Environmental Impact Statement

NAME OF APPLICANT: Pfizer, Inc.

ADDRESS: 235 East 42nd Street
New York, New York 10017

1. Describe the proposed action:

It is proposed that the applicant manufacture and market the FDA approved new animal drug morantel tartrate, an anthelmintic for cattle in a bolus and a feed formulation for the removal and control of mature gastrointestional nematode infections of cattle, without restriction against "use in dairy animals of breeding age". The chemical structure, biological composition and known pharmacological properties of the active ingredient are the same as for the currently marketed feed and bolus formulations (NADA 92-444 and 93-903, respectively). Directions for use, dosage regimen and indications for use in "dairy animals of breeding age" are the same as previously approved, i.e., administer as a single oral dose 4.4 mg/lb body weight for removal and control of the claimed parasite species.

The Morantel premix differs from most animal feed additives in that it will not be used to treat cattle continuously.

2. Discuss the probable impact of the action on the environment (including primary and secondary consequences):

Production and utilization of morantel tartrate for use in dairy cattle of breeding age would not have a significant impact on the environment for several reasons:

Manufacture of the bulk drug will occur as described in the approved NADA's (92-444 and 93-903). As deemed in the approval of those applications, the manufacture of the drug presents no adverse environmental impact. The bulk manufacturing process would be scrubbed before discharge into the atmosphere and the quantity of air pollutants would be relatively small, consisting primarily of unsubstantial amounts of hydrocarbons from the organic solvents used in the process. All such air emissions would comply fully with: 1) the Administrative regulations for the Abatement of Air Pollution of the Connecticut Department of Environmental Protection; and, 2) for U.K. production, the following U.K. legislation:

a) Site effluent tank discharges - Control of Pollution Act 1974 (Part 2)
b) Gas-scrubber emissions - Emissions into the Atmospheric Regulations 1983
c) Incinerator stack emissions - Control of Pollution Act 1974 (Part 1)
d) Waste solvent disposal - Control of Pollution (Special Wastes) Regulations 1980.

Under Sandwich, England manufacturing there are no specific effluent streams from the production of bulk morantel tartrate. The production area is designed on a total containment principle and all air leaving the process area is filtered. The filters are changed periodically, the spent filters being incinerated on site. Tank and floor washings are all aqueous and disposed of to the site effluent tanks. Air pollutants emanating from the formulation and packaging operations would be insignificant. All air emissions from such operations would comply fully with (1) the Air Pollution Control Code of the City of New York in the case of the preparation of bolus formulations at the company's Brooklyn, NY plant; and (2) the Air Pollution Control Regulations of Missouri Air Conservation Commission, in the case of the preparation of feed premixes at the company's Lee's Summit, MO plant.

Occupational exposure to air contaminants during the bulk manufacturing process would be limited, since most of the operations would be contained within a closed system. Where operator exposure would occur during the manufacturing process, appropriate personal protective equipment are prescribed. The same conditions prevail during the preparation of feed premixes. In the preparation of bolus formulations, occupational exposures are not significant, but air masks are prescribed for operations involving high particulate concentrations. Exposures in these operations are controlled within the permissible exposure limits for air contaminants established by the Occupational Safety and Health Administration.

The liquid effluent from the bulk manufacturing process contains conventional pollutants such as BOD, COD and some suspended solids. Some or all of the following substances can be expected to be present in the effluent: ammonia, dimethyl amine, 3-methyl thiophene, sodium phosphate, sodium tartrate, methyl formate, sodium formate, sodium chloride and morantel. The waste streams arising from the bulk process are: 1) Hydrogen chloride evolved from reaction - absorbed in dilute sodium hydroxide which is sewered to site effluent tanks; 2) Still heels from product fractionation - incinerated on site; 3) Ammonia evolved during reation - absorbed in dilute sulphuric acid which is sewered to site effluent tanks; 4) Still heels from product fractionation - collected with other waste solvent and disposed of via specialist contractor for incineration; 5) Reaction mother liquors are recovered to clean solvent on site - irrecoverable solvent fractions to specialist contractor for incineration - residual sludges to site effluent tanks. The maximum quantity of morantel in the effluent is expected to be approximately 1.7 gm/1,000 gal. The discharge of liquid effluent from the manufacture of morantel tartrate would comply fully with NPDES Permit No. CT00000957, issued on May 20, 1980, to the company's Groton, CT plant by the State of Connecticut Department of Environmental Protection. Normal resource recovery practices would involve recovery and recycling of isopropanol, methanol and methylene chloride.

There is no liquid effluent resulting from the formulation and packaging operations.

Standard practice with lactating dairy cattle and label directions dictate that the feed or bolus be administered as a single therapeutic dose to cattle known to have infestations of gastrointestinal worms. The low perceived need for anthelmintic treatment of mature dairy animals and the long time intervals between deworming and reinfestation with economically significant worm burdens indictate that cattle will receive morantel very infrequently. Nevertheless, the use pattern of morantel tartrate will expectedly shift as some product will be used in lactating dairy animals in addition to the lighter weight calves and stocker or beef animals. In any event, annual morantel use from the bolus and premix formulations will not exceed the originally projected 6,600 kg.

Fate in the Environment

(a) Metabolism in Cattle/Dairy Cattle:

Five lactating, Holstein dairy cattle were orally dosed at the recommended use rate with tritium labeled compound and total residues of morantel peaked at 84 ppb in milk at the second milking. Absorption of the drug was comparable to that previously observed for beef cattle. Approximately 14% of the oral dose was found in urine with no unchanged morantel present. Approximately 74% of the oral dose is recovered in feces and of this amount approximately 60% is unchanged drug. Therefore, approximately 45% of the ingested dose is excreted in feces as intact morantel, while the remainder of the dose is composed of biologically inactive metabolic products. For information concerning the environmental fate of morantel and its metabolites, refer to FR Doc. 81-29633 filed 10/15/81.

Assuming that cattle consume approximately 2% of their body weight in dry feed and given the dose rate of 2.7 mg of morantel (base) per pound of body weight, a 500 kg animal will eat about 10 kg of dry feed daily and receive 2.97 grams of morantel. If it is additionally assumed that 100% of the drug appears in the feces and urine and that dairy cattle excrete approximately 3/10 of their dry feed intake as dry manure then the concentration of morantel in manure would be 990 ppm (10 kg dry feed x 0.3=3 kg dry manure; 2.97 grams morantel divided by 3 kg dry manure = .99 grams morantel per kg dry manure = 990 ppm). However, since the animal would be treated with an anthelmintic only once during the year, the drug laden manure is further diluted with 270 days (standard lactation period) of drug-free manure. Further, in the U.K. where the treatment of lactating animals is relatively well established, only about 30% of a herd might be treated in a given year. The total manure pack therefore would contain only 1.10 ppm morantel and all possible metabolites.

It can be estimated therefore that the amount of morantel and its metabolites which would be applied to farm land in manure from the use in lactating dairy animals will be 6.08 g per acre per year.

[(2,000 pounds/ton divided by 2.2 pounds/kilograms) x 0.0011 grams morantel/kg dry manure x 6.08 tons* dry manure/acre].

For information concerning environmental fate of morantel in cattle feces refer to FR Doc. 81 29633 filed 10/15/81.

The impact of excreted Morantel on the environment can be assessed by determining its rate of photoisomerization in ultra-violet light from the biologically active trans isomer to the biologically inactive cis isomer. These studies have shown that a 10 ppm solution of Morantel isomerizes to the cis isomer with a half-life of 9.1 minutes when exposed to 366 nm light[1]. Therefore Morantel excreted from treated animals would be rapidly converted to the biologically inactive cis isomer and have little effect on the environment.

For information concerning photolability of Morantel refer to FR Doc. 81 29633 filed 10/15/81.

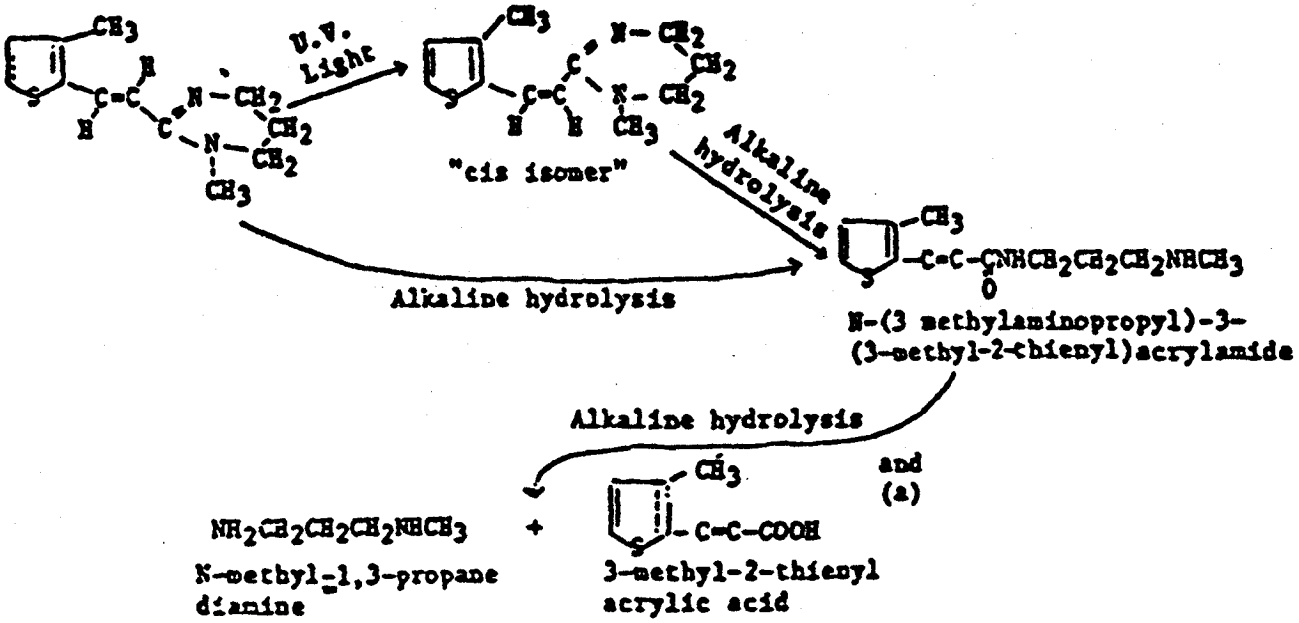
Also, under alkaline conditions such as in cattle feces, morantel is converted to the amide degradation product which is biologically inactive. The pathways for amide degradation and ultraviolet isomerization of pyrantel are shown in Figure 1.

* Maximum permitted manure spread per acre via Iowa Agricultural Extension.

1 FR Doc. 81-29633 Filed 10-15-81

Figure 1

Pathway of Environmental Inactivation of Morantel



Further degradation of both isomers can be expected through the action of bacteria and other soil saprophytes. Bacteria readily attack double bonds as well as metabolize amides.

Morantel has no activity against bacteria and fungi and is therefore susceptible to transformation reactions of either. For this analysis, morantel will be considered as a molecule containing three chemical moieties as follows:

The results from the analysis of these three moieties are:

1. Thiophene portion would be degraded by microorganisms
2. Carbon-carbon double bonds are quite susceptible to microbial degration.
3. Pyrimidine portion is also susceptible to degradation by soil microorganisms.

Each is discussed separately in the original EIAR FR Doc. 81-29633 filed 10-15-81.

The effect of morantel on aquatic species has been investigated in carp (Cyprinus carpio) and the water flea (Monia macrocopia). Each was tested at various levels of exposure to drug (Attachment A). The tests were conducted in accordance with standard protocols for the evaluation of agricultural chemicals. M. macrocopia were exposed to dissolved morantel tartrate at doses ranging from 0 to 10,000 ppm. In the water flea (M. macrocopia) the TLm was calculated as 8,400 ppm in 3 hours, 7,100 ppm in 6 hours, and 5,600 ppm in 24 hours. In carp fry (C. carpio) the calculation of the TLm was over 2,000 ppm. These values show morantel tartrate as being almost non-toxic against these aquatic species.

The metabolism reports[2] conclude that the metabolic profile of residues resulting from the administration of morantel tartrate to lactating dairy animals is not different from that which results from administration to other classes of cattle of Morantel tartrate (FR Doc. 81-29633 filed 10-15-81).

For further information on the environmental implications as of the use of morantel tartrate, please refer to FR Doc. 81-29633 filed 10-15-81.

Based on the information contained in this document and the original EIAR November 1, 1979 (FR Doc. 82-29633 filed 10-15-81) this proposed action would have negligible primary consequences and no secondary consequences on the environment.

2 Submitted May 22, 1985

3. Discuss the probable adverse environmental effects which cannot be avoided.

Based on the above, there are no significant adverse effects on the environment anticipated.

4. Evaluate alternatives to the proposed action.

Inasmuch as no significant impact on the environment is anticpated, no alternatives appear necessary.

5. Describe the relationship between local short-term uses of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity.

The local short-term uses of the proposed action would have no effect on maintenance or enhancement of long-term productivity of the environment.

6. Describe any irreversible and irretrievable commitment of resources which would be involved in the proposed action should it be implemented.

There would be no major commitment of resources with implementation of the proposed action. Only the negligible amount of energy and raw materials consumed in the manufacturing process, none of which constitute a significant commitment of resources, would be required.

7. Discuss the objections raised by other agencies, organizations or individuals which are known to the applicant.

There have been no objections by other agencies, organizations or individuals which are known to the applicant.

8. If proposed action should be taken prior to 90 days from the circulation of a draft environmental impact statement, or 30 days from the filing of a final environmental impact statement, explain why.

No such action is proposed. We submit that the agency should find that there is no requirement for an Environmental Impact Statement.

9. Analyze whether the benefit to the public of the proposed action will outweigh the action's potential risks to the environment.

Because there is a continuing need for increased efficiency in cattle/milk production in the United States, clearly the public will benefit from the proposed action. If the animal disease for which the action is proposed can be controlled, cattle would be produced more economically and efficiently. No significant risk to the environment is recognized.

Certification:

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

11/7/85
Date

(Signature of responsible official)

Director, Animal Health Research
Title

TITLE 000066
Fish Toxicity Study of PC-3203(Greenguard) in Water flea

INVESTIGATOR	LOCATION
Y. Kawashima	Chemical Product Safety Center JAPAN

REPORTED BY	CLASSIFICATION	DATE	DAY	MONTH	YEAR	PAGE
T. Hirai Y. Nakamura	☒ INTERNAL PFIZER USE ONLY ☐ GENERAL USE	START	30	Apr	'82	1 of 3
		FINISH	1	May	'82	

OBJECTIVE:

To investigate the TLm of PC-3203(Greenguard) in water flea.

MATERIALS & METHOD:

1. Compound:
   PC-3203(Greenguard) - Morantel tartrate 12%(w/v) Liquid Formula
   (MI No.
2. Test animals:
   a) Species: Water flea (Monia macrocopa)
   b) Sex/age: Female adult
3. Test Conditions:
   a) Container: Glass petri-dish [8.5cm(dia.)x5.6cm(high.)]
   b) Volume of water: 200ml
   c) Water temperature: 25°c ± 1°c
   d) Lighting: 12 hours/day
   e) Diluted water: Well aerated well-water
4. Administration:
   a) Route: Bathing
   b) Duration: 24 hours
   c) Number of fish/level: about 40
   d) No. of dose level - 10 levels: 0, 1,000, 1,350, 1,800, 2,400, 3,200, 4,200, 5,600, 7,500 and 10,000ppm
5. TLm:
   Doudoroff's method (Plotted the survival rates on the semi-logarithm graph, linked two plots closing the 50% survival line, and estimated the survival rate to be 50%.)

PROCEDURE:

The test was conducted in accordance with the standard method shown by MOAFF to evaluate fish toxicity of agricultural chemicals.

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About 40 fish were allotted into the petri dish containing 200ml of water which dissolved morantel tartrate at the dose of 0 to 10,000 ppm. Number of dead fish was checked 3, 6 and 24 hours after the start of test. The survival rates were plotted on the semi-logarithm graph, and the TLm was calculated from the graph.

RESULTS:

Table 1 TLm/Survival Rates of Water flea

DISCUSSION:

In less than 2,400ppm, the survival rate of water flea was more than 80% at 24 hours. Even in the control group, it showed 91% of survival rate. Therefore less than 2,000ppm of PC-3203 was estimated to be no effective level. In 5,600ppm, 50% of fish were alive and not alive in 7,500ppm.

CONCLUSION:

TLm of PC-3203(Greenguard) in water flea(Monia macrocopa) is calculated as 8,400ppm in 3 hours, 7,100ppm in 6 hours and 5,600ppm in 24 hours. These values show that Greenguard is almost non-toxic against fish.

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Table 1 TLm/Survival Rates of Water flea

Dose level (ppm)	No. of Fish	Survival rates		
		3 hours	6 hours	24 hours
1,000	44	100	93	80
1,350	38	97	89	89
1,800	40	95	95	90
2,400	45	98	89	82
3,200	43	91	91	53
4,200	42	95	88	57
5,600	42	90	83	50
7,500	40	82	42	0
10,000	35	0	-	-
Control	32	100	100	91
TLm (ppm)		8,400	7,100	5,600

Note) PH and Dissloved oxygen concentration(DO)(ppm) of the test water at final stage.

	Treatment	Control
PH	4.2 - 8.3	8.2
DO(ppm)	6.5 - 6.7	6.5

INTERNATIONAL AGRICULTURAL DEVELOPMENT DIVISION	EXPERIMENT REPORT	EXPERIMENT NO. 1470-06-82-011
	TITLE: Fish Toxicity Study of PC-3203(Greenguard) in Carp fry	000069

INVESTIGATOR	LOCATION
Y. Kawashima	Chemical Product Safety Center - JAPAN

REPORTED BY	CLASSIFICATION	DATE	DAY	MONTH	YEAR	PAGE
T. Hirai Y. Nakamura	[X] INTERNAL PFIZER USE ONLY [ ] GENERAL USE	START	6	May	'82	1 OF 3
		FINISH	10	May	'82	

OBJECTIVE:

To investigate the TLm of PC-3203 (Greenguard) in carp fry.

MATERIALS & METHOD:

1. Compound:
   PC-3203(Greenguard): Morantel tartrate 12%(w/v) Liquid Formula (MI NO.

2. Test animals:
   Carp fry (Cyprinus carpio)
   Average size: 6.9cm, 3.4gm
   Raised for 2 weeks at 25°c before use for test.

3. Test conditions:

   a) Container: Glass box - style bath [60(L) x 29.5(W) x 36(H)cm]

   b) Water volume: 50 l

   c) Water temperature: 25 ± 1°c

   d) Lighting: 12 hours/day

   e) Water: Well aerated well-water

4. Administration:

   a) Route : Bathing

   b) Duration: 96 hours

   c) Number of fish/level : 10

   d) Dose level: 2,000ppm

5. TLm:

   Doudoroff's method (Plotted the survival rates on the semi-logarithm graph, linked two plots clossing the 50% survival line, and estimated the survival rate to be 50%.)

PROCEDURE:

The test fish were allotted to the containers with 50 l of water. 100gm of PC-3203(Greenguard) was dissolved into 50 l of water and 2,000ppm concentrated solution was prepared.

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The survival rates were checked at 48, 72 and 96 hours respectively. Owing to the shortage in dissolved oxygen, aeration was begun after 24 hours and the drug solution was renewed at 48 hours. TLm value was calculated by Oudoroff's method.

RESULTS:

Table 1 TLm of PC-3203(Greenguard) in Carp Fry

DISCUSSION:

No death was observed through the test period. TLm of PC-3203(Greenguard) was estimated to be over 2,000ppm. In the rough test using two carp fry in 2 l of water, carp were all alive in 2,000ppm, half of them were alive in 4,000ppm, but none were alive in 5,600ppm. As this rough test result, TLm of PC-3203 will be about 4,000ppm.

CONCLUSION:

TLm of Greenguard in carp fry was over 2,000ppm. This value was over the upper limitation instructed by MOAFF for TLm of agricultural chemicals.

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Table 1 TLm of PC-3203(Greenguard) in Carp Fry

Observation (hrs)	TLm (ppm)
48 hrs	2,000
72 hrs	2,000
96 hrs	2,000

Note) PH and dissolved oxygen concentration (DO) at final stage of test.

	Treatment	Control
PH	8.5	7.9
DO (ppm)	7.8	4.9

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CENTRAL RESEARCH

PFIZER INC. EASTERN POINT ROAD GROTON, CONNECTICUT 06340

203-445-5611

November 1, 1979

Dr. William Price
Chief, Metabolic Branch
Bureau of Veterinary Medicine
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

RE: NADA #92-444 and 93-903; MORANTEL TARTRATE FOR CATTLE

Dear Dr. Price:

Thank you for arranging for the meeting between us and you, Dr. Olsen and Dr. Matheson to discuss our Environmental Impact Analysis Report for morantel tartrate. We have revised the EIAR in accord with recommendations we received at that meeting and it is herewith attached. Suggested modifications were:

1. Describe the morantel degradation pathway with chemical structures and give emphasis to probable microbial degradation routes.

2. Elaborate the discussion on potential concentrations of morantel in the environment.

3. Discuss the polar nature of morantel degradation products.

4. Include any toxicological data available from the literature on degradation products or related compounds.

5. Resubmit the attachments with no "confidential" stamp.

If you have any questions regarding this submission or wish to discuss it further, please call us.

Sincerely,

Kenneth G. Davis
Manager, Agricultural Research
Government Liaison

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ENVIRONMENTAL IMPACT ANALYSIS REPORT

Morantel Tartrate Premix - NADA #92-444

Morantel Tartrate Bolus - NADA #93-903

Environmental Information

A. Describe the Proposed Action
   1. Completely Describe the Proposed Action
   2. Chemical and Physical Properties
   3. Pharmacological/Toxicological Properties
   4. Purposes and Benefits
   5. Market Penetration, Handling, Storage, Distribution
   6. Environments Affected by the Manufacture, Distribution, Consumption and Disposal

B. Environmental Impact of the Proposed Action.
   1. Introduction into the Environment
      (a) Manufacturing and Distribution

      (b) Use
         (i) Via Cattle Feces
         (ii) Morantel Tissue Residues

   2. Analysis of the Environmental Impact of the Manufacturing Process.
      (a) Bulk Manufacturing
      (b) Premix Manufacturing
      (c) Bolus Manufacturing

   3. Fate in the Environment
      (a) Metabolism in Cattle
      (b) In Cattle Feces
      (c) Microbiologically

   (d) Photochemically

   4. Effects on the Environment

C. Alternatives

D. Effect on Maintenance of Environment

E. Irreversible Resource Commitment

F. Other Agency Objections

G. Early Action

H. Benefits vs. Risks

I. Certification

Environmental Impact Analysis Report

Morantel Tartrate Premix - NADA 92-444
Morantel Tartrate Bolus - NADA 93-903

Date: November 1, 1979
Name of Applicant: Pfizer Inc.
Address: 235 East 42nd St.
New York, N.Y. 10017

Environmental Information

A. Describe the Proposed Action:

1. Completely Describe the Proposed Action. Pfizer Inc. proposes to manufacture and market morantel tartrate, an anthelmintic that is intended to be administered to cattle for removal and control of infections of gastrointestinal nematodes. Morantel tartrate is to be administered in feed or as a bolus as a single treatment at a dose rate of 4.4 mg/lb of body weight predominantly to feedlot cattle, but also to cattle on pasture.

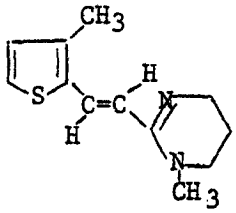
Morantel premix differs from most animal feed additives in that it will not be used to treat cattle continuously nor will the bolus be used continuously. Standard practice with cattle dictates that the feed or bolus be administered as a single therapeutic dose to cattle known to have infestations of gastrointestinal worms. The time interval between deworming and reinfestation with economically significant worm burdens dictates that cattle will receive morantel very infrequently. Anthelmintics are not used in cattle as they near market weight as the time is then too short to permit the increased weight gain and improved feed efficiency to equal or surpass the cost of deworming. It is estimated that a total of approximately 6600 kg per year for the entire United States will be introduced into the environment.

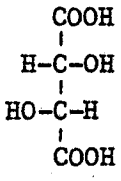
The use of morantel in cattle is so sporadic that it should, in fact, be subject to 21 CFR §25.1(f)(1)(ii)(e)(2):

> "The agency has considered the environmental effects of the following types of actions and has concluded that because these actions normally do not significantly affect the quality of the human environment, environmental impact statements, except in rare and unusual circumstances are not required:
> -----An animal drug intended:
> -----For pharmacological use:
> -----In the treatment of a disease or condition which requires individual dose administration:-----"

Morantel tartrate may be introduced into the environment during the manufacturing of the pure chemical, during formulation of the premix or bolus during administration of the drug to cattle, from the excrement of treated cattle and through morantel residues in meat consumed by man.

2. Chemical and Physical Properties. Morantel tartrate is the salt of an anthelmintic belonging to a chemical family classfied as tetrahydropyrimidines, and is prepared by chemical synthesis as described in NADA 92-444.

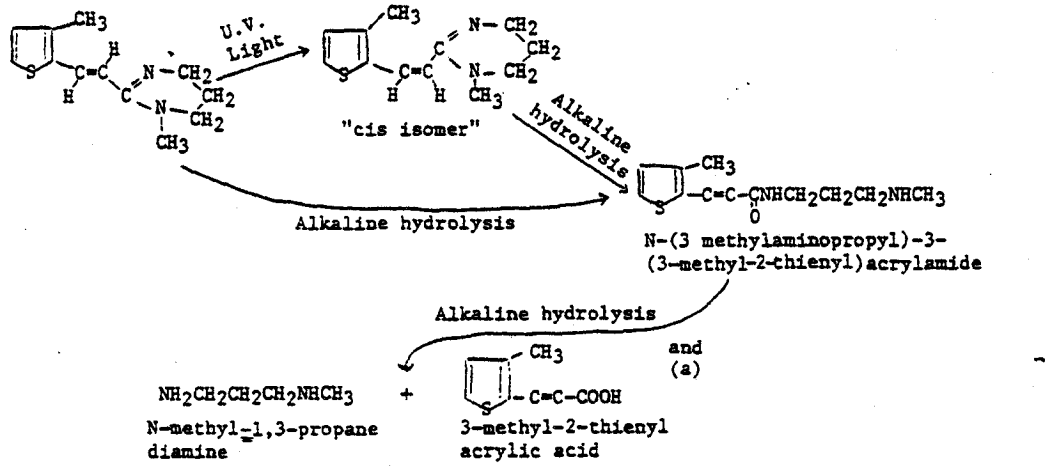




Morantel	$C_{16}H_{22}O_6N_2S$	Mol. wt. 370.4
Tartrate	RN=26155-31-7	

1,4,5,6-tetrahydro-1-methyl-2-[trans-2-(3-methyl-2-thienyl)vinyl] pyrimidine tartrate (1:1).

It is a colorless, odorless crystalline chemical with a melting point of 170°-174° C.; it is a strong base which is protonated and therefore has negligible vapor pressure. The low vapor pressure ensures that there will be no volatilization of morantel and thus no air contamination from morantel premix nor from morantel in excrement. The pH of a dilute aqueous solution is 4.4. Thirty-six different laboratory, pilot plant and production lots of morantel tartrate were analyzed. The morantel tartrate content was found to be 97.4% to 101.8% with the major contaminant being the 4-methyl isomer, ranging from <0.1% to 1.16%. Thus, morantel tartrate contains essentially no impurities which might contaminate the environment. As a crystalline material in bulk form, or in feed premix or in animal feed it is quite stable (see NADA 92-444). When morantel tartrate is dissolved in water to give a dilute solution and is exposed to sunlight or ultraviolet radiation, it is converted very rapidly to the cis isomer (Attachment 1). The cis isomer is further converted through hydrolysis of the tetrahydropyrimidine moiety under alkaline conditions to a biologically inactive amide. This is further degraded to methylthiophene acrylic acid and N-methyl-1,3-propane diamine. The degradation pathway is depicted below:



(a) It is also well-known that bacterial enzymes are capable of hydrolyzing amide bonds.

The thiophene nucleus is found in nature. Crude oils have been shown to contain a wide variety of simple and complex thiophenes; thiophene compounds of plant and fungal origin have been described.[1] Pyrimidines are widespread in nature as components of nucleic acids which are found in all living organisms. Reduced pyrimidines, such as tetrahydropyrimidines, are intermediates in the formation and degradation of nucleic acids.

By analogy with compounds reported in the literature, all chemical moieties found in morantel and its degradation products would be subject to microbiological degradation as discussed more fully in this report under B.3(c). 3-Methylthiophene was 87% degraded in 4 days by a soil Pseudomonas aeruginosa. Carbon-carbon double bonds have been shown to be rapidly transformed by various soil microorganisms by (1) hydration of the double band to produce mono-alcohols. (2) the formation of epoxides which can be readily converted to diols and (3) direct transformation to diols. The tetrahydropyrimidine, orotic acid, was shown to be rapidly converted to the naturally occurring amino acid, L-aspartic acid, carbon dioxide and ammonia by soil bacteria.

Morantel tartrate is readily soluble in water (150 mg/ml) and methanol. It is essentially insoluble in the less polar organic solvents. The degradation products are also very water soluble. Distribution of morantel tartrate from water into non-polar organic solvents is neglibible except at pH values higher than 9 (Attachment 2), where the protonated form begins to be converted to the free base. The partition coefficients for both morantel tartrate and the cis isomer in an octanol/water system are <0.1.[b] (Attachment 10). All degradation products are also very polar compounds and therefore would not be extracted from aqueous solution by octanol. The low octanol water partition coefficients ensure that there will be no bioaccumulation of morantel or of its degradation products in the environment. Thus, there will be no concentration magnification of the chemicals in water, no potential hazard to members of the aquatic food chains and no exposure of humans eating fish and shellfish. The TSCA-ITC workshop has concluded that the bio-accumulation potential of substances with an octanol/water partition coefficient of <1 is negligible.[2]

[b] This determination is normally required only for "all nonpolar organic chemicals in technical or purer form" [43 FR 29711 (July 10, 1978)].

3. Pharmacological/Toxicological Properties. Morantel tartrate has been shown to be exceedingly safe and effective in reducing the worm burden of cattle through a single oral therapeutic treatment. It has been used in countries outside of the United States since 1969 as an anthelmintic in cattle, swine and sheep. It is closely related chemically and pharmacologically to pyrantel which has FDA approval for use in man, swine, horses and dogs. The tissue residue tolerance for pyrantel in swine liver is 10 ppm.

Morantel tartrate has undergone numerous toxicological experiments including (1) rat acute oral toxicity (>900 mg/kg) and mouse acute oral toxicity (>300 mg/kg), (2) two 90 day rat studies (no-effect level-50 mg/kg), (3) two year chronic studies in rats in which the no-effect dose was found to be >20 mg/kg, (4) two year chronic studies in dogs in which the no-effect dose was found to be >10 mg/kg, (5) teratological studies in rats and (6) safety studies in cattle. In the latter studies, single doses by drench at 20 times the therapeutic dose did not cause toxic symptoms or death. With continuous dosage for 3 days, the therapeutic index was greater than 36 by feed dosage. When cattle were drenched with 20 doses over a 4 week time period the therapeutic index was 46. Details of these experiments are in NADA 92-444.

The literature has been thoroughly searched for report of toxicological data or of adverse biological effects of morantel degradation products or related compounds. A search of the computerized information available in Toxline/Toxback, RTECS and Chemical Abstracts Condensates has been conducted (Attachment 12). The only pertinent information found was the following:

1-3-Propanediamine-oral LD_{50}-rat-350 mg/kg[3]

N,N-Dimethyl,1-3-propanediamine-oral LDLo[c]-rat-1870 mg/kg[3]

2-methylthiophene-intraperitoneal LDLo[c]-mouse-500 mg/kg[4]

3-methylthiophene-intraperitoneal LDLo[c]-mouse-512 mg/kg[4]

The evidence indicates that morantel is nematocidal rather than nematostatic. It has been demonstrated that morantel is a depolarizing neuromuscular blocking agent similar to pyrantel[5][6] and is highly effective in inhibiting the fumarate reductase system of Haemonchus contortus. The fumarate reductase system functions as a respiratory chain in many helminths and in this function is specific to helminths. Its inhibition by the tetrahydropyrimidines (such as morantel) may explain the biological specificity of morantel.[7] Morantel is not an organophosphate chemical nor does it potentiate the physiological actions of the organophosphate pesticides.

Morantel is readily converted photochemically to the cis isomer which has been shown to have no antibacterial activity (Attachment 3) and exceedingly weak (if any) anthelmintic activity (Attachment 9). As the only noteworthy morantel biological activity is as an anthelmintic, it is concluded that the cis isomer is essentially devoid of biological activity.

[c] LDLo-Lethal Dose Low - the lowest dose (other than LD_{50}) of a substance introduced by any route other than inhalation, over any given period of time in one or more divided portions and reported to have caused death in humans or animals.

Using bacteriological procedures similar to those described in Attachment 3, 3-methyl-2-thienyl acrylic acid was found to be inactive against E. coli, K. pneumoniae, P. mirabilus, Ps. aerugenosa and S. aureus at 100 mcg/ml (the highest level tested). N-(3-methylaminopropyl)-3-(3-methyl-2-thienyl)-acrylamide was inactive against S. pyogenes, E. coli, Kl. aerogenes, Shigella sonei Shigella flexii, Proteus vulgaris, Proteus mirabilis and Ps. aerugenosa at 25 mcg/ml (the highest level tested).

Since morantel tartrate is the salt of a strong base, oral absorption in mammals is relatively poor and hazards due to ingestion of morantel would be minimal. Also, because of this latter property the short half-life of the active drug and the polar nature of the substance, a build up in the biological chain would not occur.

References

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4. Chemical Biological Coordination Center, Summary Biological Tests, National Research Council (Washington, D.C.), 1, 47. (1949).(d)

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7. Int. J. Parasitol. 3, 409-417 (1973).

(d) Data also given in NIOSH Registry of Toxic Effects of Chemical Substances Vol. II (1977) p. 744 and p. 906. (attached).

4. Purposes and Benefits. Morantel is proposed for use for removal and control of mature gastrointestinal nematode infections of cattle including stomach worms (Haemonchus spp., Ostertagia spp., Trichostrongylus spp.), worms of the small intestine (Cooperia spp., Trichostrongylus spp., Nematodirus spp.) and worms of the large intestine (Oesophagostomum radiatum). Morantel is currently marketed and has been proven efficacious in most of the developed countries except for the United States for the control of gastrointestinal nematodes of (1) sheep (since May, 1969), (2) cattle (since December, 1970), (3) swine (since September, 1971), (4) goats (since October, 1972) and horses (since April, 1973).

Bovine nematodes of cattle are complex host-dependent parasites, the early developmental stages of which must come in contact with the host at feeding and drinking areas frequented by cattle. Larval and adult stages of the parasite survive by feeding from the host's body reserves. Large stomach worms are blood suckers, whereas small stomach worms and nodular worms ingest tissue or products thereof. Parasitism inhibits the host's appetite and decreases digestibility of nutrients.

As much as 250 ml of blood daily can be removed from an animal by 5000 adult Haemonchus, resulting in anemia and unthriftiness and occasionally death. Trichostrongyles irritate the abomasum, thriving on mucous secretions and considerably more than 30,000 are required to produce death. A large trichostrongyle burden would be less harmful but would cause weight loss.

Although not all cattle are seriously affected, the potential loss in feed-gain performance poses a constant threat to efficient herd health programs. Most internal parasitisms are chronic and the main loss is in feed-gain efficiency. Death from acute parasitosis can occur, especially in young animals, when host resistance is completely overwhelmed. Dollar losses caused by helminths in cattle have been estimated at over 250 million dollars yearly. Various surveys conducted in the United States reveal most herds have some internal parasitism.[1]

Field trials with naturally parasitized cattle have shown the losses due to parasites and the value of various anthelmintic treatments.[2] Results in several experiments indicate economic advantages derived from treatment on the basis of increased rate of weight gain, more efficient conversion of feed into body weight, or both.[3,4,5]

The efficacy of morantel against gastrointestinal nematodes of cattle is attested to by data included in NADA 92-444 and by numerous publications (e.g. 6,7,8,9).

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7. Vet. Rec. 21: 551-554 (1973).
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9. Am. J. Vet. Res. 34: 619-620 (1973).

5. Market Penetration, Handling, Storage, Distribution.

It has been estimated that a total of 49,400,000 cattle doses of anthelmintics were used in the U.S. in 1976 (Doane Agricultural Services, Inc.; St. Louis, Missouri). It is projected that not more than 10% of these will be morantel. Also see B.2 below.

6. Environments Affected by the Manufacture, Distribution, Consumption and Disposal.

Morantel tartrate may be introduced into the environment during the manufacturing of the pure chemical, during formulation of the premix or bolus, during administration of the drug to cattle, from the excrement of treated cattle and through morantel residues in meat consumed by man.

B. Environmental Impact of the Proposed Action.

1. Introduction into the Environment.

(a) Manufacturing and Distribution.

See A-5 and A-6 above and B.2 below.

(b) Use. The use of morantel as a cattle anthelmintic may introduce morantel into the environment via cattle feces and as a possible residue in meat consumed by man.

(i) Via Cattle Feces. Standard practice with cattle anthelmintics dictates that the feed or bolus be administered as a single therapeutic dose to cattle known to have infestation of gastrointestinal worms.

The time interval between worming and reinfestation with economically significant worm burdens dictates that cattle will receive morantel very infrequently. Sporadic treatment, utilizing relatively small amounts of drug, precludes significant amounts of morantel tartrate from entering into the environment and its concentration, even in areas of dense cattle population, would be infinitesimal.

Anthelmintics are not used in cattle as they near market weight as the time is then too short to permit the increased weight gain and improved feed efficiency which is realized to equal or surpass the cost of deworming. In most feedlot situations, cattle are dewormed only once when they are brought into lot. Once adult worms are removed the high grain diet given the cattle and the extremely low exposure to reinfection in the lot combine to keep worm burdens at an extremely low level.

The age and weight of cattle entering a feedlot can be extremely variable but most range from 400 to 700 pounds and as a consequence the length of time they are fed out also varies usually from 90 to 180 days depending on the type of feed given them, weather, entering body weight and a variety of other factors.

Selecting for the most extreme potential for environmental damage the following analysis of that potential is based on 300 kilogram (660 pounds) animals entering the feedlot being administered the anthelmintic and remaining only 100 days before being shipped to market.

Morantel is used to medicate cattle at a dosage rate of 4.4 mg of morantel tartrate (2.7 mg of morantel base) per pound of body weight. For purposes of this analysis only the morantel base will be considered, since tartaric acid, the other component of the salt, is a non-toxic natural product. Assuming that cattle consume approximately 2% of their body weight in dry feed and given the dosage rate of 2.7 mg of morantel per pound of body weight, a 300 kg animal will eat about 6 kg of dry feed daily and receive 1.78 grams of morantel. If it is additionally assumed that 100% of the drug appears in the feces and that cattle excrete approximately 3/10 of their dry feed intake as dry manure then the concentration of morantel in manure is 990-ppm (6 kg dry feed x .3 = 1.8 kg dry manure; 1.78 g morantel ÷ 1.8 kg dry manure = .99 grams morantel per kg dry manure = 990 ppm). However, since the animal is administered anthelmintic only on arrival, the drug laden manure is further diluted with 99 days of drug-free manure so that the total manure pack contains only 10 ppm morantel and its metabolites.(1)

On a dry weight basis, manure may be applied to farm land up to 6.08 T/A according to the following calculations. The nitrogen content in dry cattle feces is taken to be approximately 3.7%. It is assumed that at least 50% of the nitrogen in fresh excreta is lost (most to the atmosphere as NH_3) prior to soil application of the manure and that the maximum application rate of manure is that amount containing 225 pounds of nitrogen/A.

The application of manure to 225 pounds nitrogen/A, although not practiced everywhere, is not an unreasonable assumption. The Iowa Agricultural Extension Service permits the spreading of animal wastes onto farm land up to an amount which would contain, on a yearly average, 300 pounds N/A at the time of application, not to exceed 400 pounds N/A in any given year. It can then be calculated that 450 pounds initial nitrogen/A

$$\div 0.037 \frac{\text{pounds nitrogen}}{\text{pound dry manure}} \div 2{,}000 \text{ pound/T} = 6.08 \text{ T dry manure/A.}$$

It can be estimated therefore that the amount of morantel and its metabolites which would be applied to farmland in manure will be 55.3 g per acre. [(2,000 pounds/ton ÷ 2.2 pounds/kilogram) x 0.01 gram morantel/kg dry manure x 6.08 tons dry manure/acre].

It is known from metabolic studies in cattle that only morantel metabolites are excreted in urine while 75% of the dose is excreted in feces over a four day period. Of this amount 60% is morantel;* therefore, only 45% or 25 g/A of this will be

*This estimate is based on a 0-24 hours feces sample; therefore in this calculation the amount of intact morantel excreted is overestimated.

the bioactive component, morantel. No evidence of phytotoxicity was observed following application of morantel at a rate of 15 lbs/A to tomato and cucumber plants (Attachment 4) while sugar beet and bean plants were unaffected by a level of 50 lbs/A of morantel (Attachment 5). Beans and rice were shown resistant to damage following foliar application of concentrated solutions of morantel (Attachments 4 and 6).

Cattle pens will not be cleaned until the end of the feeding cycle; therefore, it will be at least 90 days from the time of drug administration until the manure can be spread on the land and usually this period will be much longer (1). Drug build-up in the environment from excreta of morantel medicated grazing cattle is an even more remote possibility than that from feedlot cattle since the concentration per acre is infinitely less.

The total amount of morantel entering the environment in the entire United States via the feces of morantel-treated cattle is estimated to be about 3,000 kilograms per year according to the following calculations:

49,400,000 total cattle anthelmintic doses per year
10% estimated morantel doses
4,940,000 morantel doses per year

Assume 500 lb animal
2.7 mg morantel per pound
1350 mg/500 lb animal
45% dose excreted as mornatel
608 mg $\sim$ 0.61 gm

$$\frac{4,940,000 \times 0.61}{1,000} = 3103 \text{ kilos of morantel}$$

Radiotracer metabolism studies have demonstrated that the metabolism of morantel in cattle is very similar to the metabolism in the animal species used in the laboratory toxicology studies. Since both the dog and rat were auto-exposed to high levels of metabolites in their subacute and chronic testing periods, it may safely be presumed that the metabolites of morantel are also innocuous when found in the feces and urine of cattle.

The above data can be used to calculate the infintessimal amount of morantel and metabolites which would enter the environment.

Estimated morantel and metabolites introduced into the environment - 6600 kg/year.

Estimated concentration of morantel + metabolites in feces from feedlots is 10 ppm, and if spread on fields as fertilizer is calculated to be:

55.3 g/acre $\sim$ 25 g morantel
$\sim$ 30.3 g metabolites

At the rate of 55.3 g/acre, 6600 kg morantel and metabolites would fertilize ~110,000 acres per year of the 1,017,030,357 acres of farmland in the U.S.

110,000 acres equals 0.37% of the farmland in Iowa (33,044,768 acres). 6600 kg applied evenly to the farmland in Iowa would equal 0.22 mg/acre/year.

The number of feedlots in the U.S. (1974) was 210,220 and feedlots are located in each of the 50 states in the U.S. If evenly distributed to feedlots, the morantel usage would be 3.08 gms/lot/year.

The metabolites comprise approximately 55% of the excrement from cattle dosed with morantel. All available toxicological data prove there is no environmental hazard from morantel or its metabolites. The potential concentrations of the identified ultimate degradation products of morantel would be approximately:

N-methyl-1,3-propanediamine (M.W. 88)=45/100 x $\frac{88}{370}$

= 11% of total morantel in excrement = 1.1 ppm in feces.

3-methyl-2-thienyl acrylic acid (M.W. 154)=45/100 x $\frac{154}{370}$

= 19% of total morantel in excrement = 1.9 ppm in feces.

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45/100 = % of ingested dose excreted as morantel.

370 = molecular weight of morantel.

Concentration of morantel + metabolites in feces = 10 ppm.

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Thus, the concentrations and amounts of morantel and its degradation products available to contaminate the environment are exceedingly small and could not be considered to be an environmental hazard.

Since morantel does not have bacteriostatic or bacterial inhibitory properties there is no known mechanism by which it could induce transferable drug resistance to either Gram-negative or Gram-positive bacteria. It is not anticipated that morantel will be used as a human drug.

(ii) Morantel Tissue Residues. Tissue residue depletion studies were conducted in cattle which were given a single oral dose of 6.8 mg of morantel tartrate/lb. of body weight and slaughtered at 1, 3, 7, 14, 21 and 28 days after administration. Muscle, kidney and liver tissues were assayed at each drug withdrawal period for morantel residues. Results from this study indicated that drug residues are highest and most persistent in liver, and were below 0.4 ppm by 14 days following drug withdrawal.

Since low levels of morantel-related residues persist in edible tissues (liver) after 14-days of drug withdrawal, the laboratory toxicology plan included standard food additive studies. In addition to acute and subacute (90-day) studies, the safety of morantel tartrate was evaluated in chronic two-year feeding studies with rats and dogs and in a 3-generation rat teratology study.

Based on the results of these studies, FDA has determined that a drug tissue residue of 0.4 ppm is acceptable and that a 14-day withdrawal period is appropriate.

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B.2. Analysis of the Environmental Impact of the Manufacturing Process.

(a) Bulk Manufacturing. The liquid effluent from the bulk manufacturing process contains conventional pollutants such as BOD, COD and some suspended solids. No toxic pollutants are present. Some or all of the following substances can be expected to be present in the effluent: ammonia, dimethyl amine, 3-methyl thiophene, sodium phosphate, sodium tartrate, methyl formate, sodium formate, sodium chloride and morantel. The maximum quantity of morantel in the effluent is expected to be approximately 1.7 gm/ 1,000 gal. The discharge of liquid effluent from the manufacture of morantel tartrate would comply fully with NPDES Permit No. CT00000957, issued on June 9, 1978, to the Company's Groton, Connecticut plant by the State of Connecticut Department of Environmental Protection. Normal resource recovery practices would involve recovery and recycling of isopropanol, methanol and methylene chloride.

There is no liquid effluent resulting from the formulation and packaging operations.

Air emmisions from the bulk manufacturing process would be scrubbed before discharge into the atmosphere and the quantity of air pollutants would be relatively small, consisting primarily of unsubstantial amounts of hydrocarbons from the organic solvents used in the process. All such air emissions would comply fully with the Administrative Regulations for the Abatement of Air Pollution of the Connecticut Department of Environmental Protection. Air pollutants emanating from the formulation and packaging operations would be insignificant. All air emissions from such operations would comply fully with (1) the Air Pollution Control Code of the City of New York, in the case of the preparation of bolus formulations at the Company's Brooklyn, New York plant; and (2) the Air Pollution Control Regulations of Missouri Air Conservation Commission, in the case of the preparation of feed premixes at the Company's Lee's Summit, Missouri plant.

Occupational exposure to air contaminants during the bulk manufacturing process would be limited, since most of the operations would be contained within a closed system. Where incidents of operator exposure would occur during the manufacturing process, appropriate personal protective equipment would be prescribed. The same conditions prevail during the preparation of feed premixes. In the preparation of bolus formulations, occupational exposures are not significant, but air masks are prescribed for operations involving high particulate concentrations. Exposures in these operations will be controlled within the permissible exposure limits for air contaminants established by the Occupational Safety and Health Administration.

(b) Handling During Premix Manufacture and Distribution.

Morantel premix containing 88 grams of morantel tartrate per pound, packaged in 50 lb. multiwalled paper bags will serve as the dosage formulation. Morantel premix is intended for use in the manufacture of a medicated cattle feed containing 4.4 grams of morantel tartrate

per pound (100 pounds of premix/ton of medicated feed). The premix formulation marketed by Pfizer Inc. will be shipped only to manufacturers of animal feeds who hold an approved form FD 1800.

The morantel medicated feed (meal, pellets, or crumbles form) containing 4.4 grams of morantel tartrate per pound will be used as a single therapeutic treatment by feeding 0.1 pound of medicated feed per 100 pounds of body weight (4.4 mg of morantel tartrate per pound of body weight) for the removal and control of mature infections of gastrointestinal nematodes of cattle including stomach worms, worms of the small intestine and worms of the large intestine.

Written procedures are used in the training of all employees who will supervise and/or execute manufacturing or quality control operations. These procedures are available at work stations and are used to conduct training in Current Good Manufacturing Practice on a continuing basis. Each person engaged in the manufacturing process receives practical training and oral examinations to assure that they are qualified to perform their assigned functions. A certificate of qualification is placed in the service record of the employee when they have completed a training program. Refresher training is provided on a periodic basis.

The buildings have adequate lighting, ventilation, heat, washrooms, and locker facilities. The buildings are constructed of concrete block and insulated, double-wall steel siding.

The Raw Materials Warehouse is arranged to provide an orderly flow of raw materials. The subdividing area is located in the Raw Materials Warehouse adjacent to the central core processing area. The processing area consists of a basement equipment room, scale room, multi-level storage bin section, computer control room, and filling room. The manufacturing areas are maintained in a clean, orderly manner by trained personnel with the aid of separate central vacuum and dust collection systems.

Components for morantel premix will be transferred pneumatically from weighing scales to a blender. The batch is mixed for at least fifteen minutes prior to packaging. The filled premix bags are sewn closed with cotton thread and sewover tape. Fill weight is checked on an in-line, heavy duty 5-zone automatic check weigher.

A system of electrical interlocks is used to prevent raw material transfer from a silo to a storage bin above a scale not designed for that raw material. Each working storage bin and its associated screw feeder above the scale is assigned for storage of only one ingredient and cleaning of the bins would not normally be required. Equipment cleaning is performed by purging the system with diluent which is packaged and tagged for disposal in an approved land fill area.

Batching of morantel premix normally is performed by the process computer. The control room operator loads the batch program into the process computer. The computer calculates and prints the required weight of each component based on the formulation potency and the potency of that ingredient in the working bin.

Finished products, and packaging materials are stored in separate storage areas. All storage areas are arranged to permit orderly storage, dispensing, inventory control, and accountability of materials.

Normally, finished goods are shipped on a first-in-first-out basis.

Following manufacture of the medicated feed premix, it will be stored in Pfizer controlled warehouses prior to shipment to feed supplement distributors or feed manufacturing plants or mills associated with the cattle raising operations. After feed is blended containing morantel it is normally placed in feed bunks in the feedlot where it is totally consumed in a matter of hours by the cattle.

(c) Handling During Bolus Manufacture and Distribution.

The buildings used in the manufacturing, processing, packaging, labeling and storing operations for this new drug dosage form are multi-storied and of re-inforced concrete construction. The internal construction is specifically designed for the production and/or storage of pharmaceuticals. The prime considerations in design are product integrity and reliability.

The manufacturing and processing areas are designed for ease of cleaning. Adequate floor drains have been provided to facilitate washing down when required. Surfaces of walls, floors and ceilings are smooth and non-porous. Care has been exercised to minimize pockets or crevices which may harbor contamination.

In the installation of ductwork, lighting, equipment, etc., the creation of inaccessible spaces, which would be difficult to clean and maintain, has been avoided. Ductwork is either flashed or provision made for ease of cleaning. Hung ceilings are minimized.

The buildings are air-conditioned, where required, with specific areas receiving filtered, dehumidified and/or sterilized air supplies as necessary. The ventilation systems have been designed to minimize dust and provide adequate changes of air. Screening of windows and doors is provided where necessary. The buildings and work areas are equipped with adequate lighting.

The processing and packaging areas have been planned and arranged to provide adequate space for the orderly placement of equipment, the smooth orderly flow of manufacturing and packaging materials and finished products, and to facilitate cleaning and maintenance. Particular attention has been given to the adequacy of these facilities with respect to current good manufacturing practices in the pharmaceutical industry.

Where the possibility of multiple concurrent operations exists, provision has been made for adequate segregation of the units to prevent cross contaminant and/or product and labeling mix-ups.

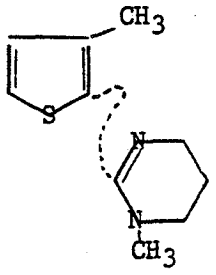
The storage areas for raw materials, labeling, packaging materials, process materials and finished goods have been arranged to permit orderly storage of the materials and to facilitate good control over the inventory, dispensing and accountability of these materials. Ambient conditions in storage areas are maintained at controlled levels where necessary to protect product and raw material integrity.

Convenient washrooms and lockering facilities have been provided for operating personnel.

Following manufacture, morantel boluses will be stored in Pfizer controlled warehouses prior to shipment to Animal Health product distributors or in certain instances directly into retail sales outlet where they will be sold as an over-the-counter medication. They will also be shipped to veterinary drug distributors, to veterinarians or directly to the livestock owners. Except for the animal handler's limited contact with the bolus during administration to the animal, no potential for environmental exposure is foreseen other than passage of the drug through the animal.

B.3. Fate in the Environment

(a) Metabolism in Cattle. When calves are dosed with radiolabeled morantel, approximately 75% of the dose is recovered in feces (0-96 hours) and of this amount approximately 60%* is unchanged morantel. Therefore, approximately 45% of the ingested dose is excreted in feces as intact morantel, while the remainder of the dose is composed of biologically inactive metabolic products. No intact morantel is detected in urine, but only biologically inactive metabolic products. On the basis of additional experimentation using radiolabeled morantel in cattle, the following metabolic scheme has been proposed for biotransformation of the thiophene and tetrahydropyrimidine moieties:

Thiophene		Tetrahydropyrimidine
a) mercapturic acid(s)		a) tetrahydropyrimidine
b) hydroxylation		b) methylthiophene acrylic acid-N-methyl-1,3-propanediamine amide
c) hydroxylation conjugated		c) N-methyl-1,3-propanediamine-conjugated

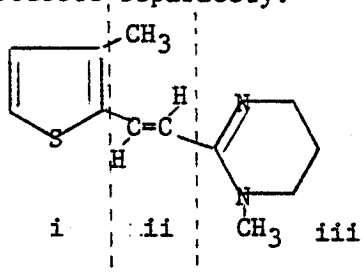
For additional details see NADA 92-444.

*Based on 0-24 hour sample.

(b) In Cattle Feces.

Cattle were medicated with morantel tartrate and the rate of degradation of the morantel excreted in the feces was determined. The rapid rate of photodecomposition observed in dilute aqueous solutions of morantel is also observed for morantel in cattle feces (Attachment 11). Morantel is quite soluble in water and is readily extracted from cattle feces so that any morantel not photodecomposed in the feces will be photodecomposed when it is exposed to the sun in the run off water. Any small amount which may not be extracted will be microbiologically degraded (See B3c).

(c) Microbiologically. Microbiological degradation studies have not been carried out with morantel, however, there is ample precedent in the literature to suggest that morantel is a suitable substrate for various soil microorganisms. Morantel has no activity against bacteria and fungi and is therefore susceptible to transformation reactions of either (Attachment 5). For purposes of this analysis, morantel will be considered as a molecule containing three chemical moieties as shown below and each will be discussed separately:



i. thiophene portion
ii. C=C portion
iii. pyrimidine portion

i. Thiophene portion. Thiophene and thiophene derivatives are found in crude oil, and microorganisms have been used to remove these sulfur compounds[1][2][3] thus improving the fuel quality of the oil fractions. The bacterial degradation of thiophene and methyl thiophenes by a Pseudomonas aeruginosa isolated from soil has been reported.[2] In an in vitro system, 2-methylthiophene was 41% degraded and 3-methylthiophene was 87% degraded within 4 days. The authors conclude that 3-methylthiophene is more rapidly and extensively degraded than BT (benzothiophene) and other related compounds. Cripps[4] has examined the mechanism of the microbiological thiophene ring cleavage in thiophene-2-carboxylate by an organism isolated from soil. The products of the degradation were SO_4 and 2-oxoglutaric acid. Thiophene acrylic acid was rapidly reduced by hydrogen in the presence of Clostridium kluyveri, a soil anerobe.[4a] Thus it is established that soil microorganisms can degrade the thiophene portion of morantel.

ii. C=C portion. Carbon-carbon double bonds are transformed by various soil microorganisms by (1) hydration of the double bond to produce a mono-alcohol, (2) the formation of epoxides which can be readily converted to diols and (3) direct transformation to diols.[5][6][7] 1-Heptene is converted microbiologically to 4-pentenoic acid,[8] 1-octene to 1,3-epoxyoctane,[8] hexadecene to 1,2-hexadecanediol[8] was shown to be metabolized by a soil

bacterium, A TCC 27042, via glycolate and glyoxylate.[9] Thus, it is established that carbon-carbon double bonds are quite susceptible to microbial degration.

iii. Pyrimidine portion. The microbial transformation of pyrimidine derivatives has been the subject of several published studies. Pyrimidine molecules are transformed by (a) hydroxylation, (b) ribosidation, (c) cleavage of the ring and (d) hydrogenation. For example, thymine is converted by cell-free extracts of Norcardia corallena to malonic acid and urea and then further to carbon dioxide and ammonia[10] 5-methylbarbituric acid is metabolized to methylmalonic acid and urea by intact cells of Corynebacterium sp.[11] Brevibacterium ammoniagenes has been demonstrated to degrade the pyrimide ring in 5-fluorouracil to α-fluoro-β-guanidinopropanoic acid, α-fluoro-β-ureidopropanoic acid, and α-fluoro-β-alanine.[12] The pyrimidine, orotic acid is degraded by cell-free extracts of the anaerobic soil microorganism Zymobacterium oroticum.[13] Thus, pyrimidines are susceptible to degradation by soil microorganisms.

(d) Photochemically. Section A-2 of this report discusses the photolability of morantel in dilute aqueous solution.

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4. Effects on the Environment

No primary or secondary impact of this action on the environment is forseen. Anthelmintics are used only sporadically in cattle. It has been estimated that only 3000-kilos of morantel per year will be released to the environment via treated cattle. Data have been presented which demonstrate that the manufacturing, formulation and distribution of morantel lead to unsubstantial amounts of morantel or any toxic pollutants being discharged to the environment. Exposure to morantel of personnel administering morantel to cattle is minimum.

Morantel has a low order of toxicity for mammals. The structurally closely related anthelmintic, pyrantel, with the same pharmacological properties and similar chemical properties as morantel, has been shown to be a well-tolerated and effective anthelmintic for poultry.* Metabolism studies using ^{14}C labeled morantel have shown that only morantel metabolites are excreted in cattle urine, while a maximum of approximately 45% of the ingested dose is excreted in feces as unchanged morantel. The effects of morantel on the environment via cattle feces are expected to be negligible.

Morantel has a narrow spectrum of pharmacological activity; the microbiological activity is restricted essentially to gastrointestinal helminths. It was inactive in the following tests: (1) in vitro against a spectrum of Gram-positive and Gram-negative bacteria (Attachment-3); (2) foliar fungicide (Attachment-6); (5) in vivo against chicken coccidiosis (Attachment-7) and a post-harvest fungicide test (Attachment-8). No phytotoxicity was observed during the foliar fungicide, soil fungicide or insecticide testing in which plant seedlings were used.

Morantel in cattle feces is degraded. Any morantel extracted from feces by rain water before it can be inactivated would rapidly be photochemically degraded when exposed to light and also be expected to be transformed by soil microorganisms.

Data demonstrating the lack of hazard to man ingesting meat from cattle treated with morantel have been presented.

C. Evaluate alternatives to the proposed action:

Inasmuch as no significant impact on the environment is anticipated, no alternatives appear necessary.

D. Describe the relationship between local short-term uses of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity:

The local short-term uses of the proposed action would have no effect on maintenance or enhancement of long-term productivity of the environment.

*Chickens have received 100 gms/ton of pyrantel tartrate in their rations continuously for 28 days without adverse affects, while 100 mg/kg of body weight administered to geese via drinking water was demonstrated to be non-toxic and efficacious against stronglyle infections.

E. Describe any irreversible and irretrievable commitment of resources which would be involved in the proposed action should it be implemented:

There would be no major commitment of resources with implementation of the proposed action. Only the negligible amount of energy and raw materials consumed in the manufacturing process, none of which constitute a significant commitment of resources, would be required.

F. Discuss the objections raised by other agencies, organizations or individuals which are known to the applicant:

There have been no objections by other agencies, organizations or individuals which are known to the applicant.

G. If proposed action should be taken prior to 90 days from the circulation of a draft environmental impact statement, or 30 days from the filing of a final environmental impact statement, explain why:

No such action is proposed. We submit that the agency should find that there is no requirement for an Environmental Impact Statement.

H. Analyze whether the benefit to the public of the proposed action will outweigh the action's potential risks to the environment:

Because there is a need for increased beef production in the United States, the proposed action would definitely benefit the public. If the animal disease for which the action is proposed can be controlled, cattle would be produced more economically and decrease the amount of time required for an animal to reach marketable size. This would result in increased beef production. No significant risk to the environment is recognized.

I. Certification.

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

_____ (Date)

_____ (Signature of responsible official)

_____ (Title)

The Photolability of Morantel

Summary

The impact of excreted Morantel on the environment can be partially assessed by determining its rate of photoisomerization in ultra-violet light from the biologically active trans isomer to the biologically inactive cis isomer. These studies have shown that a 10 ppm solution of Morantel isomerizes to the cis-isomer with a half-life of 9.1 minutes when exposed to 366 nm light. Therefore, Morantel excreted from treated animals would be rapidly converted to the biologically inactive cis isomer and have little effect on the environment.

Results and Discussion

Experimental: A solution of trans-Morantel (CP-12,009) (10 µg/ml) was exposed to long wavelength (366 nm) ultra-violet light and samples collected between 0 and 20 minutes of exposure. The solution was prepared with 0.1 N H_2SO_4 and read in this solvent. All samples were scanned with a Carey model 15 spectrophotometer from 400-220 nm in 1 cm cells. The concentration of trans - Morantel was calculated with experimentally determined extinction coefficients and by the two component method of calculation described below. A material balance for total components (trans-plus cis-Morantel) was computed by utilizing the absorbance at the isoabsorptive point.

Two Component Method of Calculation: To determine the concentration of trans - Morantel in the presence of the cis isomer (CP-12,732-18) a correction to the absorbance observed for overlapping spectra, must be made. Extinction coefficients for both isomers at 318 nm and at their isoabsorptive point are determined. Standard solutions of equal concentration of both isomers at three dilutions were prepared and scanned from 400-220 on the same chart. All solutions of trans - Morantel (CP-12,009-18) were protected from light with aluminum foil. The scans are shown in Figure 1 and the results given in Table 1. The extinction coefficients for trans-Morantel at 318 nm, cis-Morantel at 318 nm, and both isomers at their isoabsorptive point, 283 nm, were 486, 138, and 290, respectively. These values were then utilized to calculate the concentration of trans-Morantel in the presence of the cis isomer.

The mathematical expression used to calculate the concentration of trans-Morantel in the presence of the cis isomer is given in equation 1.

1)
$$[T] = \frac{A_{(318)} - \frac{E^{1\%}_{1cm}\ cis\ (318)}{E^{1\%}_{1cm}Iso_{(283)}} \times A_{(283)}}{E^{1\%}_{1cm}Trans_{(318)} - E^{1\%}_{1cm}\ cis_{(318)}} = \text{Concentration of trans-Morantel}$$

Where: $A_{(318)}$ = absorbance at 318 nm

$A_{(283)}$ = absorbance at 283 nm

$E^{1\%}_{1cm}Iso_{(283)}$ extinction coefficient of both isomers at their isoabsorptive point (283 nm).

$E^{1\%}_{1cm}Trans_{(318)}$ = extinction coefficient of trans-Morantel at 318 nm

$E^{1\%}_{1cm}cis_{(318)}$ = extinction coefficient of cis-Morantel at 318 nm.

Substitution of the appropriate extinction coefficients and expressing results in µg/ml yields equation 2.

2)
$$[T] = \frac{A_{(318)} - 0.476 \times A_{(283)}}{346} \times 10^4 = \text{Concentration of trans-Morantel in µg/ml.}$$

Determination of Total Components: The concentration of total components (trans plus cis-Morantel) may be made utilizing the absorbance at the isoabsorptive point and equation 3.

3)
$$[T] + [C] = \frac{A_{283} \times 10^4}{290}$$

Isomerization of Morantel: The isomerization of trans-Morantel to the cis isomer occurs with a half-life of 9.1 minutes as shown in Table 2. Calculations for total components (trans-plus cis-Morantel) indicates no change in concentration. Therefore, in the presence of sunlight residues of Morantel would be rapidly converted to the biologically inactive isomer and result in negligible impact on the environment.

Table 1

Calculation of Extinction Coefficients for CP-12,009-18 and CP-12,732-18.

Sample	Concentration (g/100 ml)	Absorbance (λ) 318 nm	283 nm
CP-12,732-18	0.003992	0.550	1.150
Cis-isomers	0.001996	0.275	0.583
	0.000998	0.135	0.290
CP-12,009-18	0.004008	1.940	1.150
trans-isomer	0.002004	0.978	0.583
	0.001002	0.486	0.290

Extinction Coefficients $[E^{1\%}_{1cm}\ (\lambda)\ CP\text{-}xxxxx\text{-}xx] = \frac{\text{Absorbance } (\lambda)}{\text{Concentration (g/100 ml)}}$

1) $E^{1\%}_{1cm}$ Trans (318) CP-12,009-18 = 486

2) $E^{1\%}_{1cm}$ Trans (318) CP-12,732-18 = 138

3) $E^{1\%}_{1cm}$ Iso (283) $\begin{matrix}\text{CP-12,009-18}\\ \text{CP-12,732-18}\end{matrix}$ = 290

Table 2

Isomerization of trans - Morantel Tartrate in 0.1 N H_2SO_4 upon exposure to 366 nm ultra-violet light.

Exposure Time (minutes)	Absorbance (nm)		µg/ml	
	318	283	1	2
0	0.499	0.285	10.50	9.8
0.5	0.478	0.288	9.85	9.9
1.0	0.470	0.289	9.61	10.0
1.5	0.460	0.283	9.40	9.8
2.0	0.450	0.285	9.09	9.8
2.5	0.442	0.290	8.79	10.0
3.0	0.440	0.283	8.82	9.8
5.0	0.399	0.290	7.54	10.0
10.0	0.329	0.292	5.49	10.1
20.0	0.219	0.300	2.20	10.3

$T_{\frac{1}{2}}$ = 9.1 minutes

1 Concentration of trans- Morantel calculated by the following formula:

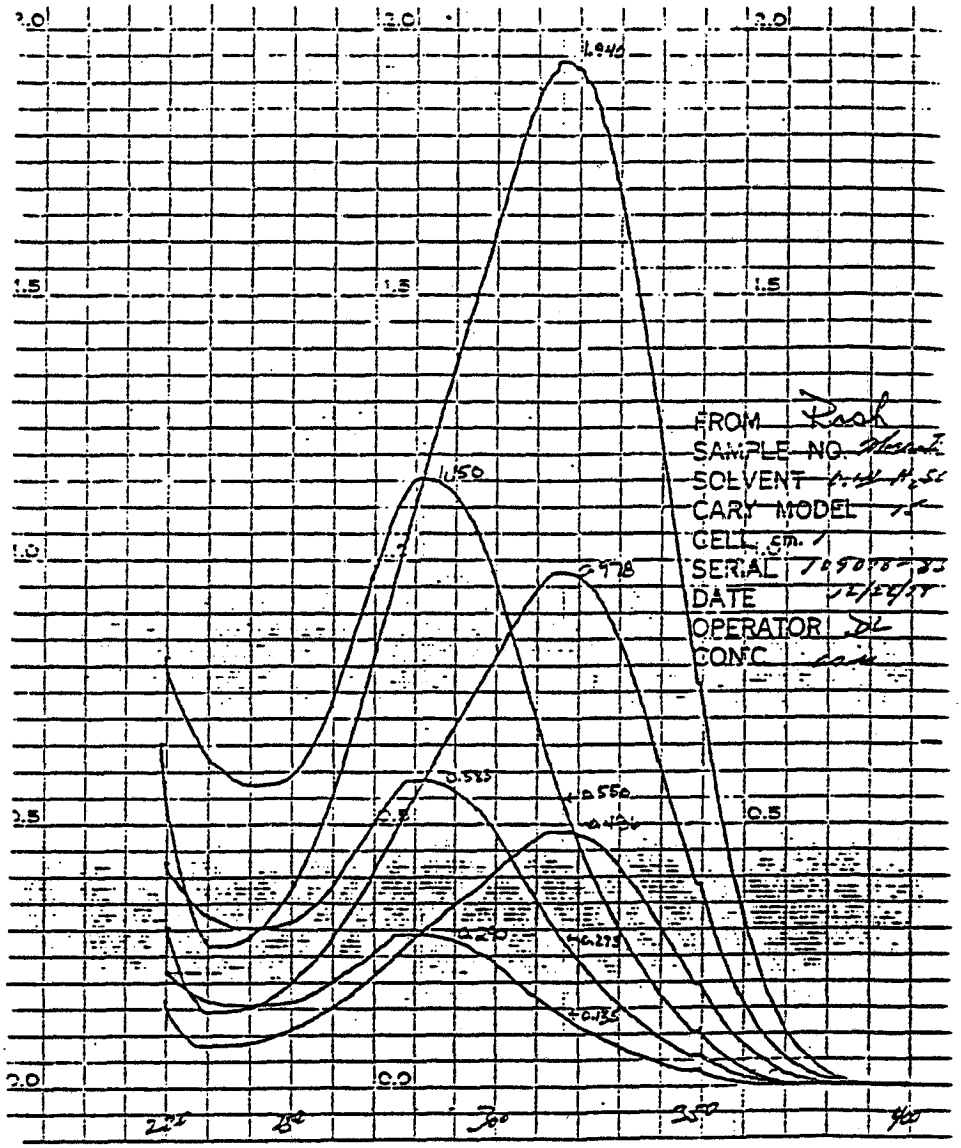
$$[T] = \frac{A_{(318)} - 0.476 \quad . \ A_{(283)}}{346}$$

2 Concentration of trans-plus cis-Morantel calculated by the following formula.

$$[T] + [C] = \frac{A_{283} \times 10^4}{290}$$

Figure 1

Calibration Curves of Morantel Isomers for Determining Extinction Coefficients



Attachment 2

Partition of Morantel Between Water and Chloroform as a Function of pH

Procedure and Results.

A series of buffer solutions at pH 2, 3, 4, 5, 6, 7, 8, 9, and 10 were prepared. Morantel solutions made from these buffers were extracted with chloroform and the concentration of morantel remaining in the aqueous buffer and that present in the chloroform was determined. The concentrations were used to calculate the partition coefficient. Figure 1 illustrates a plot of concentration of morantel in the aqueous phase and in the organic phase versus pH. At about pH 9 morantel begins to be extractable into the organic phase. Commonly found ecology conditions in the environment have a pH range of ~4-8. Therefore, morantel is not expected to be taken up by plants. Figure 2 illustrates the plot of partition coefficient versus pH and again until pH 9 the coefficient remains very small.

Conclusion. Morantel salts are not readily extractable from aqueous solution with a typical water-immiscible organic solvent, chloroform. Partitioning is not observed to any extent except above a pH of 9 at which point a portion of the morantel salt has been converted to the base form.

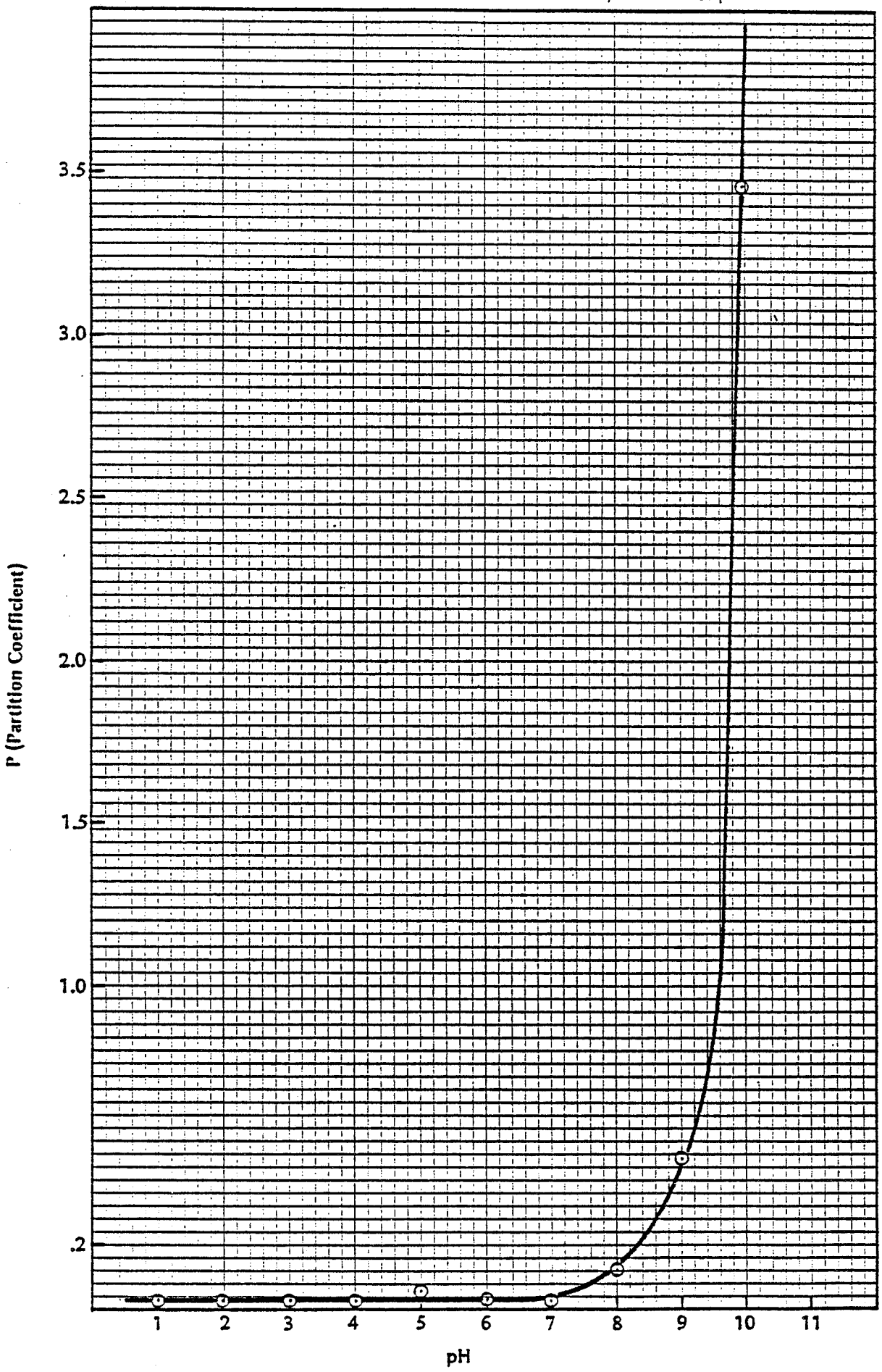
000107

MORANTEL TARTRATE

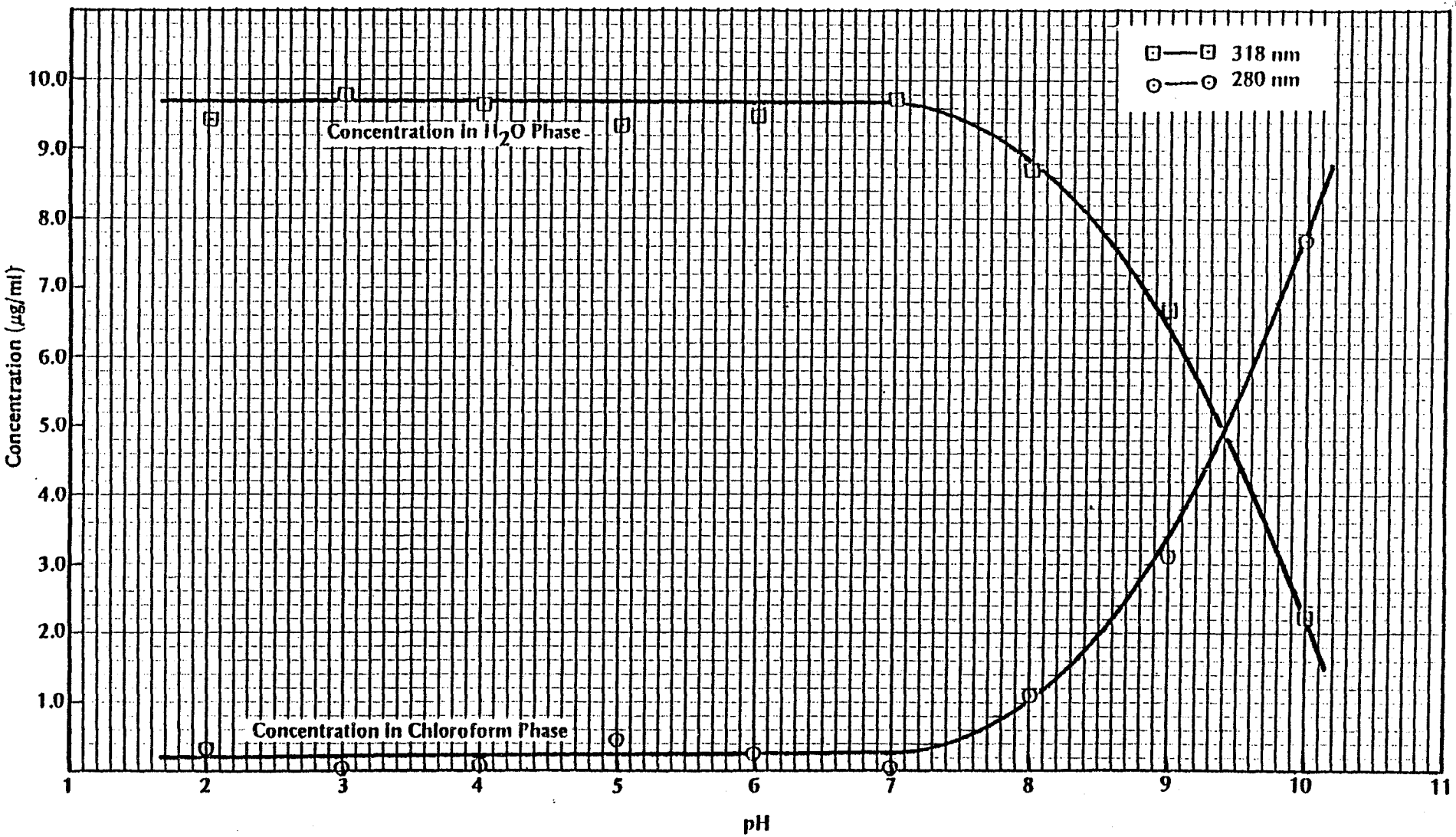
PARTITION COEFFICIENT – CHLOROFORM/WATER vs. pH

1545

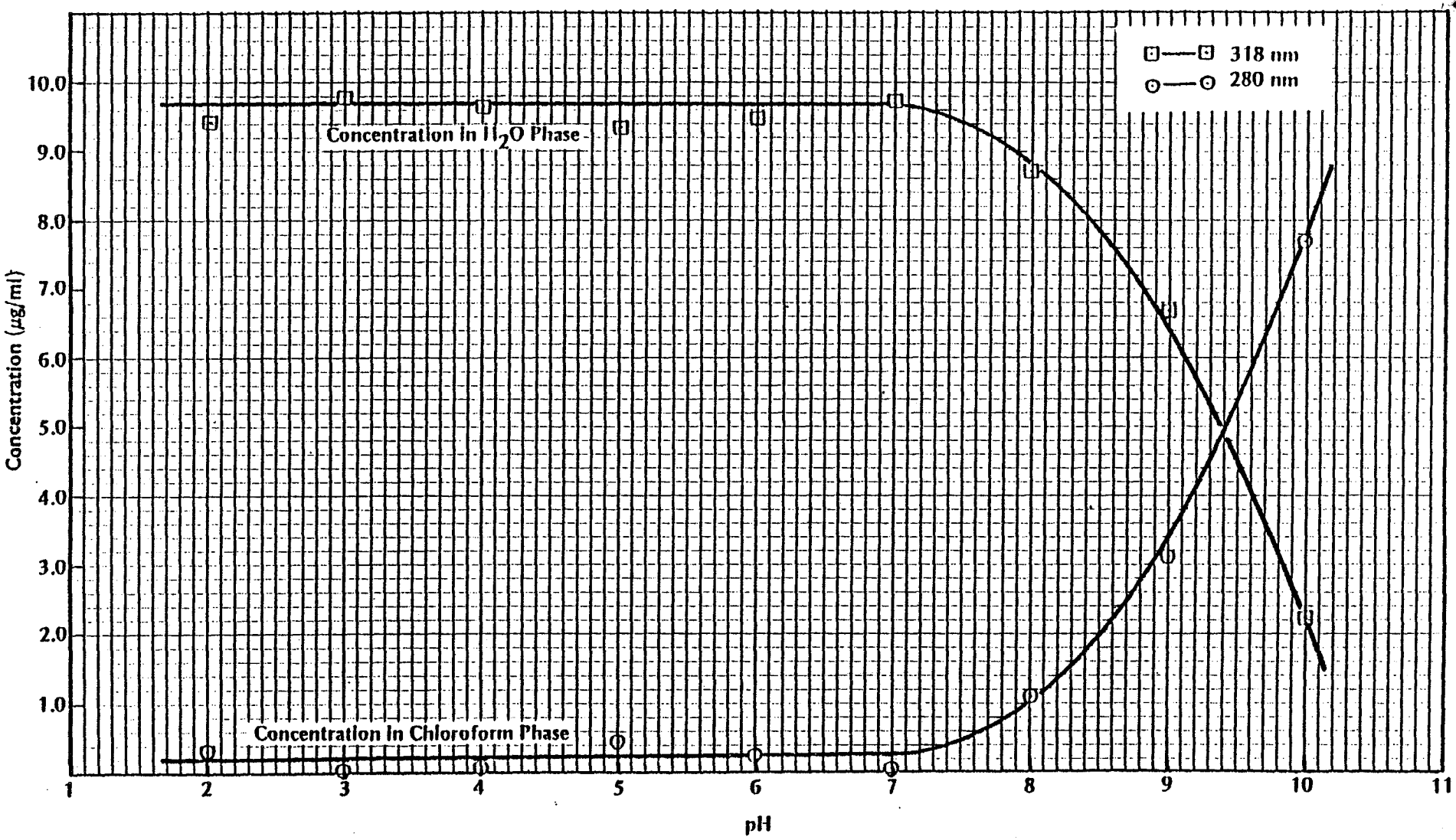
28



MORANTEL TARTRATE – CONCENTRATION vs. pH



MORANTEL TARTRATE – CONCENTRATION vs. pH



Attachment 3

Activity of Morantel and the Cis Isomer Against a Spectrum of Gram-Negative and Gram-Positive Bacteria In Vitro

Procedure:

The sensitivity of the various micro-organisms to an antibiotic is determined by the commonly accepted two-fold serial dilution technic. Final concentrations of compound per ml. range from 100 mcg. in the first tube to 0.19 mcg. in the tenth tube. The inoculum consists of 0.5 ml. of a 1×10^{-3} dilution of a standardized culture. Final volume in each tube or cup in the DisPoso tray is 1.0 ml. The tubes are incubated at 37°C for approximately 24 hours. The medium used is Witkins synthetic or Brain Heart Infusion (BHI). The sensitivity (MIC - minimal inhibitory concentration) of the test organism is accepted as the least amount of compound capable of producing complete inhibition of growth as evidenced by the absence of gross turbidity. Morantel tartrate and the cis isomer were tested by this procedure against a spectrum of Gram-negative and Gram-positive bacteria.

Results:

Microorganism	Morantel MIC (mcg/ml)	Cis Isomer MIC (mcg/ml)	Microorganism	Morantel MIC (mcg/ml)	Cis Isomer MIC (mcg/ml
Staph. aureus (1)	>200	>200	Entero. aer.	>200	
Staph. aureus (1)		>200	Serratia mar.		
Strep. pyogenes	50		Past. mult.	>200	>200
E. coli (2)	>200	>200	H. influenzae	>200	
E. coli (2)	>200		B. subtilis	>200	
Pseud. aer. (3)	>200	>200	C. perfringens	50	
Pseud. aer. (3)	>200		C. sporagenes	200	
Pseud. aer. (3)	>200		B. fragilis	>200	
Salm. typhosa	>200		Citrobacter	>200	
Klebsiella pn.(4)	>200		A. aerogenes		>200
Klebsiella pn.(4)	>200		(1),(2),(3),(4) - different strains		
Strep. pyogenes		>200			

Conclusion. Morantel tartrate and the Cis isomer are inactive against Gram-negative and Gram-positive bacteria.

Attachment 4

Efficacy of Morantel as a Foliar Fungicide; Lack of Injury to Test Plants

Procedure. The activity of morantel against a spectrum of foliar fungi and one virus was tested according to the attached protocols.

Results.

Morantel Application	Test Procedure	Fungus	% Control	Plant	Plant Injury
2000 ppm	(1)	Tobacco mosaic virus(a)	48	Bean	0
500 ppm	(2)	Rice blast disease(b)	36	Rice	0
15 lbs/A	(3)	Tomato fusarium wilt(c)	0	Tomato	0
15 lbs/A	(4)	Tomato early blight	0	Tomato	0
15 lbs/A	(5)	Cucumber powdery mildew(d)	0	Cucumber	0

(a) Positive control: CYPREX 2000 ppm was 93% effective.

(b) Positive control: BLASTOCIDIN 20 ppm was 99% effective.

(c) Positive control: BENLATE 15 ppm was 46% effective.

(d) Positive control: BENLATE 15 ppm was 100% effective.

Conclusions. Morantel has been tested either by direct application to the infected plant or by soil drench against four species of foliar fungi and one virus. Limited activity was demonstrated against the virus and one fungus; there was no activity against the other three fungi. Morantel did not injure (1) bean plants by soil drench (2) rice plants by foliar spray (3) tomato plants by soil drench, or (4) cucumber plants by soil drench.

TOBACCO MOSAIC VIRUS (TMV)

PROTECTANT: BEANS

TEST PROCEDURE - 1

Bean plants, Phaseolus vulgaris L. var. Scotia, in suitable growth stage (early first trifoliate leaves) are sprayed with candidate compound and allowed to air dry. Inoculation with virus (TMV) is then accomplished by lightly wiping the leaf surface with a cotton pad (or "Q"-tip) moistened by TMV suspension containing Carborundum. After drying, plants are maintained in the greenhouse until lesions appear, usually within four to eight days. Cyprex at 2000 PPM is currently used as a reference standard.

Soil drench systemic protectant treatments are applied two days prior to inoculation with TMV virus as above. Initial treatment rates for screening purposes are in the range of 25 ml. of a 150 PPM solution per three-inch diameter (six ounce capacity) pot. No suitable reference standard is available.

Sample requirements: 50 mg. per three replicates at 1000 PPM or
25 mg. per three replicates at 500 PPM.

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FOLIAR FUNGICIDE

TEST PROCEDURE-2

RICE BLAST DISEASE, PROTECTANT, Piricularia oryzae

Rice plants in fully developed second-leaf growth stage are mounted on a compound turntable and sprayed at 40 pounds pressure for 60 seconds with the candidate compound at concentrations indicated. Approximately 150 ml. of test solution are delivered. Candidate samples are prepared for spraying by dissolving in a suitable solvent (acetone, methyl alcohol, ethyl alcohol or other) and diluting to desired concentration with deionized water containing wetting and dispersing agents.

After drying, treated plants are spray-inoculated at 30 pounds pressure with an aqueous spore suspension of Piricularia oryzae and then immediately placed in an incubation chamber maintained at 70°F and 95% plus RH. After proper incubation time plants are removed to the greenhouse for disease development. Infection lesions are sufficiently developed within five days after inoculation to permit assessment of control. Disease severity is determined by actual count of the number of infection lesions developing on untreated inoculated controls. Effectiveness of treatment is determined by direct comparison of the number of infection lesions appearing on the respective treated plants compared directly with those lesions appearing on untreated inoculated controls. All units of test include a minimum of three replicates.

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FOLIAR FUNGICIDE

TEST PROCEDURE-3 (SOIL DRENCH)

FUSARIUM WILT OF TOMATOES, SYSTEMIC PROTECTANT, Fusarium oxysporum

Bonny Best tomato plants, Lycopersicon esculentum, approximately four weeks old, eight to ten inches tall, in true leaf stage,are used as host plants. Candidate compounds dissolved in a suitable solvent system and diluted to appropriate concentrations with deionized water are applied to the soil surface of respective containers of tomato plants which in turn are returned to the greenhouse. Forty-eight (48) hours after application of soil drench treatments, plants are removed from original containers without disturbing soil and root balls and placed overnight in new cups containing 40 ml spore suspension (2,000,000 conidia per ml). The next morning all plants are transferred and repotted in 4.0 to 4.5 inch pots and maintained under greenhouse conditions for observations of wilt development. Wilt symptoms are generally well advanced within three weeks following inoculation. All units of test include a minimum of three replicates. Effectiveness of treatments is determined by direct comparison with inoculated controls. There is no effective reference standard available. Ratings of disease control or development are recorded according to the following and converted to equivalent percentage disease control:

Disease Rating	Disease Incidence
0	No symptoms
1	Trace Wilt
2	Slight Wilt
3	Moderate Wilt
4	Severe Wilt
5	Complete Wilt

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FOLIAR FUNGICIDE

TEST PROCEDURE-4 (SOIL DRENCH)

EARLY BLIGHT OF TOMATOES, SYSTEMIC PROTECTANT, Alternaria solani

Bonny Best tomato plants, Lycopersicon esculentum, approximately five to six weeks old, in five-leaf growth stage, are used as host plants. Candidate compounds dissolved in a suitable solvent system and diluted to appropriate concentrations with deionized water are applied to the soil surface of respective containers of tomato plants which in turn are returned to the greenhouse.

Two days after treatment subject plants are spray-inoculated with a spore suspension of Alternaria solani and immediately placed in an incubation chamber maintained at 70°F and 95% plus RH. After 40 hours in the incubation chamber, plants are removed and observed for total infection lesions of the top three leaves. Effectiveness of treatments is determined by direct comparison with inoculated controls. There is no effective reference standard available. All units of test include a minimum of three replicates.

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FOLIAR FUNGICIDE

TEST PROCEDURE-5 (SOIL DRENCH)

POWDERY MILDEW OF CUCUMBERS, SYSTEMIC PROTECTANT, Erysiphe cichoracearum

Straight-eight cucumber plants in first true leaf stage, approximately 14-18 days old, are used as host plants. Candidate compounds dissolved in a suitable solvent system and diluted to appropriate concentrations with deionized water are applied to the soil surface of respective containers of cucumber plants which in turn are returned to the greenhouse.

Two days after treatment subject plants are placed among diseased (Erysiphe cichoracearum) cucumber plants, (see diagram below), subjected to an initial spore shower by dusting with spores from diseased plants and then left undisturbed in place for approximately ten days. By this procedure treated plants are subjected to the cited initial spore shower as well as to continuing natural infection pressure from surrounding inoculum. Observations ten days after initial inoculation determine duration of effectiveness of treatments. Untreated controls will reflect 75% to 100% leaf area diseased at this time. Effectiveness of treatment is determined by direct comparison with untreated inoculated controls. Benlate is used as a reference standard. All units of test include a minimum of three replicates.

M M M
X X
M M M
X X
M M M
X X
M M M

M: Diseased Plants

X: Sprayed Plants

Attachment 5

Efficacy of Morantel Against Soil Fungi; Lack of Injury to Test Plants

Procedure. Morantel was evaluated by soil incorporation tests for efficacy against Rhizoctonia Solani, Sclerotum Rolfsii, Pythum ulfimium and Fusarium solani according to the attached procedures.

Results; Conclusions.

Morantel was used at the rate of 50 pounds per acre. There was no effect on the soil fungi nor on suitable plant species with which the infected soils were seeded (sugar beets and beans). Captan, Dexon and PCNB used as positive controls were effective.

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SOIL FUNGICIDE

TEST PROCEDURE

DAMPING OFF ORGANISMS, PROTECTANT, PYTHIUM SPECIES

Oospore suspensions of Pythium ultimum (or Pythium aphanidermatum), examined with haemocytometer for oospore (plus chlamydospore) numbers per ml., are blended with dry sterilized soil in concentration to effect twice the desired fungal concentration (1000 oospores per gram of soil on a dry weight basis).

Candidate compounds dissolved in a suitable solvent are added at concentrations to effect twice the desired chemical concentration to a unit of sterilized soil in a sealed soil blender and blended therein for a specified period of time. Chemically-treated soil is then added to an equivalent unit of Pythium-inoculated soil, placed in the soil blender and blended therein for a specified period of time. Resulting blend of Pythium-inoculated and chemically-treated soil is divided equally into suitable containers, seeded with suitable plant species, water sealed to prevent loss of potential chemical vapor phase and removed to the greenhouse.

Controls include sterile soil, sterile soil plus chemical, sterile soil plus Pythium inoculum and Dexon-treated Pythium-inoculated soil as a reference standard.

Disease severity is determined by actual count of surviving plants in Pythium-inoculated soil compared to equivalent counts in sterile soil. Control effectiveness of candidate compounds is determined by actual count of surviving plants in respective treatments compared to equivalent counts in Pythium-inoculated soil. All units of test include a minimum of three replicates.

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SOIL FUNGICIDE

TEST PROCEDURE

FUSARIUM SOLANI, PROTECTANT

Spores of Fusarium solani in sufficient concentration to effect twice the desired fungal concentration are added on a dry weight basis to sterile soil, placed in a sealed soil blender and blended therein for a specified period of time.

Candidate compounds dissolved in a suitable solvent are added at concentrations to effect twice the desired chemical concentration to a unit of sterilized soil in a sealed soil blender and blended therein for a specified period of time. Chemically-treated soil is then added to an equivalent unit of Fusarium-inoculated soil, placed in the soil blender and blended therein for a specified period of time. Resulting blend of Fusarium-inoculated and chemically-treated soil is divided equally into suitable containers, seeded with suitable plant species, water sealed to prevent loss of potential chemical vapor phase and removed to the greenhouse.

Controls include sterile soil, sterile soil plus chemical, sterile soil plus Fusarium inoculum and Captan-treated Fusarium-inoculated soil as a reference standard.

Disease severity is determined by actual count of surviving plants in Fusarium-inoculated soil compared to equivalent counts in sterile soil. Control effectiveness of candidate compounds is determined by a disease rating based on a zero (no disease present) to five (equivalent to 100% diseased) scale. All units of test include a minimum of three replicates.

SOIL FUNGICIDE

TEST PROCEDURE

RHIZOCTONIA SOLANI, PROTECTANT

Sufficient Rhizoctonia solani inoculum to effect twice the desired fungal concentration is added on a dry weight basis to sterile soil, placed in a sealed soil blender and blended therein for a specified period of time.

Candidate compounds dissolved in a suitable solvent are added at concentrations to effect twice the desired chemical concentration to a unit of sterilized soil in a sealed soil blender and blended therein for a specified period of time. Chemically-treated soil is then added to an equivalent unit of Rhizoctonia-inoculated soil, placed in the soil blender and blended therein for a specified period of time. Resulting blend of Rhizoctonia-inoculated and chemically-treated soil is divided equally into suitable containers, seeded with suitable plant species, water sealed to prevent loss of potertial chemical vapor phase and removed to the greenhouse.

Controls include sterile soil, sterile soil plus chemical, sterile soil plus Rhizoctonia inoculum and PCNB-treated Rhizoctonia-inoculated soil as a reference standard.

Disease severity is determined by actual count of surviving plants in Rhizoctonia-inoculated soil compared to equivalent counts in sterile soil. Control effectiveness of candidate compounds is determined by a disease rating based on a zero (no disease present) to five (equivalent to 100% diseased) scale. All units of test include a minimum of three replicates.

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SOIL FUNGICIDE

TEST PROCEDURE

SCLEROTIUM ROLFSII, PROTECTANT

Sufficient sclerotia of Sclerotium rolfsii to effect twice the desired fungal concentration are added on a dry weight basis to sterile soil, placed in a sealed soil blender and blended therein for a specified period of time.

Candidate compounds dissolved in a suitable solvent are added at concentrations to effect twice the desired chemical concentration to a unit of sterilized soil in a sealed soil blender and blended therein for a specified period of time. Chemically-treated soil is then added to an equivalent unit of Sclerotium-inoculated soil, placed in the soil blender and blended therein for a specified period of time. Resulting blend of Sclerotium-inoculated and chemically-treated soil is divided equally into suitable containers, implanted with two carrot slices, water sealed to prevent loss of potential chemical vapor phase and removed for subsequent observation.

Controls include sterile soil, sterile soil plus chemical, sterile soil plus Sclerotium inoculum and Captan-treated Sclerotium-inoculated soil as a reference standard.

Disease severity is determined by actual count of infection loci on carrot slices in Sclerotium-inoculated soil compared to equivalent counts in sterile soil. Control effectiveness of candidate compounds is determined by actual count of infection loci on carrot slices in chemically-treated soil compared to equivalent counts on carrot slices in Sclerotium-inoculated soil. All units of test include a minimum of three replicates.

Attachment 6

Efficacy of Morantel Against Miscellaneous Insects: Lack of Injury to Test Plants

Procedure. The activity of morantel against a spectrum of insects was tested according to the attached protocols.

Results.

Morantel Application (ppm)	Test Procedure	Insect	% Insect Control	Plant	Plant Injury
500(A)	(1)	Mexican bean beetle	0	Cranberry bean	0
500(A)	(1)	Southern army worm	0	Cranberry bean	0
500(A)	(2)	Two-spotted spider mite-contact	0	Cranberry bean	0
500(A)	(2)	Two-spotted spider mite-ovacidal	0	Bean	0
500(A)	(3)	Housefly	0	-	
50(B)	(4)	Two-spotted spider mite-systemic	0	Cranberry bean	0
50(B)	(5)	Pea aphids		Broad bean	0

(A)Azodrin at 200 ppm was used as a positive control and was 100% effective in all tests except in the ovacidal test against the two-spotted spider mite. (B) Azodrin at 10 ppm was used as a positive control and was 100% effective in all tests.

Conclusion. Morantel was ineffective against all insects tested and caused no injury to the host plants.

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INSECTICIDES

TEST PROCEDURE-1

FORMULATION

Candidate compounds for greenhouse insect and mite evaluations are dissolved in acetone or other suitable solvents and diluted to appropriate concentrations in deionized water containing wetting and emulsifying agents. Compounds for housefly contact spray evaluation are dissolved in acetone or other suitable solvent and then diluted to required concentration in Cyclohexanone-refined kerosene base.

MEXICAN BEAN BEETLE AND SOUTHERN ARMYWORM--Stomach Poison

Foliar portions of cranberry bean plants in first true leaf growth stage are dipped in agitated (magnetic stirrer) test solution, allowed to air dry and removed to holding racks provided with subterranean water source. Three test plants are used for each test unit.

Ten third-instar larvae each of Mexican Bean Beetle and Southern Armyworm are respectively caged on treated plants for 48 hours. Observations for insect mortality, feeding and phytotoxicity are made 48 hours after treatment.

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MITICIDES

TEST PROCEDURE-2

TWO-SPOTTED SPIDER MITE, Tetranychus urticae

A. Foliar Treatment

Potted horticultural beans at growth stage when primary leaves are approximately one inch long are infested with two-spotted spider mites twenty-four (24) hours prior to treatment, insuring establishment of adults and egg deposition at time of treatment.

Candidate compounds are dissolved in a suitable solvent (acetone, methanol or other) or prepared as wettable powders and diluted to appropriate concentrations with deionized water containing wetting and/or dispersing agents as appropriate.

Infested host plants, as above, are dipped in agitated solutions of the candidate compound, allowed to air dry, provided with subterranean water source and held for observation. Three plants are used for each unit of treatment.

Initial mortality and phytotoxicity are determined forty-eight (48) to seventy-two (72) hours after treatment by removing and observing one leaf from each plant. Final observations of mortality, ovicidal action and residual toxicity to emerging nymphs are made seven (7) days after treatment by removal and observation of the second primary leaf.

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INSECTICIDES

TEST PROCEDURE-3

HOUSEFLY CHEMOSTERILANT

By a bait test procedure candidate compounds are evaluated for toxicity and anti-fertility activity, using adult houseflies (Musca domestica) as the test organism.

BAIT PREPARATION

A treated bait is prepared by mixing six (6) parts dry milk, six (6) parts granulated sugar and one (1) part dry egg yolk to which is added and thoroughly mixed with an acetone (or other suitable volatile solvent) solution of the candidate compound. Acetone or other volatile solvent is then allowed to completely evaporate, intermittent mixing of the preparation to assure complete release of acetone or other solvent. If the candidate compound is not soluble in acetone or other suitable volatile solvent, it is mixed with the bait preparation by dry grinding. As a guide, 75 milligrams of candidate compound mixed with a finished treated bait of 7.50 grams will provide a one (1) percent preparation adequate for three (3) replicate baits of 2.50 grams each.

METHOD

Using suitable individual containers a treated bait of 2.50 grams, a moisture supply and 50 mature pupae are respectively placed in a holding cage. Using pupae assures that emerging adults are restricted to treated bait as a food source, precluding opportunity of other food source. Three (3) such cages are prepared for each unit of treatment. Three (3) to

four (4) days after adult emergence observations are made to determine repellency of treated bait. Mortality observations are made seven (7) to eight (8) days after emergence. If high mortality is induced, evaluation should be repeated at lower toxicant rates.

Oviposition medium is placed in respective cages, removed 24 hours later and replaced with new medium. Removal and replacement of oviposition medium continues on a 24-hour schedule until adequate eggs are collected or until all females have died. Eggs are removed to and incubated in appropriate rearing medium and observed for hatching and development through third (3rd) larval instar or through pupal development and adult F_1 emergence, as appropriate. Compounds indicating sterility (no egg deposition) or anti-fertility (non-viable eggs) are reevaluated at reduced toxicant rates. At either of the foregoing development stages numbers of progeny are compared with those from comparable stages in untreated controls. Successive generations may be observed, as appropriate.

Untreated controls determine fecundity, length of life cycle stages, egg viability and normal life span of adults.

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INSECTICIDES

TEST PROCEDURE-4

TWO-SPOTTED SPIDER MITES, Systemic

Cranberry bean plants grown under greenhouse conditions, in first true leaf growth stage and in soil of low moisture content are infested during a two-hour period with Two-Spotted Spider Mites (Tetranychus telarius). Twenty ml. of an aqueous solution of the candidate compound are applied to the soil as a surface drench. Twenty-four hours later plants are provided subterranean watering for 48 hours. Percentage mite mortality and plant injury are observed 96 hours after application of the candidate compound to the soil surface. All test units are in triplicate.

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INSECTICIDES

TEST PROCEDURE-5

PEA APHIDS, Systemic

Windsor broad bean plants grown under greenhouse conditions, in first true leaf growth stage and in soil of low moisture content are treated with 20 ml. of an aqueous solution of the candidate compound applied as a surface drench. Twenty-four hours later plants are provided subterranean watering for 72 hours. Forty-eight hours after application of candidate compound to soil surface, Pea Aphids (Macrosiphum pisi) adults are transferred to and caged on foliar portions of treated plants for 48 hours. Insect mortality and plant injury are then observed. By this procedure test plant is afforded 48 hours for uptake of candidate compound before insects are exposed to foliage and an additional 48 hours for uptake during which insects are exposed to feeding on the foliage. All test units are in triplicate.

Attachment 7

Activity of Morantel Against Chicken Coccidiosis In Vivo

Procedure.

Eight-day-old New Hampshire Red chickens (60 to 70 Gm.), which are highly susceptible to Eimeria tenella, were used in this experiment. The E. tenella was selected because it is ordinarily more sensitive to drug action than other species of coccidia.

The drug was administered directly into the crop in a volume of 0.25 ml. with a 2-inch, blunt-end, 18-gauge needle attached to a 2-ml. tuberculin syringe. The E. tenella inoculum was introduced in the same manner.

On the first day of the experiment, the drug was administered in the morning; on the second day, it was given in the morning and the chickens were inoculated with 50,000 sporulated oocysts of E. tenella during the afternoon. On the third, fourth, and fifth days, the drug was given to complete the five-day schedule. Test animals were held until the eighth day, when they were necropsied and examined for evidence of infection. The efficacy of the drug was judged by the prevention of mortality and by a comparison of the pathologic index with that of the unmedicated infected controls. At necropsy, the degree of pathologic involvement was expressed as the average degree of infection, based on the following scheme: 0 = no lesions in cecum; 1 = slight lesions; 2 = moderate lesions; 3 = severe lesions; and 4 = death due to infection. Five chickens were used for the test compound and the positive control, while ten were used in the infected non-medicated and non-infected, non medicated groups.

Results.

Test Groups	Dose	% Wt. Gain	Pathological Index 0 1 2 3 4
Morantel-infected	28 mg/kg	30	2 3
Infected Control		51	5 5
Non-infected Control		100	10
Positive control-infected	38 mg/kg	99	5

Conclusion. Morantel has no activity against an E. tenella coccidial infection in chickens.

Attachment 8

In Vivo Efficacy of Morantel Against Various Post-Harvest Decay Fungi

Procedure. Morantel was tested for activity against 15 fungi implicated in post-harvest decay of fruits and vegetables according to the following procedure.

Materials

A. Glassware and Culture Containers

Vials, 18 x 60 mm, screw caps with teflon liners

Bottles, milk-dilution, 160 ml capacity, with screw caps with teflon liners; each bottle calibrated at 55 and at 60 ml.

Culture tubes, 16 x 125 mm, screw caps with teflon liners; tubes calibrated at 10 ml (sterile)

Petri dishes, polystyrene, 15 x 100 mm (sterile)

Pippettes, 6 ml, serological (sterile)

Pipettes, capillary, disposable (sterile)

Droppers, calibrated at 1 ml (sterile)

B. Chemicals, solutions, and culture medium

Dimethylsulfoxide (DMSO), sterile (autoclave 15 PSI/15 min)

.01% aqueous Triton X-100, sterile, (autoclave 15 PSI/15 min)

Distilled water, sterile

Culture medium—weight 45 g.

Dehydrated potato dextrose agar (PDA) and 2 g. yeast extract

(Difco) into a 2-liter Erlenmyer flask. Add distilled water to the 1-liter mark on the flask and heat on hot plate-stirrer until agar is dissolved. While still stirring, dispense medium through bottom tap on the flask (55 ml medium per milk dilution bottle and approximately 3 ml per 18 x 60 mm vial).

C. Test Fungi

	Species	JWE No.
1.	Phytophthora citrophthora	012C
2.	Sclerotinia fructicola	D1A
3.	Botrytis cinercea	M11C
4.	Geotrichum candidum	G109
5.	Alternaria citri	M15C
6.	Diplodia natalensis	S2C
7.	Penicillium digitatum	M6B
8.	Aspergillus niger	M8A
9.	Fusarium oxysporium	M14C
10.	Phomopsis citri	S3A
11.	Thielaviopsis paradoxa	M22B
12.	Glomerella cingulata	A3F
13.	Rhizopus stolonifera	Z6A
14.	Gloesporium musarium	
15.	Thielaviopsis paradoxa	

Preparation of Inoculum for screening test

1. Dispense approximately 3 ml PDA medium into each 18 x 60 mm vial. With caps loosened, autoclave vials plus medium at 15 PSI for 15 min. Position vials with melted PDA at approx. 45° angle so that agar will solidify as a slant.

2. Place a few ml sterile 0.01% Triton X-100 in stock tubes of the test fungi and disperse spores and/or hyphal pieces with the aid of a wire loop. By means of a disposable capillary pipette (sterile) transfer a drop of spore suspension of each fungus to 6 screw cap vials with PDA slants. Replace screw caps loosely and incubate cultures at 24°C for 15 days. All fungi except Phytophthora and Diplodia should shown abundant sporulation at this time. Screw caps down tightly, place entire vial rack in a polyethylene bag and store in 1°C room until inoculum is required in screening test. These 6 vials of will provide sufficient inoculum for about 6 weeks of screening.

Procedure for Screening Test Compounds

I. Agar dilution screening (standard procedure for all test compounds)

A. Introduction of test compound into the agar medium

1. Weighing:

a) dry solids—pulverize in sample cotnainer with an alcohol-flamed rod and weigh 12.0 mg into a tared weighing boat.

b) liquids—by means of a disposable capillary pipette, weigh approximately 12 mg into a tared weighing boat. In steps 4 and 5 below, correct for departures from 12.0 mg weighing

by taking aliquots containing exactly 6.0 mg and 0.60 mg of the test compound, rather than the 5.0 ml and 0.5 ml aliquots specified.

c) semi-solids—weigh out approximately 12 mg and apply aliquot correction in steps 4 and 5 as described for liquids.

2. Position the boat containing the weighed sample in the mouth of a sterile 16 x 125 mm screw cap culture tube (calibrated at 10 ml). In the case of dry solids, transfer the major portion of the sample to the tube by tapping the boat. Wash the remaining sample from the board with the aid of 1 ml dimethylsulfoxide delivered from a calibrated dropper. Wash liquids from boat to tube in an identical manner.

3. After the test compound has dissolved in the dimethylsulfoxide, bring the contents of the tube to 10 ml by the rapid addition of sterile distilled water from a polyethylene wash bottle.[1/] Immediate shake the tube to disperse any of the test compound that may precipitate when the dimethylsulfoxide solution is flooded with water.

[1/] If the test compound is known or suspected to be strongly hydrophyllic, then the weighing boat should be rinsed with sterile water. Also some compounds of this type may not be soluble in dimethylsufloxide, but will dissolve when this solvent is flooded with water.

4. When the dimethylsulfoxide-water dispersion of the test compound appears to be homogeneous, draw up 5 ml in a 5 ml serological pipette and discharge by blowing into 55 ml of melted PDA medium (55-60°C). Rock the bottle to disperse the test compound in the medium and pour approximately 15 ml of the melted agar into each of 4 petri dishes. The concentration of the test compound is 100 γ/ml.

5. As in (4) above, transfer a 0.5 ml aliquot of the homogeneous dispersion of the test compound to another bottle containing 55 ml agar, bring to the 60 ml graduation with sterile distilled water dispensed from a wash bottle, and rock the bottle to disperse the test compound. Pour 15 ml of medium into each of 4 petri dishes. Concentration of the test compound is 10 γ/ml.

6. Control.—Pipette 0.5 ml of sterile dimethylsulfoxide into a third bottle containing 55 ml melted agar and bring to the 60 ml graduation with sterile distilled water. After mixing, pour 15 ml of this "control" medium into each of 4 petri dishes.

B. Seeding the Agar Plates with the Test Organisms

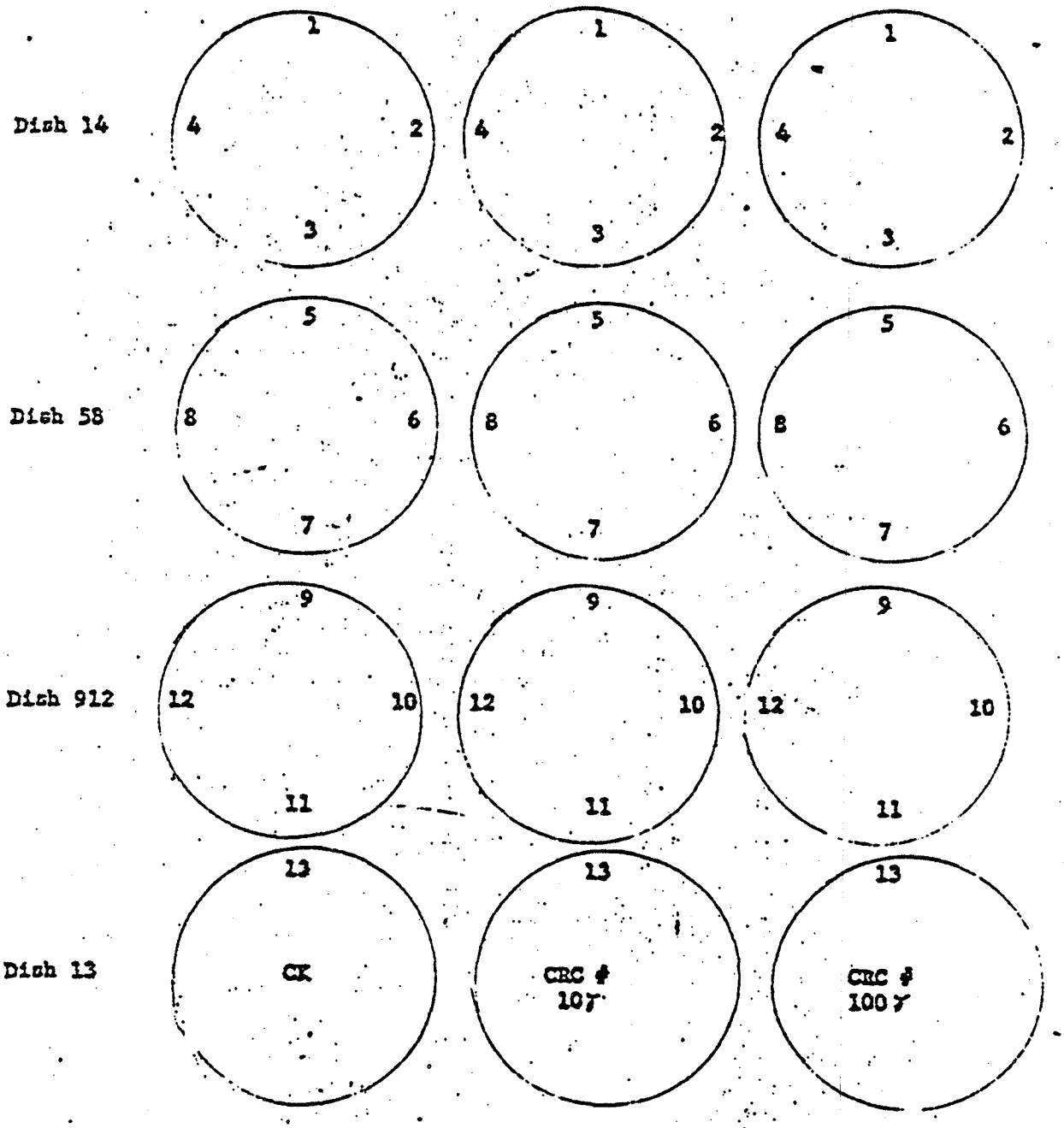
1. Arrange in a column on the bench top the 4 "control" petri dishes. In parallel columns, line up the 4 dishes containing the medium at 10 γ/ml and at 100 γ/ml. Continue in like manner with dishes containing other test compounds. A maximum of 10 compounds can be conveniently run on a single occasion.

2. With a black felt ink pen, mark our CBC accession number for the test compound in the center of the dish, and the concentration of the compound in the medium beneath. Mark the number of 4 test fungi around the periphery of each petri dish. The layout for the petri dishes is illustrated on the next page.

3. Inoculum of test fungi.—Add approximately 2 ml of sterile 0.01% Triton X-100 solution to one vial of inoculum of each of the test fungus species. Shake the vial vigorously to suspend the spores. For Diplodia and Phytophthora scrape the surface of the agar to break loose hyphal pieces which will serve as inoculum for these fungi.

4. Dip a flamed loop into the inoculum vial of test fungus 1 (Phyto phthora citrophthora). Lift up the bottom of petri dish "14" and stab the agar at position 1 with the loop carrying mycelial pieces of Phytophthora. Dip the loop in the inoculum vial once again and stab the agar within several millimeters (on an arc) of the first inoculation site.[2/]

[2/] This dual inoculation procedure gives greater assurance that each inoculation site did receive viable inoculum and that complete lack of growth at that site is due entirely to the inhibitory properties of the test compound.



Layout for Petri Dishes for

Inoculation with Test Fungi

Both inoculations should be made immediately next to the wall of the petri dish.

5. Repeat the inoculation procedure described above for the remainder of the petri dishes in the first row. Continue in like fashion with the remaining 13 fungi.

C. Incubating Test Fungi and Assessing Fungitoxic Activity of Test Compounds

1. Stack each row of petri dishes (those with same fungi) in a tray, with "control" dish on top. Separate stacks a reasonable distance so that slightly volatile compounds do not affect neighboring petri dishes.

2. Incubate the plates at 25°C and measure the centripetal radius (perpendicular to the wall of the dish) of the fungus colonies after the prescribed time periods:

Dish 14 .... 5 days

Dish 58 .... 5 days

Dish 912 .... 3 days

Dish 13 .... 3 days

Results.

No.	Fungi	100 mcg/ml.	10 mcg/ml.
1	Phytophthora citrophthora	Tr	+++
2	Sclerotina fructicola	Tr	+
3	Botrytis cinera	+++	++++
4	Geotrichum candidum	+	+++
5	Alternaria citri	-	+++
6	Dipoldia natelensis	++	++++
7	Penicillium digitatum	+	+++
8	Aspergillus niger	+	+++
9	Fusarium oxysporum	++	+++
10	Phomopsis citri	Tr	+
11	Thielaviopsis paradoxa	Tr	+
12	Glomerella cingulata	+	++
13	Rhizopus stolonifera	++	+++
14	Gloesporium musarium*	Tr	+
15	Thielaviopsis paradoxa*	+	++

*(U.S. Fruit isolate)

Control = ++++ (each + reported = 25.0% of control)

Tr = trace of activity.

Conclusion. Morantel has only limited activity against 15 species of fruit/vegetable decay fungi at a concentration of 100 mcg/ml and is essentially inactive at a concentration of 10 mcg./ml.

Attachment 9

In Vivo Anthelmintic Activity - Morantel vs. the Cis Isomer

Procedure.

Twenty gram, albino, male mice (four to eight/group) with infections of mature parasites were treated p.o. with a single dose of drug. Twenty-four hours after the treatment the animals were sacrificed and examined for worms present. The results obtained were compared with those from untreated, infected controls (four to eight/group). Activity is expressed as number of mice cleared/number of mice infected. Pfizer diluent is the standard medium used in dosing the mice with compound.

Activity against the parasites used in this test is predictive of activity against parasites of domestic animals and man and is used as an anthelmintic primary screen. Morantel and its cis isomer were evaluated in the test.

Results.

Compound	Drug Level Mg/Kg P.O.	No. Mice Cleared/No. Infected		
		H. nana	N. dubius	S. obvelata
Morantel	6.25x1	0/4	4/4	0/4
	3.125x1	0/4	4/4	1/4
	1.56x1	0/4	2/4	0/4
	0.78x1	0/4	0/4	0/4
Cis isomer of Morantel	250x1	0/5	1/5	0/5
Test 1	125x1	0/8	1/8	0/8
	62.5x1	0/4	0/4	0/4
Test 2	250x1	0/4	0/4	0/4
	125x1	0/4	0/4	0/4

Neither compound is active against H. nana or S. obvelata. Against N. dubius morantel is active at a dose of 1.56 mg/kg while the cis isomer shows questionable activity at 250 mg/kg.

Conclusion. The only noteworthy biological activity shown by morantel is as an anthelmintic. The cis isomer of morantel is at least 150 times less active as an anthelmintic than is morantel and is probably less active than that.

000150

Attachment 10

DRUG METABOLISM

December 12, 1978

TO: Dr. I. A. Solomons

FROM: M. J. Lynch

SUBJECT: Octanol/water partition coefficients for the cis- and trans-isomers of morantel tartrate

At your request, I have examined the partition coefficients of morantel tartrate (CP-12,009-18) and its cis-isomer (CP-12,732-18) in octanol/water and octanol/0.1NHCl. The partition coefficients were determined by a standard procedure utilizing uv absorption spectrophotometry.

Partition Systems	Partition Coefficient (p)	
	cis-isomer	trans-isomer
Octanol/Water	<0.1	<0.1
Octanol/0.1NHCl	<0.1	0.14

M. J. Lynch

MJL:hgn

cc: Dr. M. von Schach

PFIZER ORGANIC LABORATORY

ASSISTANT'S MANUAL

Compiled and edited by: Drs. L. J. Czuba, H. Faubl, J. J. Hamsher, C. A. Harbert, D. E. Kuhla, and J. K. Larson.

August 1970

XVII. DETERMINATION OF PARTITION COEFFICIENT

A. Purification of n-Octanol

n-Octanol: Bp 194-5°, mp 16-17°, d=0.827, n_D^{20} = 1.430, insol. H_2O, miscible with ROH, $CHCl_3$, Ether.

Wash octanol 2x with small volume of 3N KOH (Note: octanol and water layers separate slowly); wash with water until neutral; wash 2x with sat'd NaCl; dry over Na_2SO_4.

Distill at 12 mm (first cut 30-40° is an azeotrope if octanol not completely dry). Octanol distills at 88°C (12 mm) or at 94°C (17 mm).

Saturate octanol with solvent to be used - usually 0.1N HCl in water. Shake desired volumes of octanol and solvent together vigorously for about one minute and let stand overnight. If temperature changes shortly before using, solutions may become cloudy otherwise they are usually clear.(1)

B. Stock solution of unknowns

In general use solutions whose concn. = 10^{-4}M unless the ε value of the peak to be used is small (<2,000) or if a very large P value is expected. A concn. of 10^{-3}M will be concn. enough for most work.

1 x 10^{-3}M solution: 0.10 mmole of compound dissolved in 100 ml of 0.1N HCl solution saturated with octanol.

Where compound is not soluble, the saturated solution is filtered and diluted with 20 ml. additional saturated solvent.

C. Procedure

Place in each of four glass-stoppered bottles 20 ml of the above stock solution.(2) The solutions are now overlayed with the following volumes of saturated octanol: 20, 10, 5, & 2.5 ml to provide HCl/oct ratios of 1:1, 2:1, 4:1 and 8:1, respectively. Larger ratios are necessary when a large P is expected. This will provide 4 curves and the consistency of P will serve as a check as pertains to stability of compound, manipulation factors, limiting solubility in octanol, and/or "freak" partitions.

The two phase solutions are shaken for 10 min. in a vertical shaker. It is recommended that the glass stoppers be taped with adhesive tape.

The octanol that settles is removed. The most efficient method employs house vacuum. A filter flask is set up with a hose from the house vac connected to the joint provided on the flask. A rubber stopper is inserted into the top of the flask with a hole bored to accommodate a glass tube. To the outside end of the glass tube is attached vacuum tubing. Disposable pipettes are ideal for the removal of solvent. If the pipettes are placed in EtOH after use, octanol odor is reduced to a minimum.

Centrifuge one hour at maximum speed (w/o stoppers) and carefully remove the remaining octanol by the above mentioned method.

Pipette about 10 ml of each solution into suitable containers (generally 15 ml cent. tubes) and submit for UV. Have the baseline set using unsaturated HCl in the ref. cell and saturated HCl in the sample cell. This way, dilutions may be made with unsaturated HCl. Prepare about 1 liter of unsaturated HCl for the UV operator's use. Have one series run at a time, beginning with the most dilute sample (20/20) and working progressively thru until the last sample, the stock solution, is run. (Stock is, of course, the most concentrated.)

D. Calculations

Use O.D. values (corrected for dilution factor, if diluted before running UV) instead of concentrations; O.D. = optical density = concentration x constant.

$$P = \frac{\frac{\text{conc. in octanol}}{\text{volume of octanol}}}{\frac{\text{conc. in 1N HCl}}{\text{volume of .1N HCl}}} = \frac{\text{volume } H_2O}{\text{volume oct.}} \cdot \frac{OD_{stock} - OD_{sample}}{OD_{sample}}$$

Footnote 1. Saturate octanol and HCl at least one day before you plan to assay. Use a volume of HCl (ca. 500 ml.) sufficient for 4 or 5 runs. A larger ratio of octanol to HCl provides faster separations. (e.g. 2 liters octanol shaken w/500 ml 0.1N HCl). Because only relatively small volumes of 0.1N HCl can be obtained in this manner, it should be used only when time is the primary factor! Herein, all HCl referred to will be 0.1N saturated w/octanol unless otherwise indicated.

Footnote 2. To run a UV on a solution without dilution 8 ml is needed; therefore a minimum amount of 10 ml stock solution is used for each curve.

Footnote 3. Be sure rubber plugs are in metal centrifuge tubes!

Attachment 11

Fecal Degradation of Morantel

Summary: The degradation of morantel in cattle feces was investigated with feces collected from cattle after treatment (as a top-dressing) with morantel tartrate. After exposure to simulated sunlight under controlled laboratory conditions the photoisomerization of trans-morantel[1] to the biologically inactive cis-isomer was determined. Morantel degraded with a half-life of 4.2 hours. This rapid conversion of the biologically active trans-morantel to the biologically inactive cis-isomer in feces demonstrates that excreted morantel will have little adverse effect on the environment.

Results and Discussion

Experimental: Four Angus/Holstein cross steers weighing ~400 pounds were given 9.7 mg/kg morantel tartrate as a top dressing at the a.m. feeding for 4 consecutive days. Samples of feces were collected after the final dose on the fourth day of treatment. The depletion of morantel was determined.

A 150 g sample of feces was mixed with 150 ml distilled water in a Waring blender for 2 minutes. Ten gram aliquots of feces were placed in disposable petrie dishes and exposed to 366 nm ultra-violet light. Duplicate samples were taken at 0, 0.5, 1, 2, 4, and 8 hours exposure times and assayed for morantel.

Each sample was exposed to 366 nm ultra-violet light by placing on a 4' x 2' plate glass panel light by three 48" F-40 blacklights above and three below. The overhead lights were 2' above the samples and the lights below the plate were 0.5' away from the plate. The temperature of the room was 30°C.[1]

Analysis of Feces for Morantel: Individual petrie dishes containing 10 g of feces as described above were assayed. The contents of each dish were quantitatively transferred to 100 ml Nalgene test tubes with 50 ml 0.5 N $HClO_4$, mixed vigorously, and centrifuged at 1500 rpm for 5 minutes. A 20 ml aliquot of the $HClO_4$ layer was placed in a 250 ml separatory funnel and extracted three times with 50 ml $CHCl_3$. The extract was placed in a clean separatory funnel and the combined extracts washed with 10 ml 0.1 N NaOH. The $CHCl_3$ layer was transferred to a 3rd separatory funnel and the drug back extracted into 10 ml 0.1 N H_2SO_4. The 0.1 N H_2SO_4 was scanned with a Carey spectrophotometer from 400-220 nm in 1 (standards) or 2 cm cells. The concentration of trans-morantel was calculated as described below.

Method of Calculation: To determine the concentration of trans-morantel in the presence of the cis-isomer (CP-12,732-18) a correction for overlapping spectra of the two isomers must be made. Before this correction can be made the extinction coefficients for both isomers at 318 nm and at the isoabsorptive point (283 nm) are determined by scanning three dilutions of each isomer containing equal concentrations from 400-220 nm. All scans are recorded on the same strip chart. All solutions of trans-morantel (CP-12,009-18) were protected from light with aluminum foil. The scans are shown in Figure 1 and results summarized in Table 1. The extinction coefficients for trans-morantel at 318 nm, cis-morantel at 318 nm, and for both isomers at the isoabsorptive point 283 nm, were 486, 138, and 290, respectively.

The mathematical expression used to calculate the concentration of trans-morantel in the presence of the cis-isomer is given in Equation 1.

1.)
$$[T] = \frac{A_{(318)} - \frac{E^{1\%}_{1\ cm}\ cis\ (318)}{E^{1\%}_{1\ cm}\ Iso_{(283)}} \times A_{(283)}}{E^{1\%}_{1\ cm}\ Trans_{(318)} - E^{1\%}_{1\ cm}\ cis_{(318)}} \times 4.72 \times 10^4 = \text{concentration trans-morantel in } \mu g/g \text{ feces.}$$

where: $A_{(318)}$ = absorbance at 318 nm

$A_{(283)}$ = absorbance at 283 nm

$E^{1\%}_{1\ cm}Iso_{(283)}$ = extinction coefficient of both isomers at their isoabsorptive point (283 nm).

$E^{1\%}_{1\ cm}Trans_{(318)}$ = extinction coefficient of trans-morantel at 318 nm.

$E^{1\%}_{1\ cm}cis_{(318)}$ = extinction coefficient of cis-morantel at 318 nm

4.72×10^4 = dilution factor (2.5×10^4 for standards).

Substitution of the determined extinction coefficients in Equation 1 derives Equation 2. The concentration of both trans- and cis-morantel is calculated by Equation 3.

2.)
$$[T] = \frac{A_{(318)} - 0.476 \times A_{(283)}}{346} \times 5 \times 10^4$$

3.)
$$[T] + [C] = \frac{A_{(283)}}{290} \times 4.72 \times 10^4 = \text{Combined concentration of both isomers in } \mu g/g \text{ feces.}$$

where: $A_{(283)}$ = absorbance at 283 nm

290 = extinction coefficient at 283 nm

4.72×10^4 = dilution factor.

Therefore, the rate of isomerization of trans-morantel to its cis-isomer can be determined as well as a material balance of both isomers.

Standards: Process standards containing 25, 12.5, and 6.25 ppm morantel tartrate were prepared to determine the recovery of drug in the assay procedure. The results are shown in Table 2 and indicate that morantel is recovered in 99% yield.

Control feces from non-medicated cattle were fortified with 25, 12.5, and 6.25 ppm morantel tartrate and assayed to determine the recovery of the method in the presence of the biological matrix. In the presence of feces the extraction step is 53 percent efficient (Table 2). This figure was used to correct the final results given in Tables 3 and 4.

These results demonstrate that the method can accurately and reproducibly measure the amount of trans-morantel in the presence of feces thereby affording a method by which the rate of photoisomerization of morantel in feces can be determined.

Photodegradation of Morantel in Cattle Feces: Fecal samples obtained from medicated cattle as described earlier were assayed for trans-morantel and total isomer concentration by the method as presented. The results are presented in Table 3. Morantel photoisomerizes in cattle feces with a half-life of 4.2 hours as summarized in Table 4.

Excretion of morantel into the environment by treated cattle would have very little impact on the environment since the biologically active isomer (trans-morantel) would be rapidly degraded to its inactive isomer by sunlight.

References

[1]FR, Vol. 43 No. 132 page 29717.

Table 1

Calculation of Extinction Coefficient for CP-12,009-18 and CP-12,732-18.

Sample	Concentration (g/100 ml)	Absorbance (λ) 1 cm Cells 318	283
CP-12,732-18	0.003992	0.550	1.150
cis-isomer	0.001996	0.275	0.583
	0.000998	0.135	0.290
CP-12,009-18	0.004008	1.940	1.150
trans-isomer	0.002004	0.978	0.583
	0.001002	0.486	0.290

Extinction Coefficients $[E^{1\%}_{1\ cm}\ (\lambda)\ CP\text{-}XXXXX\text{-}XX] = \frac{\text{Absorbance } (\lambda)}{\text{concentration (g/100 ml)}}$

1.) $E^{1\%}_{1\ cm}(318)$ CP-12,009-18 = 486

2.) $E^{1\%}_{1\ cm}(318)$ CP-12,732-18 = 138

3.) $E^{1\%}_{1\ cm}(283)$ CP-12,009-18 / CP-12,732-18 = 290

Table 2

Recovery of Morantel Tartrate From Fortified Fecal Standards and Process Standards.

Sample	Fortification Level (ppm)	Absorbance 318 nm	Absorbance 283 nm	ppm[1] Recovered	% Recovery
Process St'd	25.0	0.494	0.310	25.0	100
Process St'd	12.5	0.246	0.160	12.3	98
Process St'd	6.25	0.126	0.088	6.1	99
				Average =	99
Fortified St'd	25.0	0.240	0.149	12.2	49
Fortified St'd	12.5	0.117	0.082	8.0	64
Fortified St'd	6.25	0.060	0.045	2.8	45
				Average =	53

[1] $[T] = \frac{A_{(318)} - 0.476 \times A_{(283)}}{346} \times 2.5 \times 10^4$ = Concentration of trans-morantel in µg/g.

Table 3

Degradation of Morantel by Photoisomerization
in Feces Excreted by Cattle After Medication

Exposure Time to[1] 366 nm Light (Hours)	Absorbance 318 nm	Absorbance 283 nm	ppm *trans*-morantel[2]	ppm *trans*-plus *cis*-morantel[3]
0	0.615	0.370	59.9	60.2
0	0.600	0.370	57.8	60.2
0.5	0.567	0.372	53.2	60.5
0.5	0.500	0.361	44.8	58.8
1	0.533	0.399	46.8	64.9
1	0.493	0.398	41.4	64.8
2	0.426	0.425	30.5	69.2
2	0.425	0.412	31.2	67.1
4	0.360	0.462	19.1	75.2
4	0.375	0.445	22.3	72.4
8	0.280	0.389	12.9	63.3
8	0.335	0.430	17.8	70.0

[1]Duplicate samples assayed at each time point.

[2]ppm *trans*-morantel $= \frac{A_{(318)} - 0.476 \times A_{(283)}}{346} \times 4.72 \times 10^4$

[3]ppm *trans*-plus *cis*-morantel $= \frac{A_{(283)}}{290} \times 4.72 \times 10^4$

Table 4

Summary of the Degradation of trans-morantel in Cattle Feces by Photoisomerization

Exposure Time (Hours)	ppm trans-morantel
0	58.8
0.5	49.0
1	44.1
2	30.8
4	20.7
8	15.4

T 1/2 = 4.2 hours

000163

October 22, 1979

TO: Dr. I. A. Solomons

FROM: J. L. Sardinas

SUBJECT: Toxline/Toxback, RTECS and Chemical Abstracts Condensates Computer-readable Files

In accordance with your request*, the contents of the following databases are outlined:

TOXLINE/TOXBACK

These databases are the National Library of Medicine's collection of computerized toxicology information containing over 540,000 references to published human and animal toxicity studies, effects of environmental chemicals and pollutants, adverse drug reactions and analytical methodology. Older information is in the Toxline Backfile, Toxback (which contains over 379,000 references). Dates covered range from as long ago as 1940 to 1979 (most, however, date from the 1960's to the present).

RTECS

This database, also available from the National Library of Medicine, represents the online file of the printed version of the Registry of Toxic Effects of Chemical Substances. RTECS is an annual, printed compilation prepared by the National Institute for Occupational Safety and Health (NIOSH), as mandated by the Occupational Safety and Health Act of 1970 (PL 91-596). The file contains much chemical information, such as, chemical formulas, CAS Registry Numbers and nomenclature, as well as, toxicological effects on humans and animals, threshold limit values, recommended standards in air, aquatic toxicity, etc. Over 32,000 compounds are listed.

CHEMICAL ABSTRACTS CONDENSATES

There are 3 databases provided by Lockheed, i.e., Files 2, 3 and 4 which store computer-readable references corresponding to the printed Chemical Abstracts. Coverage includes chemistry and chemical aspects of the life sciences. The files contain well over 4 million references. Coverage extends from 1967 to 1979.

*Morantel degradation products

J. L. Sardinas

JLS:njm