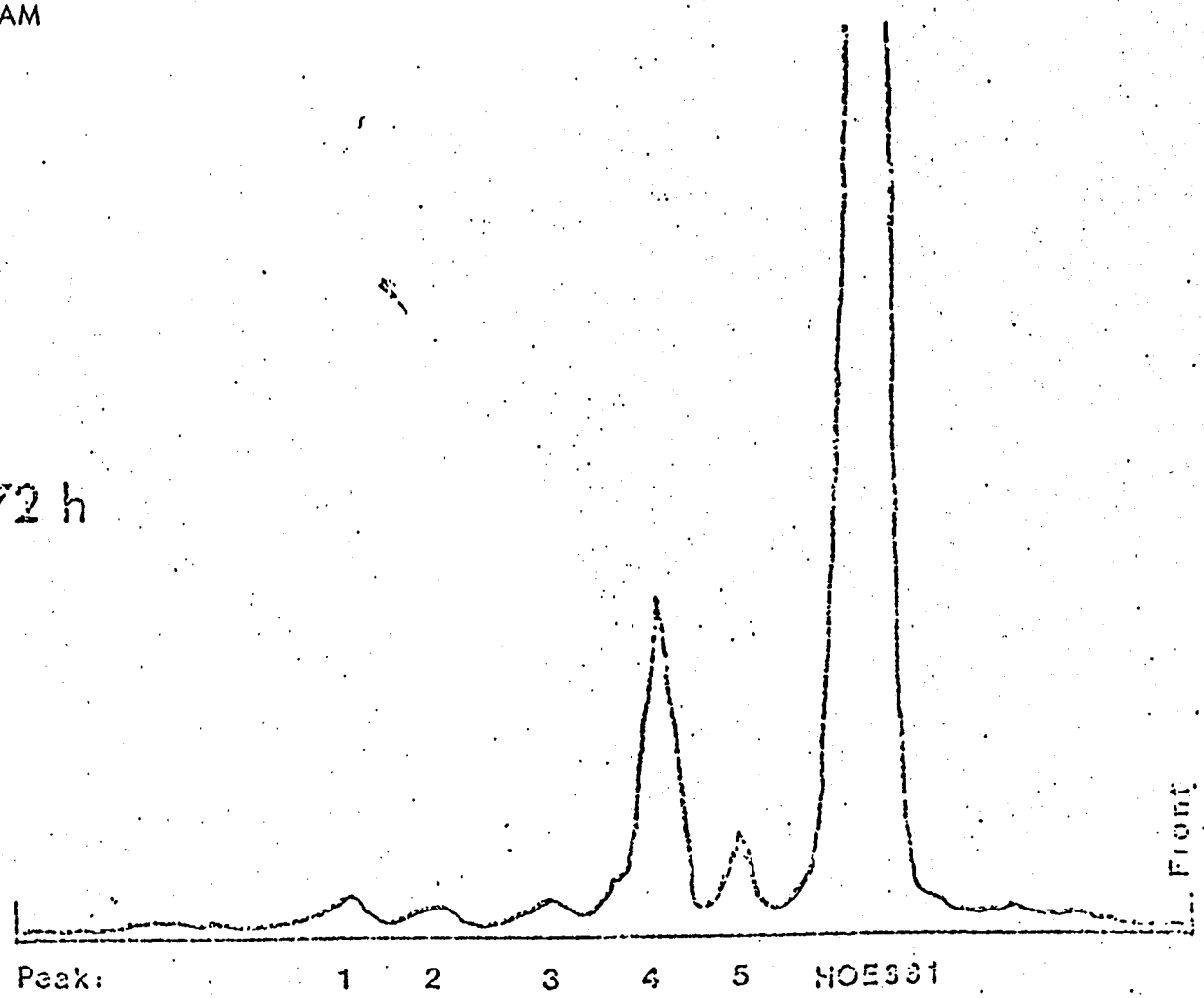


Fig. 6

THIN-LAYER RADIOCHROMATOGRAM

PIG NO. 3

Fecal extract 24 - 72 h



Dr. G. Klöpffer
BIOCHEMIE SÜD H 821

27. November 1975/Bl

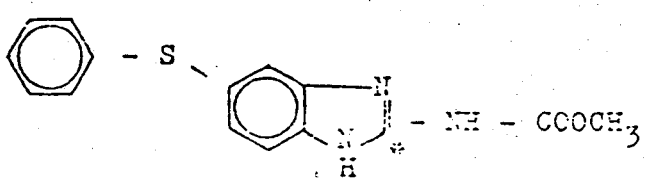
Untersuchungen zur Metabolisierung von S 71 1881 in Schaf, Rind und Schwein

Zusammenfassung

Nach einmaliger oraler Gabe von je 5 mg/kg Hoe 881-^{14}C wurde in Harn und Kot von Schaf, Rind und Schwein ein quantitatives Metabolitmuster erstellt. Aus Tierversuchen mit inaktiver Substanz wurden die Hauptmetabolite isoliert und ihre chemische Struktur aufgeklärt (Schema 1). Als wichtigster Metabolit im Schaf- und Rinderharn wurde die Verbindung 2-Carbmethoxy-amino-5-(4'-hydroxy-thiophenoxy)-benzimidazol ermittelt. Im Schweineharn sind 4 Metabolite in größerer Konzentration vorhanden, darunter ebenfalls die Hydroxyverbindung. Im Kot aller Tiere wird hauptsächlich unverändertes Präparat ausgeschieden, daneben 4 - 5 Metabolite in geringerer Konzentration. Einer von diesen wurde als das von der Originalsubstanz abgeleitete Sulfoxyd identifiziert.

Präparat:

S 71 1881



*) bezeichnet die Markierung mit Kohlenstoff-14

/ 2

Tierversuche:

Die Versuche mit markierter Substanz wurden im Radiochemischen Laboratorium durchgeführt. Die spezifische Aktivität betrug je nach verwendeter Charge 10,8 mCi/g, 7,1 mCi/g bzw. 10,1 mCi/g. 5 Schafe, 3 Schweine und 1 Kuh wurden oral mit 5 mg/kg Hoe 881-^{14}C behandelt. 1 Schaf wurde i. v. mit 0,51 mg/kg Hoe 881-^{14}C behandelt. Für die metabolischen Untersuchungen wurden Harn und Kot beim Schwein von 0 - 72 Stunden, beim Schaf und Rind von 0 - 120 Stunden nach der Applikation verwendet. Sämtliche Proben wurden bis zur Aufarbeitung tiefgefroren aufbewahrt. Die genaue Versuchsbeschreibung ist in den Berichten der Herren Dr. Kellner/Dr. Christ vom 29. 6. 1973, 2. 9. 1974 und 25. 4. 1975 enthalten.

Die Tierversuche mit nicht radioaktiv markierter Substanz zur Isolierung der Metabolite wurden in Zusammenarbeit mit Herrn Dr. Düwel (Labor für Helminthologie) durchgeführt. Schafe wurden mit 500 mg/kg Hoe 881, Schweine mit 100, 500 bzw. 1000 mg/kg und Rinder mit 500 bzw. 2000 mg/kg oral behandelt. Harn und Kot wurden bis zu 120^{h} gesammelt.

Analytische Methoden:

Vor der Extraktion mit organischem Lösungsmittel wurden die Proben mit einer Enzymmischung aus ß-Glucuronidase/Arylsulfatase im Acetatpuffer (pH = 5,5) 16 Stunden bei 37 ^{o} inkubiert um vorhandene Konjugate zu spalten.

Aus den Harninkubationslösungen wurde nach mehrmaligem Extrahieren mit dem fünffachen Volumen an Chloroform 85 - 95 % der im Harn vorhandenen Radioaktivität ins organische Lösungsmittel gebracht. Die Kotproben konnten nahezu quantitativ extrahiert werden.

/ 3

Die Auftrennung der einzelnen Substanzen erfolgte mittels Dünnschichtchromatographie auf Kieselgelfertigplatten PF 254 + 366. Als Fließmittel wurden verwendet:

A : Äthanol-Aceton-Benzol-25 % Ammoniaklösung = 5-45-45-1
B : Chloroform-Essigsäureäthylester-Essigsäure = 50-50-4
C : Methylenchlorid-Methanol = 9-1

Beim sauren Fließmittel wurden Varianten verschiedener Zusammensetzung verwendet.

Nach Entwicklung der Chromatogramme wurden die Platten getrocknet und die Aktivitätsverteilung mit einem Radiodünnschichtscanner aufgezeichnet. Die Anzahl und die Lage der Peaks ließ das qualitative Metabolitmuster erkennen. Die Konzentration der einzelnen Metabolite wurde durch Elution der in 6 mm breite Streifen aufgeteilten Kieselgelschicht mit Dimethylsulfoxyd und nachfolgender Messung der Radioaktivität im Liquid Scintillation Counter ermittelt.

Die präparative Dünnschichtchromatographie (Fließmittel A, B und C) der nicht aktiven Harn- und Kotextrakte ermöglichte die Isolierung und Reinigung der einzelnen Metabolite für die chemisch-physikalische Strukturaufklärung. Die Lage der in Frage kommenden Banden wurde durch Vergleich der Fluoreszenzlöschung im UV mit dem Leerwert und durch Vergleich der Rf-Werte mit den radioaktiven Peaks festgestellt. Die Identifizierung erfolgte durch

- chromatographische Auftrennungsversuche an Mischungen mit den von Herrn Dr. Loewe (Pharma Synthese) synthetisch hergestellten Metaboliten in mindestens 3 verschiedenen Fließmitteln;
- gruppenspezifische Färbereaktionen auf der Dünnschichtplatte, z. B. Blaufärbung phenolischer OH-Gruppen mit dem [illegible]-Reagens oder Gelbfärbung sulfidisch gebundenen Schwefels mit Palladiumchlorid;

- Aufnahme von Massenspektren (durchgeführt und ausgewertet von Herrn Dr. Fehlhaber, Pharma Synthese);
- Vergleich der UV-Spektren der natürlichen und der synthetischen Metabolite;
- Mischen des aktiven und inaktiven Extraktes und Vergleich der dünnschichtchromatographischen Rf-Werte.

Ergebnisse:

Schaf

Die Ausscheidung der radioaktiven Substanzen im Harn beträgt bei den 5 oral behandelten Schafen in den ersten 120 Stunden 6 - 9 %, im Kot 76 - 87 %.

Die Abbildungen 1 und 2 zeigen die qualitativen Metabolitmuster von Schaf 5 im Fließmittel A. In Tabelle 1 ist die quantitative Zusammensetzung der Harn- bzw. Kotmetabolite von allen Schafen aufgeführt.

Tabelle 1

	Schaf 1	Schaf 2	Schaf 4	Schaf 5	Schaf 6
Harnmetabolit 1	1 %	1 %	1 %	1 %	≤ 1 %
" 2	1 %	1 %	1 %	1 %	2 %
" 3	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %
" 4	3 %	3,5%	4 %	3,5%	5 %
Originalsubstanz	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %	≤ 1 %
Kotmetabolit 1	11 %	11 %	8 %	10 %	9 %
" 2	9 %	16 %	5 %	11 %	9 %
" 3	14 %	4 %	20 %	9 %	13 %
Originalsubstanz	58 %	61 %	46 %	46 %	40 %

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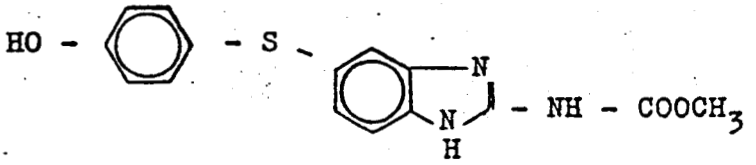
Der Harnextrakt des i. v. behandelten Schafes 3 zeigte ein praktisch identisches Metabolitmuster verglichen mit dem der oral behandelten Tiere.

Der Hauptmetabolit im Schafharn (Harnmetabolit 4) ist mit durchschnittlich 4 % die Hydroxyverbindung.



Die Lage der spezifisch anfärbbaren OH-Gruppe ergab sich durch massenspektrometrische Untersuchungen. Dünnschichtchromatographische Vergleiche mit synthetisierter p- und o-Hydroxyverbindung ergab vollkommene Übereinstimmung der Rf-Werte von Metabolit und p-Hydroxyverbindung, aber Trennung von der o-Hydroxyverbindung. Die m-Stellung ist aufgrund der allgemein geringen Reaktionsfähigkeit dieser Position und ihrer sicher unterschiedlichen Wanderungsgeschwindigkeit auf der Kieselgelplatte auszuschließen. Die Originalsubstanz findet man im Harn nur spurenweise.

Fast die Hälfte des applizierten Präparates wird als Originalsubstanz im Kot ausgeschieden. Kotmetabolit 3 konnte durch dünnschichtchromatographischen Vergleich mit synthetisierter Substanz als Sulfoxyd der Originalsubstanz identifiziert werden. Dieser Metabolit wird im Abschnitt über das Schwein ausführlicher behandelt.

Rind

Die Ausscheidung der ersten 5 Tage betrug im Harn 13,6 %, im Kot 75 %. Das Metabolitmuster besteht in beiden Fällen aus jeweils 4 Metaboliten, deren Konzentration in Prozent der applizierten Dosis in Tabelle 2 angegeben ist. Beim Vergleich mit anderen Tierarten muß beachtet werden, daß gleiche Metabolitnummern nicht chemische Identität bedeutet.

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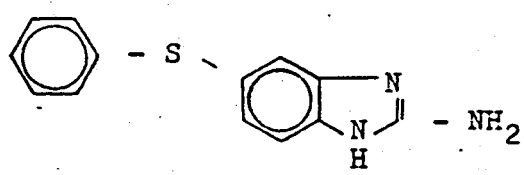
Tabelle 2

METABOLITMUSTER VOM RIND

Harn			Kot		
Harnmetabolit	1	3 %	Kotmetabolit	1	5 %
"	2	2,5 %	"	2	2 %
"	3	0,5 %	"	3	8 %
"	4	6,5 %	"	4	10 %
Originalsubstanz		0,5 %	Originalsubstanz		48 %

In den Abbildungen 3 und 4 ist die Auftrennung des Harn- und Kotextraktes im Fließmittel A dargestellt.

Die Identifizierung ergab folgende Resultate: Harnmetabolit 4 ist das p-Hydroxyphenylderivat (= Harnmetabolit 4 beim Schaf). Aus einem Versuch mit inaktiver Substanz wurde ein weiterer Metabolit isoliert und als 2-Amino-5-thiophenoxybenzimidazol erkannt. Er ist seinem Rf-Wert nach Harnmetabolit 1 oder 2 zuzuordnen.



Kotmetabolit 3 ist das bereits beim Schaf erwähnte Sulfoxyd; es wurde durch Mischungsversuche mit dem radioaktiven Extrakt identifiziert. Auch beim Rind wird die Hälfte des applizierten Präparates unverändert im Kot ausgeschieden.

Schwein

Die Ausscheidung der radioaktiven Verbindungen von den 3 Schweinen beträgt in den ersten 3 Tagen im Harn 30 - 35 % der applizierten Menge, und 50 - 58 % im Kot. Das Radiodünnschichtchro-

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matogramm des Harnes läßt 6, das des Kots 5 verschiedene Metabolite und zusätzlich die Originalsubstanz erkennen.

In Tabelle 3 ist die Zusammensetzung der Metabolite in Harn und Kot der einzelnen Tiere aufgeschlüsselt.

Tabelle 3

	Schwein 1	Schwein 2	Schwein 3
Harnmetabolit 1	6 %	8 %	5 %
" 2	10 %	6 %	4 %
" 3	1 %	1 %	2 %
" 4 + 5 + 6	13 %	10 %	16 %
Originalsubstanz	1 %	1 %	1 %
Kotmetabolit 1	1 %	<1 %	<1 %
" 2	<1 %	<1 %	<1 %
" 3 + 4 + 5	8 %	4 %	8 %
Originalsubstanz	42 %	52 %	37 %

Die Abbildungen 5 und 6 zeigen Radiodünnschichtchromatogramme von Schwein 3 mit Fließmittel A. Die Peaks 4 + 5 + 6 im Harn und die Peaks 3 + 4 + 5 im Kot sind zwar zu erkennen, liegen aber zu eng benachbart, um quantitativ abgetrennt und bestimmt zu werden. Daher wurde jeweils ihre Summe gemessen und angegeben.

Hoe 881 wird im Schwein, verglichen mit den Wiederkäuern, quantitativ unterschiedlich metabolisiert. Während bei Schaf und Rind rund 10 % im Harn ausgeschieden werden, sind es beim Schwein etwa 30 %. Außerdem sind die 4 Harnmetabolite in annähernd gleicher Konzentration vorhanden. Durch dünnschichtchromatographischen Vergleich mit synthetischer Substanz und durch Ninhydrinfärbungen wurde Harnmetabolit 2 als das beim Rind ausführlicher beschriebene 2-Amino-5-thiophenoxybenzimidazol identifiziert. Aus

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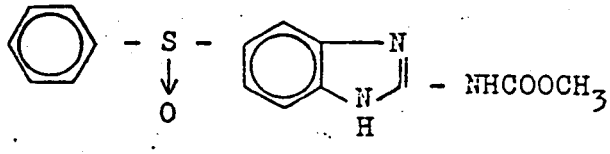
einem Versuch mit inaktiver Substanz wurde ein weiterer Metabolit isoliert und als die p-Hydroxyphenylverbindung ermittelt. Dieser Metabolit ist dem Rf-Wert nach Harnmetabolit 4, 5 oder 6 zuzuordnen.

Von den Kotmetaboliten 3 + 4 + 5 entfällt der größte Aktivitätsanteil auf eine Verbindung, die als Sulfoxyd identifiziert wurde (= Kotmetabolit 4).

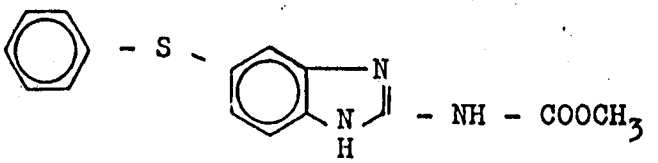


Auch beim Schwein wird 40 - 50 % im Kot als Originalsubstanz eliminiert.

Versuche mit nicht inkubiertem Harn ergaben eine geringere Extraktionsausbeute (Schaf 61 %, Rind 39 %, Schwein 75 %).

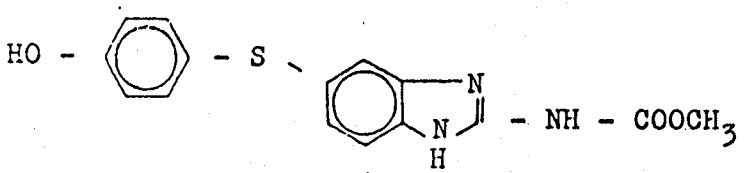
Schema 1

Metabolisierung von S 71 1881

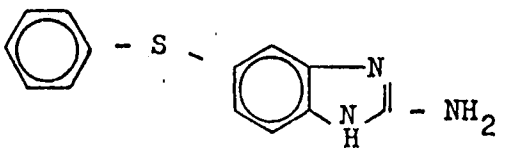


S 71 1881

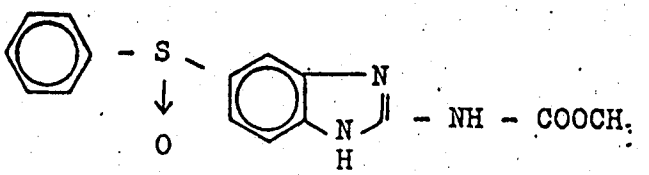
Schaf : Harn ≤ 1 %; Kot 50 %
Rind : Harn ≤ 1 %; Kot 48 %
Schwein : Harn 1 %; Kot 44 %



Schafharn : 4 %
Rinderharn : 6 %
Schweineharn : < 13 %



Rinderharn : 3 %
Schweineharn : 7 %



Schafkot : 12 %
Rinderkot : 8 %
Schweinekot : < 7 %

000140

0016175

Abb. 1

Radiodünnschichtchromatogramm

Schaf 5

Harnextrakt 0 - 120 h

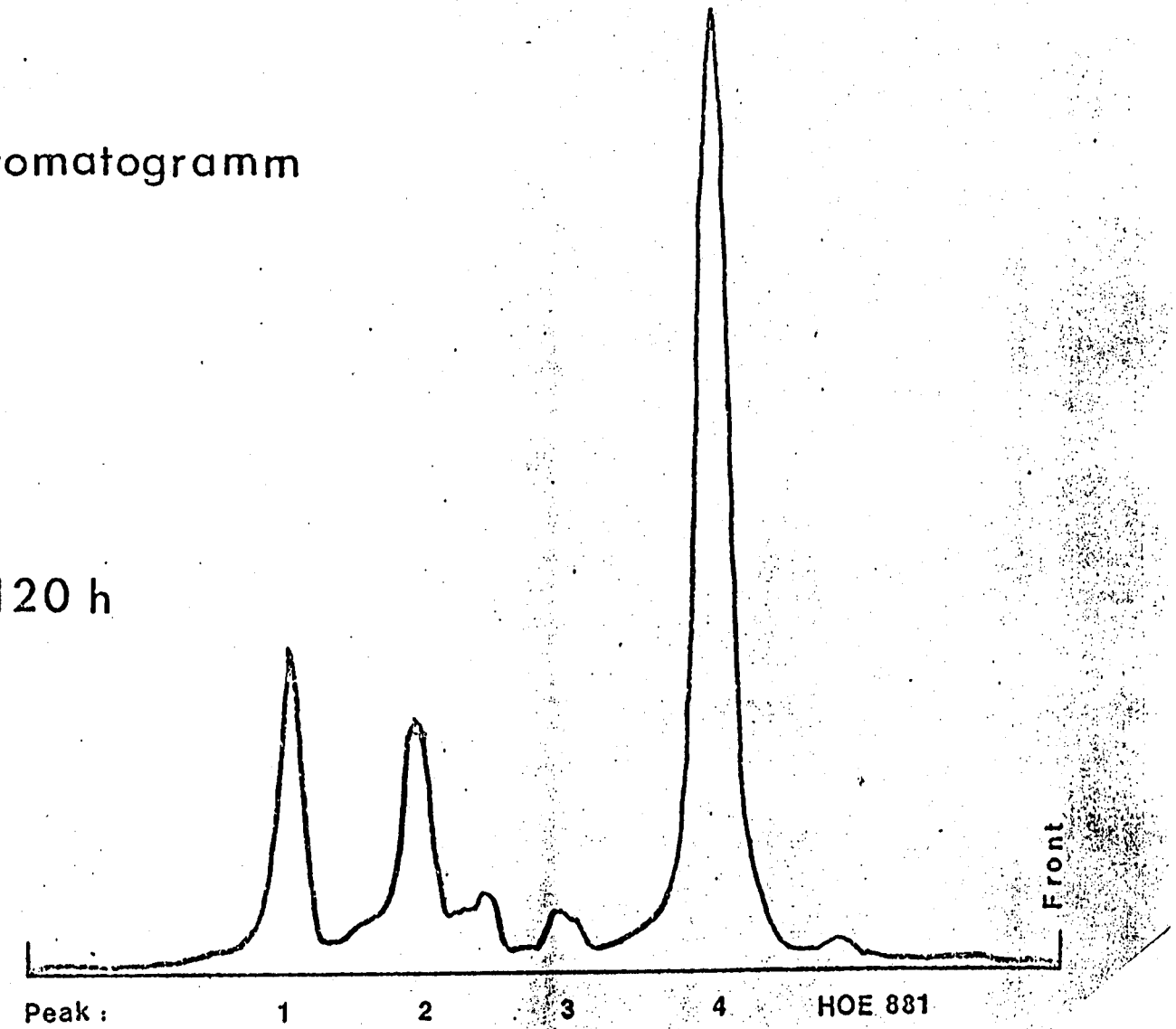


Abb. 2

Radiodünnschichtchromatogramm

Schaf 5

Kotextrakt 0-120 h

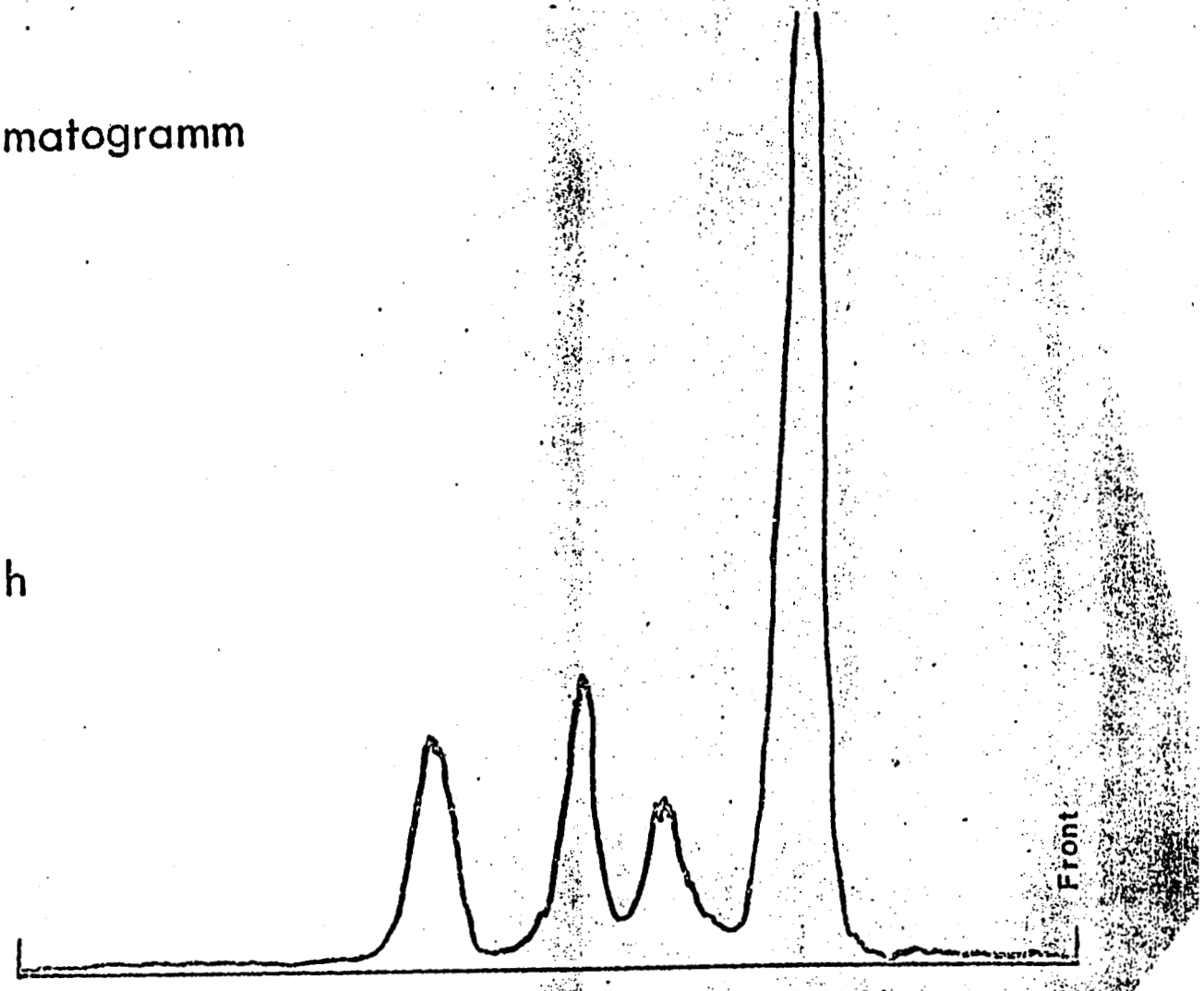


Abb. 3

Radiodünnschichtchromatogramm

Rind 1

Harnextrakt 0 – 120 h

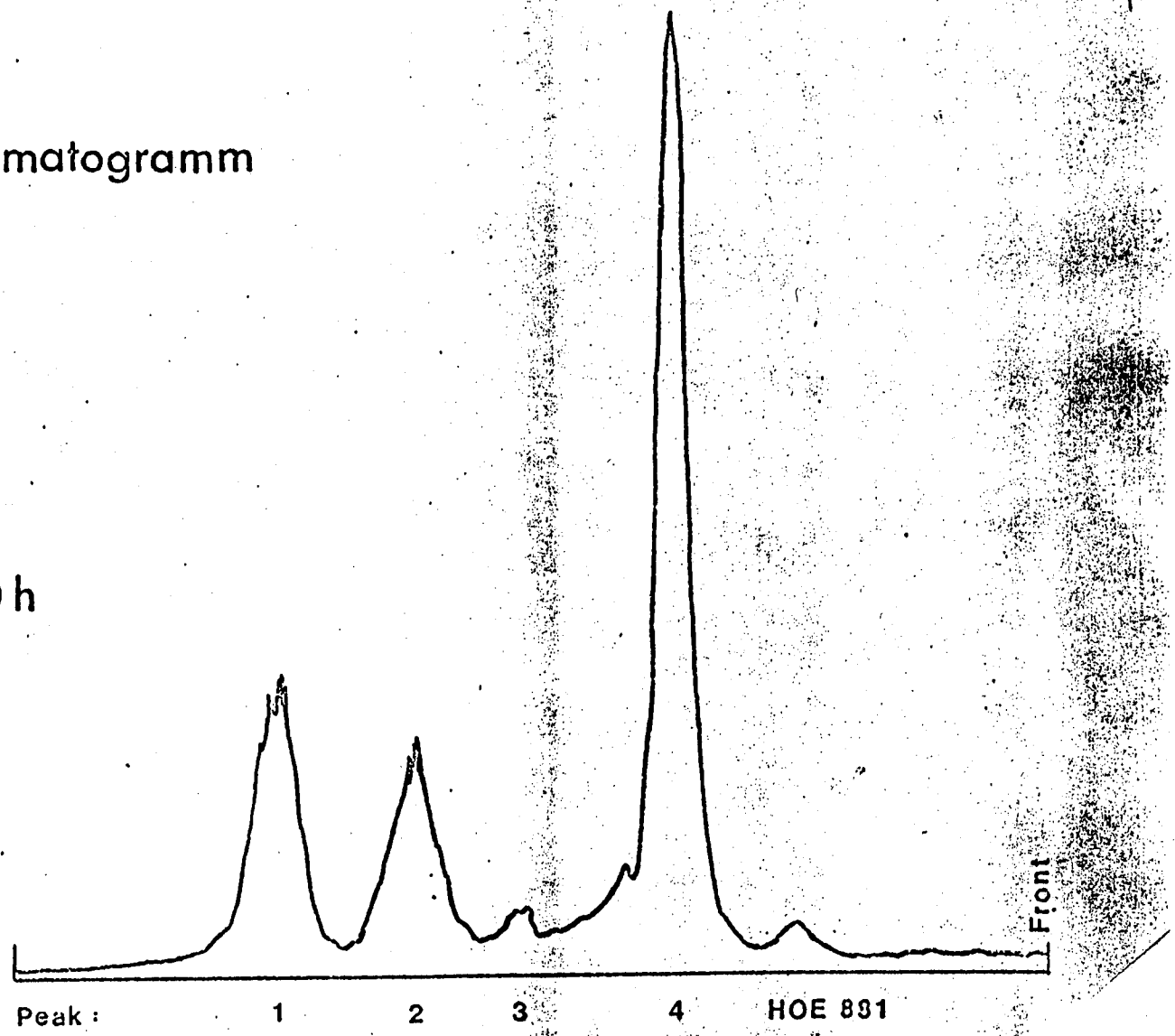


Abb. 4

Radiodünnschichtchromatogramm

Rind 1

Kotextrakt 0 - 120 h

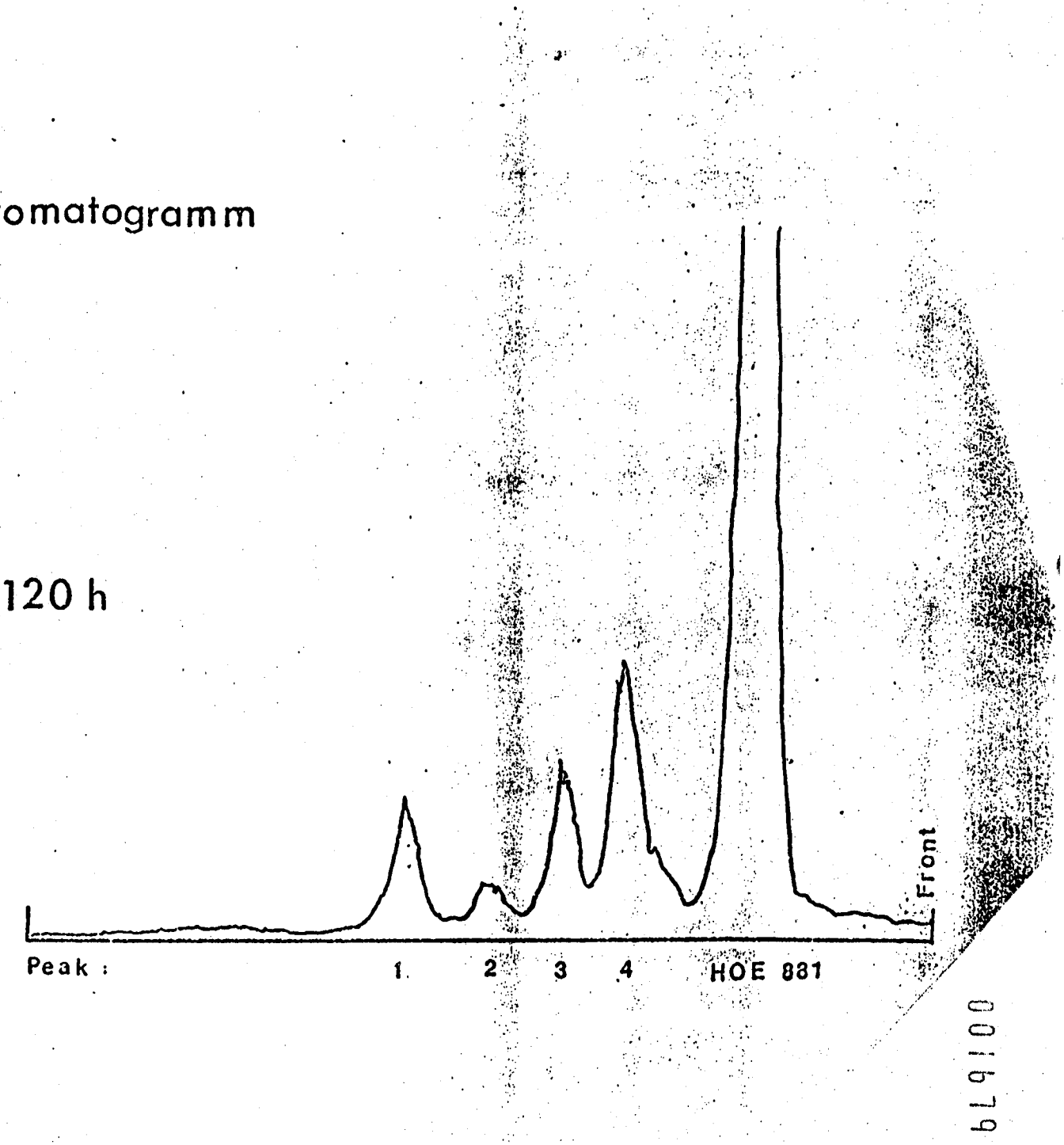


Abb. 5

Radiodünnschichtchromatogramm

Schwein 3

Harnextrakt 0 - 72 h

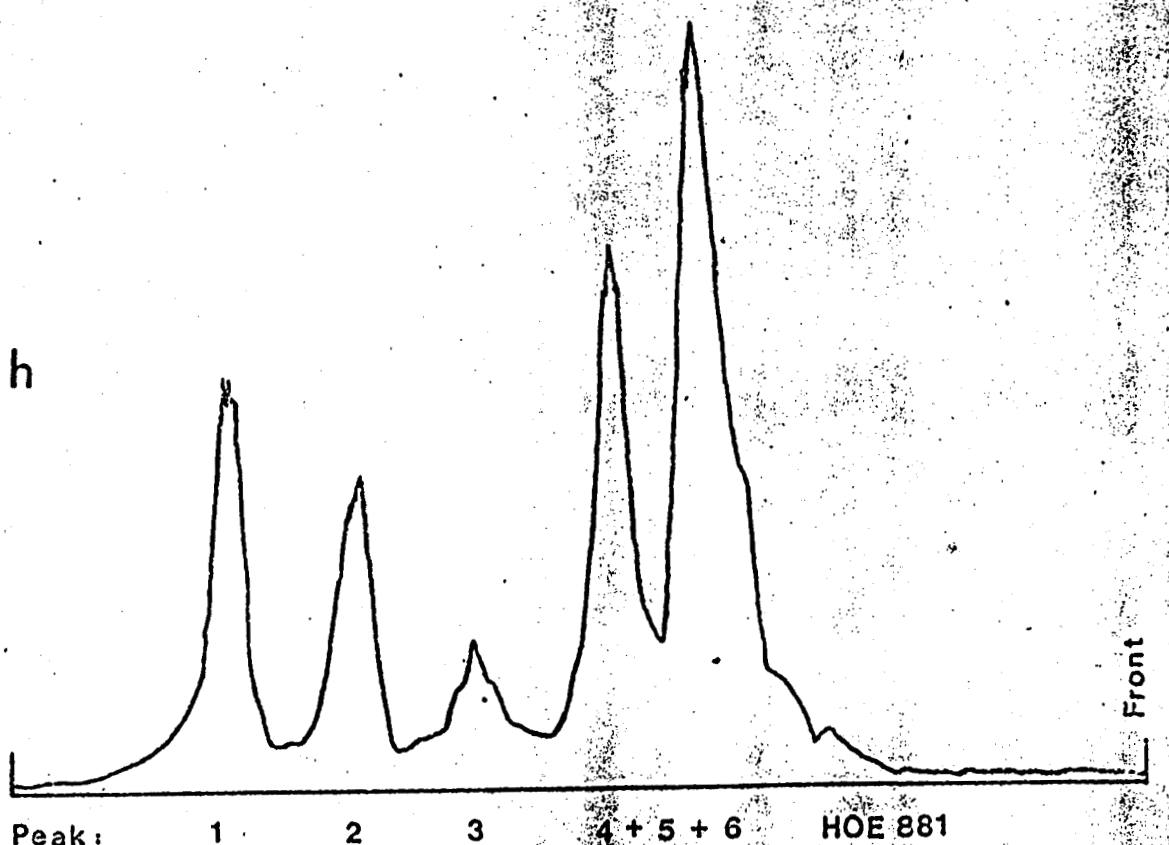
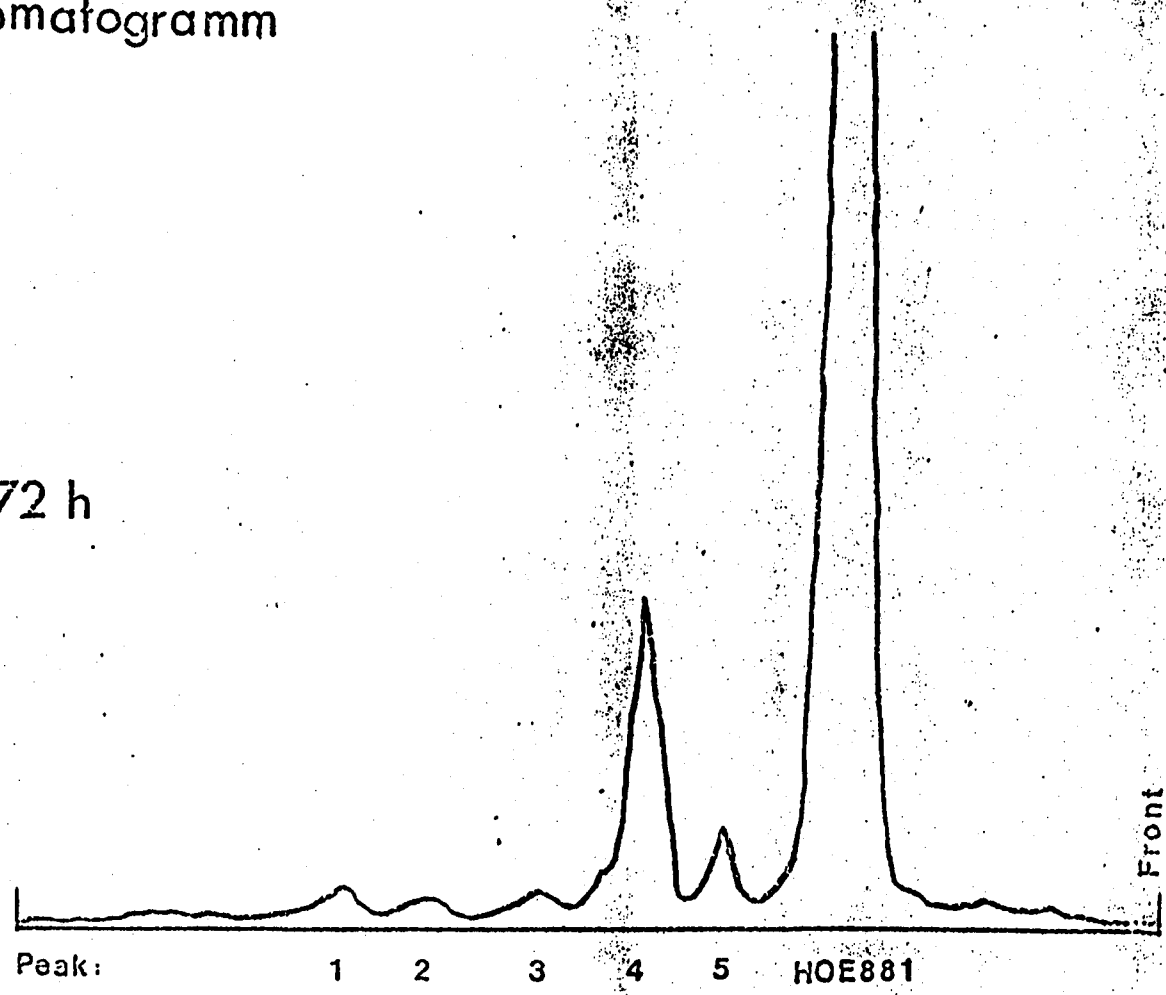


Abb. 6

Radiodünnschichtchromatogramm

Schwein 3

Kotextrakt 24 - 72 h



APPENDIX 8

Fenbendazole - Subacute Toxicity to Earthworms (*Lumbricus terrestris*)

SPONSOR:	Hoechst-Roussel Agri-Vet Company
PROTOCOL TITLE:	"Fenbendazole - Conducting an Earthworm Subacute Toxicity Test Following FDA Technical Assistance Handbook, Document # 4.12", Springborn Laboratories Protocol #091294/HRAV/EW.FDA 4.12 and Protocol Amendments #1 #2 dated 7 December 1994 and 15 February 1995, respectively.
REPORT NUMBER:	95-2-5715
STUDY NUMBER:	1719.0994.6239.635
SPONSOR PROTOCOL/ PROJECT NUMBER:	1684-01-16-94-SP
TEST ARTICLE:	Fenbendazole, Lot No. Y-10911, CAS Registry No. 4321-67-9, a white powder with a purity of 100.4% and expiration date of August 1995 reported by the Study Sponsor, was received from Hoechst-Roussel Pharmaceuticals, Inc. on 22 September 1994
TEST DATES:	6 December 1994 to 3 January 1995
SPECIES:	*Lumbricus terrestris* Source: Johnson's Earthworm Supply, Wareham, Massachusetts
TEST MEDIUM:	Artificial soil medium
TEST TEMPERATURE:	11 to 14 °C (waterbath surrounding test vessels) 12 to 13 °C (individual test vessels)
NOMINAL TEST CONCENTRATIONS:	63, 130, 250, 500 and 1000 mg/kg
MEAN MEASURED CONCENTRATIONS:	56, 120, 240, 500 and 960 mg/kg; (90 to 100% of nominal)

RESULTS:

Based on the results of this test, the 28-day LC50 for earthworms exposed to fenbendazole was calculated using mean measured chemistry values, by moving average angle analysis to be 180 mg/kg (corresponding 95% confidence interval of 150 to 210 mg/kg). The Lowest-Observed-Effect Concentration (LOEC) for this study was determined to be 120 mg/kg, based on survival at termination (day 28).

The corresponding No-Observed-Effect Concentration (NOEC) for the 28-day exposure was 56 mg/kg (mean measured).

Mean measured concentrations tested, percent survival, mean live weight of organisms at test initiation and termination and percent weight change during the 28-day toxicity test exposing earthworms (*Lumbricus terrestris*) to fenbendazole.[a]

Mean Measured Concentration (mg/kg)	Rep	Mean Survival (%)				Individual Mean Live Weight (g)		Percent Weight change[b] (%)	Mean Weight change (%)[SD][c]
		Day 7	Day 14	Day 21	Day 28	Day 0	Day 28		
Control	1	100	100	100	100	4.6	5.1	12	
	2	100	100	100	100	4.3	5.5	29	
	3	100	100	100	100	4.2	6.3	48	
	4	100	100	100	100	5.2	6.4	24	
	Mean				100				+ 28 [15]
56	1	100	100	100[d]	100[d]	4.8	6.3	31	
	2	100	100	80[f,g]	80[f,g]	4.6	5.3	16	
	3	100	100	90[j]	90[j]	4.5	5.5	23	
	4	100	100	100[j]	100[j]	3.9	5.1	30	
	Mean				93				+ 25 [6.7]
120	1	100[e]	80	60[e]	50[h]	4.4	5.7	28	
	2	100	100	90[j]	60[j]	3.8	5.0	30	
	3	100	100	60[j]	20	4.0	4.3	7	
	4	100	100	90[j]	80[j]	5.3	7.0	33	
	Mean				53[k]				+ 25[l] [12]
240	1	80	70[h]	40[g]	30[f]	3.5	5.3	51	
	2	100	80[d]	70[h]	50[d]	4.7	6.1	30	
	3	100[f]	90[h]	70[h]	40[j]	3.7	4.4	19	
	4	90[f]	70[h]	40[h]	20[j]	3.9	5.0	28	
	Mean				35[k]				+ 32[l] [14]
500	1	100[e]	90[j]	30[j]	30[j]	4.6	5.1	11	
	2	100[e]	80[h]	40[h]	40[j]	4.6	5.3	14	
	3	100[e]	80[h]	40[d]	30[f]	4.9	7.0	41	
	4	100[f]	60[j]	20[j]	0	4.9	NA[m]	NA	
	Mean				25[k]				+ 22[l] [17]
960	1	100[f,g]	60[j]	0	0	3.7	NA	NA	
	2	100[f]	80[j]	20[j]	0	5.1	NA	NA	
	3	90	60[j]	30[j]	0	3.2	NA	NA	
	4	100[f]	50[j]	0	0	4.0	NA	NA	
	Mean				0[k]				NA[l]

[a] Values presented are based on actual unrounded values, not the rounded (2 significant figures) values presented in this table.
[b] (((mean weight, day 28 - mean weight, day 0)) / mean weight, day 0) x 100.
[c] The standard deviation for mean percent weight change is presented (SD).
[d] Two of the earthworms exhibited segmental constriction.
[e] Two of the earthworms were observed to have lesions (i.e., white blisters).
[f] One of the earthworms was observed to have lesions (i.e., white blisters).
[g] One of the earthworms exhibited segmental constriction.
[h] Several of the earthworms exhibited segmental constriction.
[i] All of the earthworms exhibited segmental constriction.
[j] Several of the earthworms were observed to have lesions (i.e., white blisters).
[k] Significantly different from control data based on Fisher's Exact test ($p \leq 0.05$).
[l] Omitted from statistical analysis due to significant effect on survival.
[m] NA = Not applicable.

APPENDIX 9

Fenbendazole - Determination of Effects on Seed Germination and Root Elongation of Six Plant Species

SPONSOR:	Hoechst-Roussel Agri-Vet Company
PROTOCOL TITLE:	"*Fenbendazole* - Seed Germination and Root Elongation Test Following FDA Technical Assistance Document 4.06", Springborn Protocol #: 011094/FDA/600, and Protocol Amendments #1 and #2 dated 23 November 1994 and 21 December 1994, respectively (Appendix I)
REPORT NUMBER:	95-2-5700
STUDY NUMBER:	1719.0994.6242.600
SPONSOR PROTOCOL/ PROJECT NUMBER:	1684-04-16-94-SP
TEST ARTICLE:	Fenbendazole, Lot No. Y-10911, CAS Registry No. 4321-67-9, a white powder with a purity of 100.4% and expiration date of August 1995 reported by the Study Sponsor, was received from Hoechst-Roussel Pharmaceuticals, Inc. on 22 September 1994
STUDY DESIGN:	50 seeds per replicate, 6 replicates per concentration or control, 300 seeds per treatment or control
STATISTICAL END POINTS:	percent germination and radicle length
EFFECT CRITERIA:	No-Observed-Effect Concentration (NOEC), Lowest-Observed-Effect Concentration (LOEC) and treatment-related morphological abnormalities were determined for each species

TEST SPECIES:

corn (*Zea mays*)
cucumber (*Cucumis sativus*)
perennial ryegrass (*Lolium perenne*)
soybean (*Glycine max*)
tomato (*Lycopersicon esculentum*)
wheat (*Triticum aestivum*)

TEST DATES:

1 to 6 December 1994 (corn, cucumber, perennial ryegrass)
30 December 1994 to 4 January 1995 (wheat)
30 December 1994 to 5 January 1995 (soybean, tomato)

NOMINAL TEST CONCENTRATIONS:

63, 130, 250, 500 and 1000 mg/L (corn, cucumber and perennial ryegrass)

0.31, 3.1, 31, 63, 130, 250, 500 and 1000 mg/L (soybean, tomato)

63, 130, 250, 500 and 1000 mg/L (wheat)

MEASURED TEST CONCENTRATIONS:

61, 110, 240, 480 and 970 mg/L; 85 to 98% of nominal (corn, cucumber and perennial ryegrass)

0.36, 3.6, 36, 61, 150, 310, 530 and 1000 mg/L; 97 to 123% of nominal (soybean, tomato)

61, 150, 310, 530 and 1000 mg/L; 97 to 123% of nominal (wheat)

RESULTS:

NOEC and LOEC for seed germination and root elongation of six plant species exposed to mean measured concentrations of fenbendazole.

Species	Germination		Root Elongation	
	NOEC (mg/L)	LOEC[a] (mg/L)	NOEC (mg/L)	LOEC[a] (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

Based on the lack of observed effects for all species, it was determined that percent germination and root elongation were unaffected by the exposure to fenbendazole at a measured concentration as high as 970 mg/L for corn, cucumber and perennial ryegrass and 1000 mg/L for soybean, tomato and wheat. The maximum required test concentration according to the study guideline is 1000 mg/L. The highest measured concentrations tested in this study achieved or closely approximated this value.

APPENDIX 10

Fenbendazole - Determination of Effects on Seedling Growth of Six Plant Species

SPONSOR:	Hoechst-Roussel Agri-Vet Company
PROTOCOL TITLE:	"*Fenbendazole* - Seedling Growth Toxicity Test Following FDA Technical Assistance Document 4.07", Springborn Protocol #: 012494/FDA/620, and Protocol Amendment #1 dated 21 December 1994 (Appendix I)
REPORT NUMBER:	95-2-5721
STUDY NUMBER:	1719.0994.6243.620
SPONSOR PROTOCOL/ PROJECT NUMBER:	1684-03-16-94-SP
TEST ARTICLE:	Fenbendazole, Lot No. Y-10911, CAS Registry No. 4321-67-9, a white power with a purity of 100.4% and expiration date of August 1995 reported by the Study Sponsor, was received from Hoechst-Roussel Pharmaceuticals, Inc. on 22 September 1994
STATISTICAL END POINTS:	Survival, shoot length, shoot dry weight and root dry weight
EFFECT CRITERIA:	No-Observed-Effect Concentration (NOEC), Lowest-Observed-Effect Concentration (LOEC) and treatment-related morphological abnormalities were determined for each species
TEST SPECIES:	corn (*Zea mays*) cucumber (*Cucumis sativus*) perennial ryegrass (*Lolium perenne*) soybean (*Glycine max*) tomato (*Lycopersicon esculentum*) wheat (*Triticum aestivum*)

TEST DATES: 11 January to 1 February 1995 (corn, cucumber and perennial ryegrass) in-life exposure

8 to 9 February 1995, dry weight data collection

12 January to 2 February 1995 (soybean, tomato and wheat) in-life exposure

15 to 16 February 1995, dry weight data collection

NOMINAL TEST CONCENTRATIONS: 47, 94, 190, 380, 750 and 1500 mg/kg

MEASURED TEST CONCENTRATIONS: 36, 64, 150, 360, 810 and 1600 mg/kg (68 to 110% nominal)

RESULTS:

The Lowest-Observed-Effect Concentration (LOEC) and No-Observed-Effect Concentration (NOEC) for the seedling growth test of six plant species exposed to mean measured concentrations of fenbendazole.

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

[a] LOEC and NOEC based on the most sensitive parameter measured (i.e., 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 high measured concentration tested.
[c] LOEC and NOEC based on root weight.

* Exceeds guideline limit of 1000 mg/kg.

APPENDIX 11

Fenbendazole - Acute Toxicity to Dung Beetles (*Onthophagus gazella*)

SPONSOR:	Hoechst-Roussel Agri-Vet Company
PROTOCOL TITLE:	"Fenbendazole - Acute Toxicity to Dung Beetles (*Onthophagus gazella*)", Springborn Laboratories Protocol #120194/Hoechst/Dung beetle and Protocol Amendment #1 dated 24 March 1995.
REPORT NUMBER:	95-4-5788
STUDY NUMBER:	1719.0994.6240.169
SPONSOR PROTOCOL/ PROJECT NUMBER:	1684-05-16-94-SP
TEST ARTICLE:	Fenbendazole, Lot No. Y-10911, CAS Registry No. 43210-67-9, a white powder with a purity of 100.4% and expiration date of August 1995 reported by the Study Sponsor, was received from Hoechst-Roussel Pharmaceuticals, Inc. on 30 September 1994
TEST DATES:	24 to 31 March 1995
SPECIES:	*Onthophagus gazella* Source: Kailua, Oahu, Hawaii
TEST MEDIUM:	Artificial soil medium
TEST CONDITIONS:	26 to 29 °C (waterbath surrounding test vessels), relative humidity of 58 to 66% and a light intensity of 60 footcandles
NOMINAL TEST CONCENTRATION:	1000 mg/kg
MEAN MEASURED CONCENTRATION:	770 mg/kg (77% nominal)
RESULTS:	Based on the results of this test, the 7-day LD50 for dung beetles exposed to fenbendazole was empirically estimated to be greater than 770 mg/kg, the mean measured concentration tested. The No-Observed-Effect Level (NOEL) for this study was determined to be 770 mg/kg.

Temperature and relative humidity measured in the exposure chamber during the acute toxicity test exposing dung beetles (*Onthophagus gazella*) to fenbendazole.

Test Day Day	Temperature (°C)		Relative Humidity (%)
	Min	Max	
0	26	26	60
1	26	29	60
2	28	29	66
3	28	29	58
4	28	29	64
5	28	29	64
6	28	29	63
7	27	29	65

Nominal and measured concentrations of fenbendazole in the treated cattle manure during the acute toxicity test with dung beetles (*Onthophagus gazella*).

Nominal Concentration (mg/kg dry weight)	Measured Concentration (mg/kg)[a]									Mean Measured Concentration (SD)[c]	Percent of Nominal
	Day 0			Day 7[b]			Day 7				
	1	2	3	1	2	3	1	2	3		
Control	ND[d]	ND	ND	NA[e]	NA	NA	ND	ND	ND	NA	NA
1000	780	770	870	640	620	610	760[f]	730[f]	730[f]	770(51)	77
QC #1[g]		—[h]					1440[i] (1830)				
QC #2		—[h]					1330[i] (1870)				
QC #3		2440[i] (4440)[j,k]					1320[i] (1840)				
QC #4		3700[i] (4460)					4060[m] (4410)				
QC #5		4160[i] (4510)					4090[m] (4440)				
QC #6		—[h]					4340[m] (4740)				

[a] Values presented represent the total amount (mg) of fenbendazole measured per kg (dry weight) in the treatment manure.
[b] These data were not normalized.
[c] Calculated using unrounded analytical results and not the rounded results presented in this table. Mean measured concentration presented reflects the mean of the Day 0 measured concentrations and the normalized Day 7 concentrations.
[d] ND = Not detectable.
[e] NA =Not Applicable.
[f] Due to a decrease in the percent moisture of manure on day 7, analytical results were normalized by multiplying the result by the decimal percent recovery difference (0.18) of QC samples (1-6), then adding the analytical result. (i.e., (0.18 x analytical result) + analytical result.
[g] QC = Quality Control sample.
[h] QC #1 and 2 were prepared incorrectly and therefore were not analyzed; QC #6 was not prepared at this interval.
[i] QC sample was prepared using the same manure used to prepare exposure concentrations (i.e., 77% moisture)
[j] Value in parentheses represents the corresponding nominal fortified concentration for each QC sample.
[k] Percent recovery for this QC sample was outside of the standard acceptable range established by this laboratory (i.e., within three standard deviations of the mean percent recovery determined during the method validation/recovery study, Appendix V).
[l] QC sample was prepared using a composited control manure from the exposure system (percent moisture of 47%).
[m] QC sample was prepared with manure used at test initiation which had been stored under refrigerated conditions to preserve the moisture content (resultant moisture content was 78%).

Mean measured concentration tested, percent survival and cumulative percent survival during the 7-day toxicity test exposing dung beetles (*Onthophagus gazella*) to fenbendazole.

Mean Measured Concentration (mg/kg)[a]	Replicate	Percent Survival
Control	A	100
	B	100
	C	100
	D	100
	E	100
	Mean	100
770	A	100
	B	100
	C	100
	D	100
	E	100
	Mean	100

[a] Mean measured concentration reflects the mean of the Day 0 measured concentrations and normalized Day 7 concentrations